UNIVERSIDAD COMPLUTENSE DE MADRID

FACULTAD DE OPTICA Y OPTOMETRÍA

Departamento de Bioquímica y Biología Molecular IV

TESIS DOCTORAL

Estudio del mercado de futuros medicamentos y biomarcadores oculares, basado en análisis de datos de propiedad intelectual y de ensayos clínicos

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

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Director

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UNIVERSIDAD COMPLUTENSE DE MADRID

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TESIS DOCTORAL

Dirigida: Dr. Jesús Pintor Just y Dra. Almudena Crooke Álvarez

D. JESÚS PINTOR JUST, Y Dra. ALMUDENA CROOKE ÁLVAREZ, PROFESORES DEL DPTO. DE BIOQUÍMICA Y BIOLOGÍA MOLECULAR IV DE LA FACULTAD DE ÓPTICA Y OPTOMETRÍA DE LA UNIVERSIDAD COMPLUTENSE DE MADRID,

CERTIFICAN QUE:

D. Basilio Colligris, licenciado en Ingeniería de Telecomunicaciones por la Academia Militar de Atenas Grecia, ha realizado en el Dpto. de Bioquímica y Biología Molecular IV de la Facultad de Óptica y Optometría y bajo la dirección del que suscribe, el trabajo titulado "Estudio del mercado de futuros medicamentos y biomarcadores oculares, basado en análisis de datos de propiedad intelectual y de ensayos clínicos", que cumple las condiciones exigidas para optar al título de Doctor.

Madrid, a 30 de junio de 2016

D. Jesús Pintor Just
Catedrático de Universidad

Dra. Almudena Crooke Álvarez
Profesor Contratado Doctor

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CONTENIDOS

ABREVIATURAS

5-MCA-N AT	5-MetoxI-carbonIlamino-N-acetIltryptaminA
Ap_4A	Diadenosina tetrafosfato
ADDE	Ojo Seco Acuo-Deficiente
ADPIC	Aspectos de los Derechos de Propiedad Intelectual
AINE	Anti-Inflamatorios No Esteroideos
AMPc	Adenosina Monofosfato Cíclico
ARN	Ácido Ribonucleico
ARNm	ARN Mensajero
ASH	Hibridación Específica de Alelo
ASPE	Alelo de Extensión del Cebador Específico
BAFF	Factor Activador de Célula B perteneciente a la familia del TNF
CA	Anhidrasa Carbónica
CAI	Inhibidor de Anhidrasa Carbónica
CCI	Coeficiente de Correlación Intraclase
CFS	Tinción con Fluoresceína
CLEK	Longitudinal Evaluation of Keratoconus
CM	Músculo Ciliar
CMC	Carboximetilcelulosa
ConA	Concanavalina A
CsA	Ciclosporina A
DARC	Detección de Células Apoptóticas en la Retina
DEWS	Taller Internacional de Ojo Seco-Subcomité Diagnóstico
DRZ	Dorzolamida
EDE	Ojo Seco Evaporativo
FDA	Food and Drug Administration
GMPc	Guanosina Monofosfato Cíclico
GPCR	G-Protein-Coupled Receptor
GTN	Glaucoma de Tensión Normal
GTPasa	Guanosina Trifosfatasa
HCQ	Hidroxicloroquina
HPLC	Cromatografía Líquida de Alta Presión

HTM	Malla Trabecular Humana
I+D	Investigación y Desarrollo
ICAM-1	Integrina de Molécula Pequeña
IL-1	Interleucina-1
ipRGC	Células Ganglionares de la Retina Intrínsecamente Fotosensibles
JAK	Janus Quinasa
JOAG	Glaucoma Juvenil de Ángulo Abierto
LGCS	Lisamina de Tinción Conjuntival Verde
LOCF	Última Observación Realizada
mmHg	Milímetros de Mercurio
MMP	Metaloproteinasa de la Matriz
MT	Malla Trabecular
MYOC	Miocilin Trabecular Meshwork Inducible Glucocorticoid Response
NGF	Factor de Crecimiento Nervioso
NO	Óxido Nítrico
NR	Receptor Nuclear
OPTN	Optineurin
OTRI	Oficina de Transferencia de Resultados de Investigación
P2Y2	Receptor Purinérgico P2Y2
PG	Prostaglandina
PIO	Presión Intraocular
POAG	Primary Open-Angle Glaucoma
PPADS	Ácido Piridoxalfosfato-6-azofenilo-2',4'-disulfonico
RGC	Células Ganglionares de la Retina
RhoA	Miembro de Familia de Homólogos Genes Ras
ROCK	Rho-quinasa asociada
RZR/ROR	Retinoid Z Receptor/Related Orphan Receptor
SAA	Suero Amiloide A
SARM	Modulador Selectivo del Receptor de Andrógenos
SCID	Síndrome de Inmunodeficiencia Combinada Severa
SERM	Modulador Selectivo del Receptor Estrogénico
siRNA	RNA de Interferencia de Pequeño Tamaño
SNP	Polimorfismos de Nucleótido Único
STATS	Transductor de Señal y Activador de la Transcripción
TFBUT	Tiempo de Ruptura de la Película Lagrimal

TIMP-1	Inhibidores Hísticos de Metaloproteinasas-1
TNF-α	Factor de Necrosis Tumoral-alfa
TrkB	Tirosina Quinasa del Receptor B
TRPV1	Receptor Transitorio Potencial del Vaniloides-1
TYK2	Tirosina-Quinasa 2 no Receptora
VEGF	Factor de Crecimiento Endotelial Vascular
WDR36	WD Repeat Domain 36
XFS	Síndrome de Exfoliación

1. RESUMEN

Resumen en español

Introducción

Durante esta tesis doctoral, hemos buscado nuevas vías de investigación, que podrían ayudar a la industria farmacéutica-oftalmológica a organizar su estrategia a largo plazo, adaptándose a la evolución de las tendencias internacionales en I+D. En este sentido, hemos investigado la dirección y el futuro del mercado de medicamentos y biomarcadores a nivel ocular, basándonos en información adquirida del análisis de las patentes publicadas y de ensayos clínicos en curso.

En la realización de este estudio se ha investigado la aparición de posibles biomarcadores de glaucoma, con potencial para el diagnóstico, seguimiento y evaluación de esta patología y nuevos tratamientos que están actualmente en desarrollo.

El glaucoma es una enfermedad ocular, que causa la degeneración progresiva de las células ganglionares y daño en el nervio óptico. Como consecuencia de estos eventos se produce una pérdida progresiva e irreversible del campo visual que desemboca en una ceguera permanente no tratable. Frecuentemente el avance de la enfermedad es imperceptible para el paciente, que acude al especialista cuando la patología se encuentra en un estadio avanzado. La causa exacta del glaucoma es incierta. Existen indicios de que la presión intraocular elevada está relacionada con la mayoría de los casos de glaucoma. Por lo tanto, el control de la presión intraocular sigue siendo el principal aspecto tratable en la terapia antiglaucomatosa. La mayoría de los casos de glaucoma pueden ser controlados y detener la pérdida de la visión mediante el tratamiento.

Objetivos

Este trabajo centra su estudio en la innovación farmacológica específica de las neuropatías glaucomatosas, segunda causa de ceguera a nivel mundial, y el síndrome del ojo seco, patología de la superficie ocular cuyo número de casos está aumentando significativamente en la actualidad.

Resultados y Conclusiones

El análisis de la información recabada revela la aparición de moléculas novedosas con las que actualmente se trabaja en los centros de investigación, las nuevas perspectivas del sector y los esfuerzos económicos y humanos de las investigaciones en curso. Los resultados muestran prometedoras estrategias a corto y largo plazo para fomentar la investigación y desarrollo de nuevos medicamentos oculares.

La recién implementación de nuevas técnicas en las áreas de proteómica y metabolómica ha generado una intensa expectativa en la búsqueda de nuevos biomarcadores en la detección y diagnóstico de las patologías glaucomatosas. Prueba de este creciente interés es el gran número de estudios básicos encontrados en la literatura actual y el gran número de métodos diagnósticos patentados últimamente. Sin embargo, a pesar de la intensidad de esta búsqueda los datos obtenidos son vagos y no han cristalizado aún en una biomolécula capaz de diagnosticar o predecir la aparición de esta patología. A pesar de la expectativa causada por las innovaciones acontecidas en estas nuevas áreas, la investigación en el campo del diagnóstico molecular no se ha detenido, con la incorporación de prometedores biomarcadores. No obstante, los hallazgos realizados en este campo, la transferencia de los descubrimientos y las técnicas a la práctica clínica no se ha materializado aún en la actualidad. Este proceso cuenta con numerosas dificultades y obstáculos, haciendo impredecible la repercusión real y la practicidad de estas nuevas herramientas moleculares.

Por otra parte, en el diseño y la innovación farmacológica de esta enfermedad hemos constatado la existencia de tres líneas fundamentales o estrategias básicas encaminadas principalmente a reducir las desventajas asociadas a las terapias farmacológicas actuales. La mejora de los medicamentos existentes, el desarrollo de nuevas combinaciones de fármacos y el diseño de nuevos fármacos con mecanismo de acción innovador.

De entre éstas, la de mayor potencial e interés es el desarrollo de fármacos de acción novedosa. Entre estos, una de las líneas más destacadas es el estudio de la melatonina y sus derivados. Los datos hallados en la bibliografía estudiada demuestran que existe un interés considerable en el diseño y la síntesis de nuevos análogos, no sólo más estables metabólicamente, sino también más selectivos. Por consiguiente, un gran número de nuevos análogos de la melatonina han sido recientemente patentados. Una de las propiedades más interesantes de estos análogos es su capacidad para potenciar los efectos de fármacos hipotensores ya existentes, lo que permitiría una reestructuración de las terapias de combinación de combinación de fármacos ya existentes.

Existe además otro grupo de fármacos basados en inhibidores de las Rho quinasas. La vía de acción de esta nueva clase de fármacos se dirige al aumento en el drenaje del humor acuoso y el aumento del flujo sanguíneo en el nervio óptico, reportándose además retrasos en la apoptosis del nervio óptico.

A pesar de los esfuerzos realizados, la falta de conocimiento de los desencadenantes y los factores subyacentes a esta enfermedad, dificultan el avance en la incorporación de biomarcadores y fármacos que cumplan con las expectativas de especificidad y efectividad requeridos. Esta situación responde en última instancia a la gran complejidad de esta enfermedad, por lo que estimamos necesaria la creación de redes globales multidisciplinarias entre clínicos e investigadores, capaces de aunar esfuerzos en el esclarecimiento de las causas subyacentes de la enfermedad. Sin el adecuado impulso en estos avances, entendemos que la mejora en las terapias sería en cualquier caso sólo parcial y extremadamente costosa.

Otro caso de interés en el desarrollo de nuevos fármacos lo presenta el tratamiento del ojo seco. Esta patología está adquiriendo una gran relevancia debido al importante aumento en el número de casos. Frente a los tratamientos tradicionales, que corregían parcialmente la sintomatología de esta patología, durante los últimos años el desarrollo de nuevos fármacos para un tratamiento más específico, se ha convertido en un objetivo clave para la industria farmacéutica. El “pipeline” del ojo seco es fuerte, con 49 moléculas en diferentes fases de desarrollo en el año 2015. A diferencia de los tratamientos antiguos, los nuevos compuestos en estudio o desarrollo recogidos en este trabajo, centran su acción principal en el control de la inflamación, o la restauración de los niveles normales de cantidad y calidad de lágrima y mucina endógena.

La nueva dirección tomada por las compañías farmacéuticas, es la sustitución de los tratamientos basados únicamente en lágrimas artificiales, por el tratamiento de fenómenos relacionados con la patología, como es la inflamación. A pesar de estos avances, las causas que subyacen a esta enfermedad siguen sin ser abordadas farmacológicamente. En este sentido la búsqueda de estrategias eficaces en el tratamiento del ojo seco se ve irremediablemente frenada por la ausencia de un conjunto aceptado de criterios definitivos de evaluación de gravedad de la enfermedad.

La falta de consenso en la comunidad clínica y científica, derivada de la complejidad en la etiología de esta enfermedad, refleja de igual forma la ausencia de una prueba diagnóstica objetiva que resulte además efectiva en la evaluación de la gravedad de la enfermedad, y en la efectividad de los tratamientos.

En este sentido creemos que la investigación de biomoléculas que resuelvan de forma eficaz el papel de biomarcador, debe ser establecido como estrategia básica y fundamental en el futuro tratamiento de esta patología. La aparición de nuevos estudios sobre moléculas como el Ap_4A, revela la existencia de potenciales biomarcadores, disponibles para el diagnóstico objetivo, no invasivo y con capacidad para ser trasladado al uso clínico; aumentando las expectativas sobre la mejora en el desarrollo de los futuros tratamientos.

Summary in English

Introduction

During this thesis, we have sought new avenues of research that could support pharmaceutical ophthalmological industry to organize their long-term strategy, adapted to the changing trends in Research & Development. In this sense, we have explored the course and future of the ophthalmological pharmaceutical and biomarkers market, based on analysis of information acquired through published patents and ongoing clinical trials. The analysis of information revealed the development of novel molecules and new prospects for the ongoing research of the sector. The results demonstrate the existence of promising strategies in the short and long term for the development of new ocular drugs.

Objectives

This thesis is focused on the study of pharmacological innovation of glaucomatous neuropathies, which is the second leading cause of blindness worldwide, and the dry eye syndrome, which is an ocular surface pathology where the number of cases is rising significantly today.

Results & Conclusions

Glaucoma is an eye disease that causes progressive degeneration of ganglion cells leading to optic nerve damage. As a result of these events occurs a progressive and irreversible loss of visual field, leading to a permanent untreatable blindness. Often the disease progression is imperceptible to the patient who comes to the specialist when the disease is at an advanced stage. The exact cause of glaucoma is uncertain, even though there is evidence that elevated intraocular pressure is associated with most cases of glaucoma. Therefore, control of intraocular pressure remains the main aspect treatable in antiglaucoma therapy today. Most cases of glaucoma can be controlled and stop vision loss by treatment.

In conducting this study, we additionally investigated the development of potential biomarkers of glaucoma diagnosis. These biomarkers could monitor and evaluate the disease progress and the application of new treatments. The recent implementation of new techniques in the areas of proteomics and metabolomics has generated intense expectation in the pursuit for new biomarkers for the early detection and diagnosis of glaucomatous pathologies. Proof of this growing interest is the large number of basic studies in current literature and the large number of patented diagnostic methods. However, despite these progressive data, after their analysis,

it becomes evident that for the moment is not existing a definitive biomolecule capable of diagnosing or predicting the onset of the disease. It is obvious that, the transfer of discoveries and techniques into clinical practice has not yet materialized. This process has many difficulties and obstacles, making unpredictable the actual impact and practicality of these new molecular tools.

Concerning the glaucoma treatment, we have found that there are three basic lines and strategies aimed primarily at reducing the disadvantages associated with current drug therapies. These strategies include the improvement of existing drugs, the development of new drug combinations and the design of new compounds, applying innovative mechanisms of action.

Of these new strategies, the one with greatest potential and interest is the development of drugs with novel action. Among these, one of the most important lines under study, is the development of melatonin and its derivatives. The data found in the literature demonstrates a considerable interest in the design and synthesis of new melatonin analogues, not only more metabolically stable, but also more selective. Therefore, a large number of new melatonin analogues have recently been patented. One of the most interesting properties of these analogues, is its ability to potentiate the effects of existing antihypertensive drugs, which would allow a restructuring of combination therapies combination of existing drugs. Another strong candidate for glaucoma treatment is a group based on Rho kinase inhibitors. The course of action of this new class of drugs is directed to the increase of the aqueous humour outflow, the increase of the optic nerve blood flow and a reported delay in optic nerve apoptosis.

Despite the efforts, the lack of knowledge of the triggers and the underlying disease factors, is hindering the progress in incorporating new biomarkers and drugs, meeting the expectations of specificity and effectiveness required. For these issues we could blame the complexity of this disease, so we estimate as necessary the creation of multidisciplinary global networks among clinicians and researchers capable of joining forces in elucidating the underlying causes of disease. Without proper momentum in these advances, we believe that improved therapies would be only partial and in any case extremely costly.

Another case of interest in the development of new drugs for the treatment of dry eye syndrome. This condition is becoming more relevant due to the significant increase in the worldwide number of cases. Compared to traditional treatments, which partially correct the symptoms of this disease, in recent years the development of new drugs for more specific treatment, it has become a key target for the pharmaceutical industry. The dry eye "pipeline"

is strong, with 49 molecules in various stages of development only in 2015. Unlike traditional treatments, the new compounds under study or development studied in this work, focus their main action on the inflammation control, or the restoration of normal levels of quantity and quality of endogenous tear and mucins.

The new direction taken by pharmaceutical companies, where traditional treatment was based solely on artificial tears, is the cure of inflammation phenomena. Despite these advances, the underlying causes of the disease remain unaddressed pharmacologically. In this sense the search for effective strategies in the treatment of dry eye is inevitably hampered by the absence of an accepted set of definitive criteria for assessing disease severity.

The lack of consensus in clinical and scientific community, is derived from the complexity in the aetiology of this disease, reflected similarly from the absence of an objective diagnostic test, effective in evaluating disease severity, and treatment effectiveness.

In this regard we believe that the research of biomolecules acting effectively as biomarkers, should be established as a basic and fundamental strategy in the future treatment of this disease. The emergence of new studies on molecules like Ap_4A, reveals the existence of potential biomarkers available for disease diagnosis, being non-invasive and capable for clinical use, and is increasing the expectations for improvement in the development of future treatments.

2. INTRODUCCIÓN

La continua innovación es una de las características más substanciales de la industria farmacéutica. Los nuevos fármacos pueden influir en la calidad y la duración de la vida humana en formas que hace años era impensable [1]. Por otra parte, la sostenibilidad de esta industria depende de la introducción de nuevos fármacos exitosos que tengan continuidad. En este contexto el potencial de las ventas mundiales de fármacos es inmensa: La estimación del mercado farmacéutico internacional para el año 2015 fue de 1.100 millones de dólares [2].

Sin embargo, la innovación farmacéutica no es un proceso totalmente ordenado, ni predecible. Depende considerablemente de los avances científicos y casi siempre es difícil predecir los resultados y la adecuación de los nuevos fármacos al organismo humano [3]. La invención de nuevos fármacos es un proceso de negocio con necesidad de una disciplina fiscal estricta y decisiones de gestión estratégica, organizativa y eficaz. Además, es necesaria una competencia tecnológica, décadas de rigurosa y profunda investigación y sobre todo es imprescindible la comprensión de las necesidades insatisfechas de los clientes-pacientes [4]. Por ello, parece necesaria y oportuna una revisión global de los procesos, las estrategias y de las prácticas relacionadas con la I+D de la industria farmacéutica.

Actualmente, en muchos países, el procedimiento de los derechos de la propiedad intelectual es considerado como un mecanismo que proporciona los necesarios incentivos para la comercialización de los resultados de investigación farmacéutica. Los últimos datos internacionales muestran un importante incremento en el número de solicitudes de patentes presentadas por las universidades y la industria [5]. Además se han promulgado políticas de promoción de la transferencia de tecnología entre la universidad y la industria, y las universidades han adoptado políticas de propiedad intelectual y transferencia de tecnología, estableciendo oficinas para la gestión de los derechos de su propiedad intelectual [6].

Las universidades juegan un papel importante en el avance de las fronteras de la ciencia y de la tecnología. Asumiendo el hecho de que resultados relevantes en investigación quedan sin ser transferidos a la sociedad, se ha gestado un creciente interés en la búsqueda de los marcos más adecuados para promover la colaboración entre la universidad y la industria con el fin de facilitar el trasvase de conocimiento y tecnología entre ambas. Por consiguiente los últimos años la riqueza del conocimiento generada dentro de las universidades se puede transferir a la

industria para que la sociedad en general, y las empresas en particular, puedan beneficiarse de los conocimientos científicos y tecnológicos de la universidad [7].

En España las oficinas de transferencia de la propiedad intelectual de las universidades se llaman Oficinas de Transferencia de Resultados de Investigación (OTRI) y están creadas con el objeto de promover la colaboración y la transferencia de tecnología entre la Universidad y el sector empresarial [8]. Dentro de sus labores se encuentran las relacionadas con la creación de empresas basadas en la investigación universitaria (spin-off) [8]. Esta función se considera como una vía importante para la comercialización de las nuevas tecnologías y en particular cuando la naturaleza de la tecnología no permite asumir el riesgo de la comercialización de una determinada invención [9].

La existencia de estas pequeñas-medianas empresas, aumenta significativamente la cantidad de nuevos fármacos en desarrollo, teniendo en mente que las empresas farmacéuticas, poseen un número muy reducido de proyectos en sus departamentos de I+D.

Esta reducción del número de proyectos dentro de las empresas farmacéuticas responde a un nuevo marco económico en el que los nuevos medicamentos destinados al mercado internacional parecen carecer del potencial de ingresos de sus predecesores. Durante los años 1990-94, 11 nuevos medicamentos alcanzaron el “top 100 de medicamentos” en términos de ventas globales [10]. A partir de 1995-99, 10 nuevos medicamentos aprobados se hicieron con la categoría del “top 100 de medicamentos". Sin embargo, durante el período comprendido entre 2000-04, sólo 2 nuevos compuestos irrumpieron en el “top 100” de los medicamentos más rentables [11]. De forma global, la industria farmacéutica está bajo una creciente presión y con importantes pérdidas de ingresos debido a la expiración de patentes, los recortes presupuestarios de los sistemas de salud y los requisitos regulatorios cada vez más exigentes [12].

Estos hechos sugieren que las compañías farmacéuticas, biotecnológicas y de biomedicina necesitan reducir el tiempo de permanencia de sus potenciales fármacos en el “pipeline” y deben centrarse en una mejor planificación de su I+D [13]. Por el contrario, la mayoría de las grandes empresas farmacéuticas, con el afán de economizar en tiempos de crisis, han reducido sustancialmente sus laboratorios de I+D e incluso en algunos casos los han suprimido totalmente [14].

Normalmente el tiempo que transcurre desde el descubrimiento en el laboratorio del principio activo, hasta alcanzar la comercialización del fármaco oscila de 10 a 15 años generalmente [15].

Además, según la FDA sólo uno de cada 1.000 compuestos candidatos, consigue pasar del laboratorio a los ensayos clínicos. Y de estos últimos sólo uno de cada cinco se aprueba y se comercializa [16].

En conclusión, el proceso del desarrollo farmacéutico es demasiado caro e ineficiente para resistir en el entorno competitivo actual. Las razones de esta recesión en el terreno del I+D se deben, fundamentalmente al tremendo coste que supone la amortización de la investigación básica y preclínica. Hay que añadir la aparición de nuevas y complicadas normativas, el creciente coste del trabajo y la presión del mercado sobre los precios de los fármacos, debido a la aparición de los medicamentos genéricos [17].

2.1 BIOMARCADORES PARA EL DIAGNÓSTICO DE LAS ENFERMEDADES

Un marcador biológico (biomarcador) se ha definido como una alteración bioquímica, molecular o celular que se puede medir en medios biológicos, tales como tejidos, células y fluidos [18]. Un biomarcador representa un indicador de procesos ya sean normales o patológicos, o de una respuesta a la intervención terapéutica. Los biomarcadores se ven afectados por la enfermedad, lo que los hace útiles como marcadores de diagnóstico o de la progresión del tratamiento. Los biomarcadores moleculares en el glaucoma se asocian generalmente con variaciones individuales, gran dinámica del espectro de posibles concentraciones de moléculas específicas, y la cada vez mayor colección de especies moleculares.

En el caso de las patologías glaucomatosas, Aronson y sus compañeros están dividiendo los biomarcadores en dos grupos principales, los extrínsecos y más importantes aún, los intrínsecos a la patología [19]. Estos engloban tanto los datos clínicos (como la presión intraocular) como los bioquímicos relacionados directamente con la enfermedad [20, 21].

Los biomarcadores, han de ser necesariamente y objetivamente medibles y evaluables; y deben ser capaces de discernir entre situaciones normales o patológicas, así como la eficacia de un nuevo medicamento. En la evaluación de los biomarcadores es necesario discernir entre los de carácter cualitativo (la detección de mutaciones para una patología) de aquellos cuantitativos (basados en la medición de los niveles diferenciales de expresión) [22].

Durante los últimos años, el aumento de la sensibilidad y de la precisión de las técnicas en el campo de la genómica y la proteómica, y la adquisición de técnicas novedosas en el campo de la metabolómica, han permitido identificar entidades moleculares que pueden servir como marcadores potencialmente útiles en las patologías glaucomatosas, incluyendo:

- Marcadores de la detección inicial del glaucoma;
- Marcadores para predecir la gravedad de la enfermedad;
- Marcadores para predecir la tasa de progresión de la enfermedad.
- Marcadores que servirán como predictores de la respuesta al tratamiento [20].

2.2 PATENTES-PROPIEDAD INTELECTUAL

Las patentes son derechos de propiedad exclusivos de las creaciones tangibles de la mente humana [23]. Estos derechos de propiedad se recogen en forma de leyes en los diferentes países, y pueden ser aplicadas sólo en la medida en la cual una patente cubre el territorio de un estado individual. Por su parte, las invenciones tienen condicionada su protección a la demostración de la originalidad, el nivel inventivo y la posibilidad de explotación comercial entre otros. Los derechos de patente se limitan en su duración, con el estándar mundial de 20 años a partir de la fecha de aplicación [24]. El nuevo producto, artículo de fabricación o proceso que se describe en la solicitud de patente debe tener algo que nunca se ha dado a conocer con anterioridad en cualquier parte del mundo y cuyo carácter no sea evidente. Las determinaciones de si se cumplen estos requisitos se hacen mediante la comparación de las pretensiones del solicitante de la patente contra el cuerpo de la literatura publicada en el campo, incluyendo las patentes emitidas anteriormente. Este proceso se llama examen, y asegura que nadie es capaz de reclamar los derechos de la patente de forma arbitraria [25].

Las patentes funcionan de manera diferente según el tipo de industria. En las industrias farmacéuticas, químicas y biotecnológicas, la patente normalmente es igual al producto, y protege la amplia inversión en la investigación y los ensayos clínicos necesarios antes de colocarlo en el mercado [26]. La protección de patentes de productos químicos y farmacéuticos es especialmente importante en comparación con otras industrias debido a que el proceso de fabricación real es a menudo fácil de replicar y se puede copiar con una fracción de la inversión de la requerida para la investigación y los ensayos clínicos [25].

El extenso coste que es requerido para producir un nuevo producto farmacéutico ha hecho que la inversión del sector privado en la innovación farmacéutica haya sido desproporcionadamente dirigida a productos que satisfacen las necesidades de los pacientes en los países desarrollados, particularmente en los Estados Unidos, que combina una fuerte protección de patentes con un mercado libre de controles de precios [27].

Hasta el acuerdo ADPIC (Organización Mundial del Comercio sobre los Aspectos de los Derechos de Propiedad Intelectual) del 1994, muchos países en desarrollo no reconocían la protección de patentes para los productos farmacéuticos [28]. Generalmente las patentes farmacéuticas se globalizan a través del acuerdo ADPIC y a continuación, se refuerzan aún más a través de acuerdos bilaterales y regionales con los llamados acuerdos ADPIC-Plus [29].

Mientras que los países que se han adherido a la Organización Mundial del Comercio (OMC) firmaron un convenio para proporcionar dicha protección, las autoridades de los países desarrollados no están obligadas a cumplir con este compromiso hasta el año 2016 [25]. Esta continua falta de protección de las patentes para los productos farmacéuticos, hace que sea muy difícil establecer industrias basadas en la investigación en la mayoría de los países en desarrollo y la investigación médica en estos países se lleva a cabo en el sector público. La falta de cualquier medio de patentar estos inventos y la falta de experiencia relacionada con la concesión de licencias al sector privado, suprime el desarrollo de empresas comerciales centradas en el alivio de enfermedades comunes en los países en desarrollo [30].

La controversia sobre la disponibilidad de terapias patentadas para el tratamiento de la enfermedad del VIH ha provocado un renovado interés en la concesión de licencias obligatorias de productos farmacéuticos [31]. Sin embargo, hasta la fecha, en realidad no hay licencias obligatorias emitidas, a pesar de que se ha utilizado la amenaza de las licencias obligatorias como un medio de búsqueda de precios más bajos [31].

Uno de los peligros de las licencias obligatorias es que acarrearán nuevas problemáticas a la I+D comercial necesaria para el desarrollo de nuevos medicamentos que permitan combatir epidemias globales como por ejemplo el Ebola [32]. Otro peligro es que la obligatoria concesión de licencias se puede utilizar para bajar los niveles de precios por debajo de lo que el mercado nacional es capaz de soportar, concentrando aún más la carga de la financiación de la innovación farmacéutica en los consumidores de los países desarrollados y desalentando el desarrollo de fármacos dirigidos a las cargas de morbilidad de los países mediante licencias obligatorias [33].

En general las empresas de investigación farmacéuticas operan bajo un competitivo modelo de negocios [34]. Considerando los escasos éxitos que tienen los futuros medicamentos es evidente que los ingresos de los pocos fármacos que mejor se venden en los mercados, deben compensar los muchos fracasos. De hecho, sólo dos de cada 10 medicamentos recuperarán la financiación gastada en su desarrollo [35].

En general la propiedad intelectual farmacéutica, como las patentes y la protección de datos, proporcionan los incentivos de estimulación de la investigación y del desarrollo [23]. Aseguran que las empresas farmacéuticas innovadoras que han invertido en el largo y costoso desarrollo de medicamentos, tengan la oportunidad de amortizar sus inversiones [36]. La protección de la propiedad intelectual también ayuda a las empresas a asegurar los recursos para futuras inversiones en la investigación de nuevos medicamentos [37].

El sistema de la propiedad intelectual ofrece mecanismos en tres puntos importantes:

- Proporciona incentivos justos y eficaces para la innovación.
- Proporciona a los innovadores certeza sobre sus derechos.
- Ofrece a los titulares de las patentes unas fuertes herramientas para defender sus patentes infringidas.

Sin derechos de propiedad intelectual, los competidores podrían simplemente copiar las innovaciones de medicamentos, ofreciendo sus propias versiones sin necesidad de invertir tiempo y dinero en el desarrollo de éstos. Los innovadores de la industria bio-farmacéutica podrían perder la posibilidad de recuperar su sustancial inversión en el desarrollo de nuevos fármacos, lo que hace más difícil encontrar financiación [38].

Sin embargo, las patentes, enfocadas primordialmente hacia la maximización de las ganancias, poseen varias consecuencias negativas. En primer lugar, se ha argumentado que el sistema de patentes provoca importantes pérdidas en el bienestar de los consumidores ya que el precio final del producto es dictado por un monopolio [39]. Adicionalmente, el sistema de patentes fomenta la producción de medicamentos falsificados que pueden representar hasta el 10% del mercado mundial de productos farmacéuticos. Otro problema de los medicamentos patentados es que se gasta en su comercialización el doble que se gastó para sus actividades de I+D. Por todo eso, es cada vez más evidente que el sistema de patentes no está funcionando bien y un nuevo enfoque es necesario, para la solución de los problemas del sector [40].

2.3 EL PROCESO DE DESARROLLO DE FÁRMACOS- "THE PIPELINE"

A continuación, analizaremos el proceso de desarrollo de los nuevos fármacos, centrándose en la investigación de medicamentos y terapias desarrolladas para las enfermedades de glaucoma y ojo seco. El proceso de desarrollo de un nuevo fármaco es complejo, largo y costoso. Se puede dividir en las siguientes Fases:

- ✓ **Descubrimiento**
- ✓ **Ensayos preclínicos en animales**
- ✓ **Ensayos clínicos**
- ✓ **Comercialización del producto**

En general las estructuras orgánicas sobre las que interfiere la farmacología son:

- ✓ **Enzimas**. Los inhibidores o activadores de una enzima, dependiendo de la función que ejerce la enzima.
- ✓ **Moléculas transportadoras**. Inhibidores de procesos fisiológicos alterando la actividad de un transportador de membrana.
- ✓ **Canales iónicos**. El fármaco regula la actividad de los canales iónicos que existen en las membranas celulares y la membrana plasmática.
- ✓ **Receptores**. El fármaco reacciona con una proteína que sirve para el reconocimiento específico de neurotransmisores, hormonas y otros mediadores.

✓

2.3.1 DESCUBRIMIENTO Y ENSAYOS PRE-CLÍNICOS

Los nuevos medicamentos están diseñados para unirse a receptores o enzimas y se prueban en células animales, tejidos y organismos enteros [41]. En su mayoría los fármacos experimentales se originan en los laboratorios de las universidades, los institutos de investigación y los laboratorios farmacéuticos de pequeñas empresas. Las pequeñas empresas tienen un papel fundamental, debido a que a menudo venden los derechos de sus descubrimientos a empresas más grandes, que tienen los recursos para ejecutar el proceso de la evolución de un nuevo fármaco en su totalidad [42].

Posteriormente a su descubrimiento, los compuestos que parecen más prometedores se someten a extensas pruebas en una Fase que se ha denominado como preclínica.

El efecto de los fármacos se produce por su interacción con componentes macro-moleculares de las células. Los fármacos, provocan modificaciones bioquímicas sobre los órganos o sistemas y la farmacocinética investiga todos los procesos de transporte en el organismo después de la administración de los fármacos. No todo lo que sucede a un medicamento en un organismo complejo como el cuerpo humano puede ser comprendido plenamente [43]. Durante el proceso de la administración de un medicamento se pueden combinar diferentes unidades funcionales, tales como tejidos y órganos responsables de la distribución y eliminación a través de las diferentes vías existentes. La farmacocinética hoy es una disciplina bastante establecida en el desarrollo de fármacos y la investigación farmacéutica. Para uso terapéutico óptimo de cualquier compuesto, es esencial conocer su destino en el cuerpo después de la administración y caracterizar su disposición [43].

Por otra parte, farmacodinamica es el estudio de la acción del fármaco en el cuerpo o en los microorganismos y otros parásitos dentro o sobre el cuerpo. Se puede estudiar a muchos niveles como es el nivel molecular, celular, en los tejidos y los órganos y todo el cuerpo *in vivo*, *ex vivo* e *in vitro* y utilizando una amplia gama de técnicas y métodos [44]. La acción de cualquier fármaco requiere que haya una concentración adecuada del compuesto y para la mayoría de los fármacos el efecto se relaciona con el tiempo entre el aumento y la disminución de su concentración en el tejido diana [45].

El primer paso de la evolución de un compuesto farmacéutico consiste en pruebas *in vitro* en el laboratorio. Los estudios *in vitro* permiten a los científicos aislar células específicas, estudiándolas sin las distracciones que tiene un organismo completo y pueden proporcionar importante información sobre los efectos de las sustancias en las células [46]. Desgraciadamente,

esto significa que muchas veces los resultados encontrados en los estudios *in vitro* no se pueden traducir en buenos resultados *in vivo*, tampoco pueden demostrar de manera concluyente si un agente es seguro o tóxico. Sin embargo, en comparación con los estudios *in vivo* los estudios *in vitro* son sustancialmente más rápidos, menos costosos, y se puede hacer con menos preocupaciones éticas y de seguridad [47].

El siguiente paso en la investigación farmacéutica son los estudios en animales *in vivo*. Típicamente, los fármacos candidatos se prueban por primera vez en animales de experimentación que incluyen entre otros, ratones, ratas, conejos, peces y a veces perros [48]. Ocasionalmente el siguiente paso es la experimentación en primates, pero los últimos años debido a la sensibilidad del público a estos animales, este tipo de experimentación se tiende a omitir. Con estas pruebas, aunque los animales no siempre tienen la semejanza deseada con el funcionamiento del organismo de los seres humanos, se puede demostrar en una primera etapa, la seguridad y la eficacia de un candidato farmacéutico [49].

La Asociación Médica Mundial (AMM) ha promulgado en junio 1964 en la 18ª Asamblea Médica Mundial, en Helsinki, Finlandia, la Declaración de Helsinki, como una propuesta de principios éticos para investigación médica en seres humanos, incluida la investigación del material humano y de información identificables. La AMM insta a otros involucrados en la investigación médica en seres humanos a adoptar estos principios. La Declaración es un importante documento en la historia de la investigación ética, como un significativo esfuerzo de la comunidad médica para autorregularse, y forma la base de muchos de los documentos subsecuentes [50].

2.3.2 GLOSARIO DE ENSAYOS CLÍNICOS

Si un candidato es prometedor pasando satisfactoriamente los estudios preclínicos, en fase posterior se administra a sujetos humanos con el objetivo de evaluar la tolerancia del organismo humano al compuesto. Este tipo de pruebas están denominadas como estudios clínicos. Un ensayo clínico es un estudio de investigación biomédica o conductual en sujetos humanos, que es diseñado para responder a preguntas específicas acerca de intervenciones biomédicas o conductuales como vacunas, fármacos, tratamientos, dispositivos o nuevas formas de utilización de medicamentos conocidos, tratamientos o dispositivos [51].

En general los ensayos clínicos se utilizan para determinar si las nuevas intervenciones biomédicas o conductuales son seguras, eficaces y efectivas. El proceso de desarrollo se considera que es lineal y consecutivo y regularmente pasa a través de cuatro fases de desarrollo (Fase I a Fase IV). A veces en la bibliografía aparece una Fase más, la denominada Fase V o trabajo de campo [41].

Según las normativas vigentes, cuando un nuevo medicamento, ensayo, dispositivo, procedimiento u otra innovación médica potencial se desarrolle, debe ser probado completamente, para afirmar su seguridad y su eficacia como tratamiento [52]. La realización de ensayos clínicos reúne diversas áreas, como la bioética, la implementación de un centro de investigación, el seguimiento del ensayo y su seguridad. Los aspectos legales que rigen los ensayos clínicos en España están establecidos en el Real Decreto 223/2004 [53] y la Ley 29/2006, de 26 de julio [54]. Estos dos documentos regulan las garantías para el uso racional de los medicamentos y productos sanitarios, y asimismo regulan los ensayos clínicos con compuestos médicos y la investigación de los medicamentos de uso humano. Adicionalmente, con la Orden SCO/256/2007, de 5 de febrero, se establecen los principios y las directrices detalladas de buena práctica clínica y los requisitos para autorizar la fabricación o importación de medicamentos en investigación de uso humano. En esta Orden se incorporó en su totalidad al ordenamiento jurídico Español la Directiva 2005/28/CE de la Comisión Europea, de 8 de abril, por la que se establecen los principios y las directrices detalladas de las buenas prácticas clínicas respecto a los medicamentos en investigación de uso humano, así como los requisitos para autorizar la fabricación o importación de estos productos [55].

2.3.2.1 GLOSARIO DE ENSAYOS CLÍNICOS

- ADME: Es el acrónimo de absorción, distribución, metabolismo y eliminación en inglés. Los estudios ADME determinan cómo un medicamento es absorbido por el cuerpo, los cambios químicos que puede pasar y cómo se elimina del cuerpo [56].
- Aleatorización: Es la asignación de los participantes a los grupos de tratamiento basados en el azar. Este generalmente se realiza mediante un programa informático de una manera que no permite a los participantes o los investigadores a elegir su grupo. La aleatorización se utiliza para reducir el sesgo en los ensayos clínicos [57].
- Base de referencia: Es un punto en el tiempo en el inicio de un ensayo clínico antes de que los participantes en el estudio reciban cualquier tratamiento. Al inicio del estudio, los participantes suelen tener ciertos tipos de pruebas. Durante y después del tratamiento, se pueden realizar las mismas pruebas y los resultados se comparan con los resultados de referencia para ver si el fármaco ha causado algún cambio [57].
- Biodisponibilidad: Es la parte de la dosis de un compuesto que alcanza la corriente sanguínea. Si el compuesto se administra por vía intravenosa, su biodisponibilidad es 100%. Si el compuesto se administra en cualquier otra forma, tal y como por vía oral, tópica, o a través de inyección intramuscular, su biodisponibilidad disminuirá debido a la absorción incompleta [56].
- Brazo: un tipo específico del tratamiento al que está asignado un grupo de participantes en los ensayos clínicos. Los ensayos clínicos pueden tener desde uno hasta varios brazos. Por ejemplo, un ensayo clínico que compara dos dosis diferentes de un fármaco en investigación en comparación con un placebo tendría tres brazos: los participantes que recibieron una mayor dosis del fármaco en investigación, los participantes que recibieron una dosis menor del fármaco en investigación, y los participantes que recibieron el placebo [58].
- Cegamiento: Es un proceso utilizado para evitar que los participantes, los investigadores o ambos, sepan si la sustancia administrada es el medicamento o el placebo. El proceso de cegamiento ayuda a reducir el sesgo, debido que los participantes del estudio y los investigadores es menos probable de ser inconscientemente influenciados, por el conocimiento que la sustancia proporcionada es en realidad el medicamento. Si están cegados sólo los participantes, el estudio se denomina estudio simple ciego. Si además están cegados los investigadores, el estudio se denomina estudio doble ciego [57].

- Cohorte: Es un grupo de participantes en el estudio que tienen ciertas características en común, como el sexo, el rango de edad o la gravedad de la enfermedad. Dividiendo los participantes en cohortes se hace a menudo como parte de los análisis de los datos del estudio [41].
- Comité de Monitorización de Datos (DMC) o Seguridad de Datos y Vigilancia (DSMB): Un comité de expertos que revisa periódicamente los datos acumulados de un ensayo clínico multicéntrico. Los miembros de un DMC/DSMB deben ser independientes, y no pueden participar como investigadores en el ensayo clínico. Basándose en su opinión, los expertos aconsejan el patrocinador con respecto a la seguridad del estudio y si los datos sugieren que el estudio debe ser modificado o detenido [57].
- Consentimiento informado: Es un proceso por el cual los investigadores médicos proporcionan la necesaria información a una persona acerca de un estudio clínico y la persona confirma voluntariamente su voluntad de participar en el estudio [41].
- Contraindicaciones: Es un factor que hace que el uso de un medicamento en particular es desaconsejable. Por ejemplo, una persona que ha tenido una reacción alérgica a la penicilina en el pasado se considera que tiene una contraindicación para el uso de la penicilina en el futuro [41].
- Criterios de inclusión/exclusión: Son los factores definidos en el protocolo de un estudio que determinan si es segura la participación de una persona en un ensayo clínico y si sus datos ayudarán a lograr los objetivos del estudio. Los candidatos del estudio se someten a evaluación durante el período de selección para determinar si cumplen con todos los criterios de inclusión y si no cumplen con los criterios de exclusión, tal como se define en el protocolo. Estos criterios generalmente son factores tales como edad, sexo, tipo de enfermedad, estadio de la enfermedad, tratamiento previo, y otras condiciones médicas para determinar la elegibilidad para el estudio [59].
- Efecto adverso grave: Es un evento adverso que es potencialmente mortal, requiere para pacientes hospitalización o alarga de la estancia hospitalaria, conduce a discapacidad sustancial, conduce a un parto defectuoso, o resulta en la muerte [59].
- Efecto secundario: Es cualquier efecto de un fármaco que no sea el efecto deseado. Los efectos secundarios son a menudo no deseados y pueden ser molestos. Otros nombres para un efecto secundario molesto son adversa reacción farmacológica o toxicidad del fármaco [41].
- Eficacia clínica: La capacidad de un compuesto de producir el efecto deseado [56].

- Eficacia o efectividad: la capacidad de un fármaco para prevenir, curar o frenar el proceso de una enfermedad o para aliviar los síntomas de una enfermedad o condición [41].
- Elegibilidad: Es una determinación que se hace durante el período de selección si un participante cumple con los requisitos del estudio [41].
- Estudio de bio-equivalencia: Es una prueba realizada para averiguar la proporción de un compuesto en la corriente sanguínea cuando se administra en diferentes formas de dosificación [41].
- Estudio de etiqueta abierta: Es un estudio en el que los participantes y los investigadores saben que se está dando tratamiento. En un estudio abierto, no hay cegamiento y ninguno de los participantes recibe un placebo [57].
- Estudio de rango de dosis: Es un ensayo clínico en el que se prueban dos o más dosis de un compuesto para determinar la dosis que ofrezca la mejor combinación de seguridad y eficacia en ensayos clínicos posteriores o en la atención médica [57].
- Estudio multicéntrico: Es un estudio realizado en más de un lugar. Los estudios se realizan generalmente en centros de ensayo clínico individuales si no existen suficientes candidatos para completar un gran ensayo [57].
- Estudio pivotal: Es un estudio que está diseñado para generar los datos requeridos por las autoridades reguladoras para decidir si aprueban o no un fármaco en investigación. Un estudio pivotal es por lo general de larga duración, aleatorizado de Fase IIb o Fase III y frecuentemente es ciego y se utiliza un placebo como control [59].
- Estudio transversal: Es un diseño de estudio con dos o más brazos, donde los participantes reciben un tratamiento por un período de tiempo y luego cambian a un segundo tratamiento por un período de hora. Tal diseño del estudio permite que los efectos de los dos tratamientos se pueda comparar en el mismo paciente [57].
- Evento adverso (EA): Es un evento que causa molestia en un participante del estudio. El EA puede ser relacionado con el tratamiento que se investiga o puede ser debido a otra causa (por ejemplo, otro tratamiento, otra condición médica, un accidente o una cirugía) [59].
- Fármaco en investigación: Es un medicamento que se está probando como tratamiento potencial para una enfermedad o condición, pero aún no ha demostrado su seguridad y efecto para ese uso.
- Farmacología clínica: Es una ciencia que estudia las propiedades de los fármacos en relación con su valor terapéutico en los seres humanos [41].

- Formulario de consentimiento informado: Es un documento que describe un estudio clínico para los participantes. El formulario incluye información sobre los objetivos del estudio, el diseño del estudio y la duración, los tipos de pruebas a realizar, los riesgos potenciales y los inconvenientes, los beneficios potenciales, los posibles gastos o pagos asociados a la participación en el estudio, la alternativa disponible de terapias, los derechos y responsabilidades de los participantes y las personas en contacto, si el participante tiene preguntas. El formulario debe ser revisado y firmado antes de que el participante tenga cualquier prueba o tratamiento de estudio, incluyendo las pruebas realizadas durante el período de selección al comienzo del estudio [59].
- Grupo control: Es un grupo de participantes que no recibieron el fármaco en investigación, pero en su lugar recibieron un tratamiento estándar para la enfermedad o recibieron un placebo. Los resultados observados en el grupo de pacientes que recibieron el fármaco en investigación se compara con los resultados observados en el grupo de control [57].
- Junta de Revisión Institucional o Comité de Ética Independiente: Es un consejo de médicos, estadísticos, investigadores, defensores de la comunidad, y otros que son responsables de garantizar la protección de los derechos, la seguridad y el bienestar de los participantes en un ensayo clínico en un centro de estudios [59].
- Patrocinador: Es la organización responsable de la financiación y coordinación de un ensayo clínico. Muy a menudo, se trata de una empresa farmacéutica o biotecnológica [57].
- Períodos de proyección: Es un punto al comienzo de un ensayo clínico cuando los candidatos para el estudio se evalúan para determinar si segura su participación y si pueden aportar datos que ayudarán a alcanzar los objetivos del estudio [41].
- Placebo: una versión inactiva de un fármaco en investigación. Un placebo tiene una parecida apariencia al fármaco en investigación, pero no tienen valor terapéutico. El placebo se utiliza como un tratamiento de comparación para reducir el sesgo en los estudios aleatorios [59].
- Protocolo: Es un documento que describe qué tipo de personas pueden participar en un estudio clínico y los objetivos, tratamientos, medidas, métodos estadísticos, oportunidades y organización de un ensayo clínico. El protocolo se debe preparar con anterioridad al estudio y debe ser revisado y aprobado por los comités de evaluación y las autoridades reguladoras antes de iniciar el estudio. Los investigadores deben seguir el protocolo para llevar a cabo el estudio [41].

- Punto de llegada: Es una ocurrencia de un resultado de la enfermedad, síntoma, signo o prueba que constituye uno de los resultados objetivos de un ensayo clínico [57].
- Relación beneficio-riesgo: Es el equilibrio del riesgo de efectos secundarios que se espera con el uso de un fármaco en comparación con el potencial de beneficio con el uso de ese medicamento. Un medicamento con una buena relación riesgo-beneficio tiene pocos efectos secundarios y es muy eficaz [41].
- Sesgo: Es un factor acerca de los efectos de los beneficios y de los riesgos de un tratamiento o la falta de equilibrio en la selección de pacientes para un estudio que reduce la probabilidad de que los resultados del estudio son ciertos. Métodos como el cegamiento y la asignación al azar se utilizan para limitar la posibilidad de sesgo [57].
- Significativo o estadísticamente significativo: Es un resultado en un ensayo clínico que resulte a partir de una diferencia real y es poco probable que sea debido a casualidad. El nivel de significación estadística se expresa a menudo en términos de un valor, lo que indica la probabilidad de que la diferencia no se debe a la casualidad. Por lo general, un valor de p menor de 0,05 se considera estadísticamente significativo [59].
- Toxicidad: Es un efecto secundario producido por un compuesto que es molesto para la persona que toma el fármaco [60].
- Toxicología: Es el estudio de los efectos adversos de los productos químicos realizado en modelos animales para predecir los posibles efectos adversos en los seres humanos. Algunos se llevan a cabo durante el desarrollo de los estudios clínicos para evaluar regímenes de dosificación [60].

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2.3.2.2 FASES DE LOS ENSAYOS CLÍNICOS

Los ensayos clínicos-biomédicos de fármacos experimentales, de tratamientos, de dispositivos o intervenciones conductuales normalmente proceden a través de cuatro fases. Como se mencionó anteriormente, unas veces el trabajo en campo se denomina como Fase V [41]:

- Fase I: En los primeros ensayos de seguridad de un compuesto experimental se prueba la nueva intervención biomédica en un pequeño grupo típicamente entre 20-80 personas para evaluar la seguridad del compuesto, para determinar un intervalo seguro de la dosificación y para identificar posibles y evidentes efectos tóxicos, efectos secundarios o eventos adversos. Los ensayos de Fase I a menudo utilizan voluntarios sanos sin padecer la enfermedad en estudio [51]. Por lo general, los pacientes son expuestos al compuesto por un corto período de tiempo, tal vez sólo unos pocos días. Estos estudios evalúan la farmacocinética de un compuesto, según el acrónimo ADME en inglés (Absorption, Distribution, Metabolism and Elimination) estudiando la absorción, distribución, metabolismo y eliminación por el cuerpo. En esta etapa los investigadores también tratan de determinar la cantidad óptima del compuesto que ofrecerá el mayor beneficio, con toxicidad aceptable, en un proceso conocido como búsqueda de dosis. Aunque puede haber algunos indicios de que un compuesto funciona, la determinación de la eficacia no es el objetivo en esta fase de ensayos clínicos [61].

- Fase II: Una vez que se compruebe que no hay problemas importantes de seguridad, se hace una prueba más para averiguar si el compuesto es seguro en un grupo mayor de personas, normalmente entre 50 hasta 500, que esta vez padezcan la enfermedad en estudio. En la Fase II de ensayos clínicos se estudia la intervención biomédica o el comportamiento en un grupo de personas y normalmente en varios cientos, para determinar la eficacia y valorar aún más la seguridad del compuesto. Estos estudios también proporcionan los datos preliminares sobre la eficacia del candidato. A veces, estas pruebas se dividen en Fase IIa o estudios experimentales y la Fase IIb, pequeños ensayos controlados. El período de estudio es más largo que la Fase I, y dura por lo general varios meses hasta dos años. En un esfuerzo para acelerar el proceso de desarrollo, las etapas de ensayo a veces se combinan (Fase I/II o Fase II/III). Esta etapa

es donde la mayoría de los candidatos a fármacos son eliminados, y sólo alrededor de un tercio de los agentes experimentales pasa con éxito los estudios de esta Fase [61].

- Fase III: El objetivo de la tercera Fase de pruebas en humanos es determinar si el agente experimental es eficaz en una población aún más grande y típicamente en varios cientos. Los estudios de Fase III investigan la eficacia de la intervención biomédica o conductual en grandes grupos de sujetos humanos (de varios cientos a varios miles) mediante la comparación de la intervención con otras intervenciones estandarizadas o experimentales, así como para supervisar posibles efectos adversos [61]. Estos ensayos suelen durar por lo menos un par de años y a menudo duran más tiempo. El tipo más riguroso de estudio es el estudio prospectivo, doble ciego, aleatorizado, controlado, que compara un fármaco candidato contra un placebo (fármaco simulado) o una terapia ya disponible actualmente. Durante esta etapa, los investigadores continúan vigilando la seguridad del agente, ya que algunas toxicidades pueden hacerse evidentes sólo después de que un fármaco se utiliza en grandes grupos o en períodos de tiempo más largos. Datos de los últimos estudios de Fase III, llamados ensayos pivotales, pueden presentarse a la agencia estatal responsable para los estudios clínicos, como parte de una solicitud de nuevo fármaco para ser considerado como evidencia para su aprobación [51].

- Fase IV: Posteriormente que un medicamento ha sido aprobado y está en el mercado, se hacen unos estudios adicionales para averiguar su funcionamiento en el mundo real y para determinar si su eficacia es de larga duración. En estos estudios llamados post-comercialización, también se buscan toxicidades poco frecuentes o de larga duración que no se presentaron en los estudios anteriores. Con el tiempo, más información puede ser revelada acerca de las interacciones con otros fármacos y el uso en diferentes poblaciones, como las personas con enfermedades coexistentes. Tras la aprobación por la agencia estatal responsable para los estudios clínicos, los fármacos comercializados sufren un escrutinio continuo, y este examen se está incrementando debido a los problemas que han surgido con algunos medicamentos después de su aprobación [51]. Entre los puntos fuertes importantes de la investigación de Fase IV son la exposición en una gama más amplia de pacientes con el compuesto en estudio, lo que resulta en más información por la seguridad de la medicina y su eficacia.

- La llamada Fase V o trabajo de campo: Unas veces se utiliza la denominación de la Fase V para la fase de determinación si el efecto terapéutico se realiza en la práctica clínica del día a día. En general, la investigación de la denominada Fase V se considera "trabajo de campo" y está diseñado para probar la generalización de la intervención a una mayor muestra y en contextos clínicos típicos y variables. El argumento central en la Fase V es si los efectos son similares a los encontrados en estudios de eficacia posteriores y la determinación de quién se beneficia con el tratamiento. Además durante esta fase se analiza la relación coste-beneficio[62].

•

2.4. EL OJO

2.4.1 CARACTERÍSTICAS Y ESTRUCTURAS

El globo ocular es una estructura esférica que en humanos puede alcanzar un diámetro aproximado de 2.5 cm y un volumen de 6.5 ml. En esta estructura se puede diferenciar una zona anterior, que es más curvada, llamada córnea y que incluye 1/6 de la circunferencia total y una zona posterior, llamada esclera o esclerótica, que es menos curvada, y que incluye los 5/6 restantes de la circunferencia del globo ocular [63] (Fig. 1).

El globo ocular está compuesto por tres capas básicas. La más externa es la capa fibrosa (cornoescleral), la capa intermedia llamada úvea o tracto uveal (compuesta por la coroides, el iris y el cuerpo ciliar) y la más interna, la capa neural (retina) [64]. Estas

tres capas crean un espacio interior que contiene tres medios transparentes: el humor acuoso,

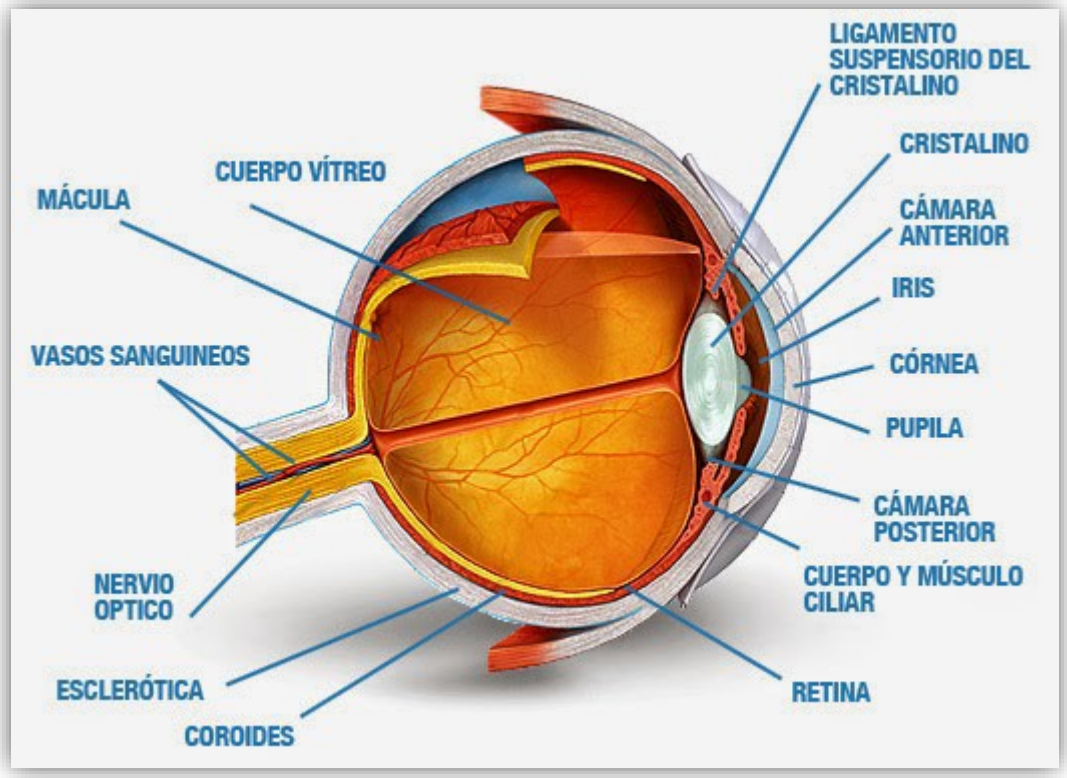

Fig. 1 El globo ocular (Imagen obtenida de http://gavetasdemiescritorio.blogspot.com.es/2015/01/funcion-de-las-estructurasinternas-del.html)

el cristalino y el cuerpo vítreo. El cristalino separa el ojo en dos cámaras independientes y así el humor acuoso está contenido en las dos cámaras (anterior y posterior) separadas por el iris (Fig. 1 y Fig. 2). La capa externa más fibrosa, compuesta por la córnea, la esclera y la unión de

ambas (limbo esclero-corneal), proporciona protección y apoyo estructural para el resto de estructuras intraoculares [63].

La función del globo ocular está relacionada con dos tipos de células especializadas de la capa neural: los conos y bastones. Estos dos tipos celulares son responsables para la fotorrecepción. Durante este proceso la luz que llega hasta la retina esta transformada en impulsos nerviosos, que se envían por el nervio óptico hacia el encéfalo donde se produce, el fenómeno de la visión [65]. El resto de las estructuras oculares, son necesarias para el correcto funcionamiento del sistema visual. En este sentido, la córnea y el cristalino son responsables para la transmisión y el correcto enfoque de la imagen en la retina, el iris y el cuerpo ciliar son necesarios para la nutrición de las estructuras avasculares intraoculares y junto con el sistema de drenaje del humor acuoso, ayudan al ojo para mantener su forma [63].

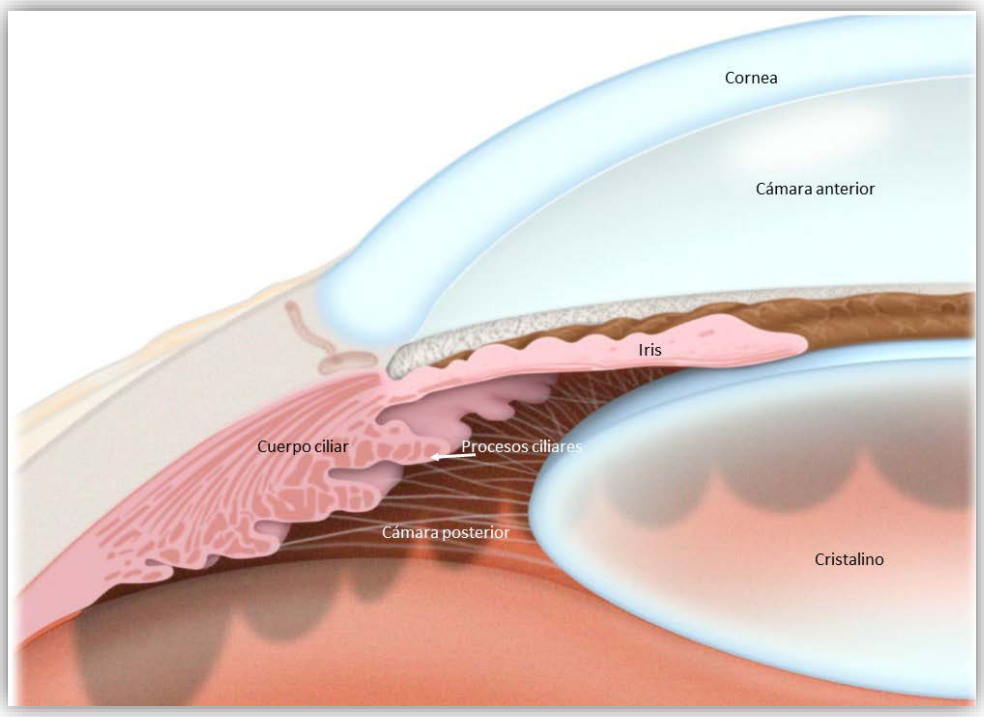

Fig. 2. Corte sagital de ojo (Imagen obtenida de http://gavetasdemiescritorio.blogspot.com.es/2015/01/funcion-de-las-estructuras-internas-del.html)

2.4.2 LA SUPERFICIE OCULAR Y LA LÁGRIMA

La superficie ocular es un conjunto de elementos del ojo, relacionados con la parte su externa. En general todo que envuelve la superficie ocular, está denominado como Unidad Funcional Lagrimal (LFU, sus siglas en inglés) [66]. La LFU está compuesta por la superficie ocular (córnea y conjuntiva), las glándulas lagrimales, las glándulas de Meibomio, los párpados y la inervación que los conecta, tanto sensorial como motora. La LFU controla y regula la lágrima respondiendo ante cualquier influencia ambiental o endocrina [67]. Los párpados ayudan a mantener la córnea húmeda y protegen al ojo frente a agresiones o frente de la luz excesiva, regulando la cantidad de la misma que llega a la retina [68]. Los párpados son esenciales para la distribución y drenaje de la lágrima. En los parpados están ubicadas las glándulas palpebrales. De esas las glándulas de Meibomio están relacionadas directamente con la película lagrimal [68].

La conjuntiva es una membrana mucosa translúcida y delgada que une el globo ocular a los párpados. Cubre los párpados por su posterior y se refleja anteriormente hacia la esclera, formando una capa continua junto con el epitelio corneal [68]. Las células Goblet pertenecen en la conjuntiva y constituyen una de las fuentes más importantes de mucinas para la capa mucosa de la película lagrimal y contienen las enzimas para la síntesis y secreción de las mucinas. La estimulación de la secreción mucosa se puede llevar a cabo por varias vías de señalización, la parasimpática, la simpática y la purinérgica [69].

La glándula lagrimal principal está situada dentro de la órbita y contribuye a la formación del componente acuoso de la lágrima. Las glándulas lagrimales accesorias son pequeñas glándulas lagrimales, que contribuyen de forma significativa a la formación de la capa acuosa de la lágrima secretando proteínas, electrolitos y agua [68].

La película lagrimal forma una barrera natural que separa los ojos húmedos de los medios externos. Esta película se forma principalmente a partir de tres capas, la acuosa, la mucosa, y de los lípidos que proporcionan el equilibrio necesario para mantener sana la superficie ocular [70]. Las funciones principales de la película lagrimal son: lubricación de la superficie ocular, transferencia de elementos nutritivos a la córnea, eliminación de materias extrañas y restos de células generadas en la superficie ocular por el flujo de lágrimas y el proceso de abrir y cerrar, y que actúa como la primera línea de defensa contra infecciones de la superficie ocular [71].

2.4.3 EL HUMOR ACUOSO

El humor acuoso (HA) es un fluido transparente, parecido al plasma sanguíneo, compuesto principalmente por agua, electrolitos y substancias de bajo peso molecular. Su composición iónica varía entre las diferentes especies [72].

Algunas estructuras intraoculares como el iris, el cristalino, y el endotelio corneal, contribuyen a la composición final del HA, sin embargo su formación se da fundamentalmente como un proceso activo de secreción en los procesos ciliares (Fig. 2) [63].

Tras su secreción en los procesos ciliares hacia la cámara posterior, el HA circulará, a través de la pupila, hacia la cámara anterior, donde será drenado [73] (Fig. 2).

El ojo humano tiene un volumen de HA de 250 µl aproximadamente [74], y el volumen medio que se forma por minuto es de 2,75 µl [75].

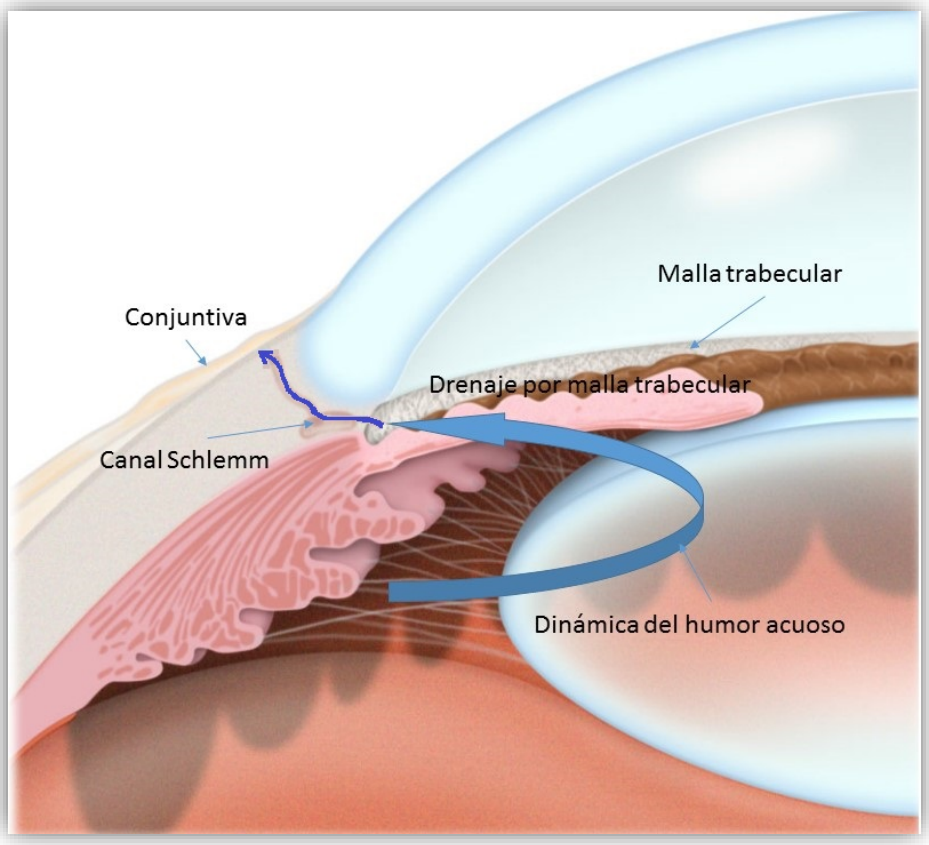

Fig. 3 Flujo del humor acuoso (Imagen obtenida de http://gavetasdemiescritorio.blogspot.com.es/2015/01/funcion-de-las-estructuras-internas-del.html)

La formación del HA es un proceso complejo en el cual los diferentes compuestos que lo forman son transferidos de forma regulada de la sangre hacia la cámara posterior. Este

fenómeno tiene lugar principalmente en los procesos ciliares. El HA, después de ser secretado, atraviesa el iris y pasa a la cámara anterior, donde será drenado a nivel del ángulo iridio-corneal por las vías de evacuación (Fig. 3) [73]. Existen dos vías principales de evacuación del HA hacia la circulación venosa: la vía trabecular (convencional) y la vía uveoescleral (no convencional).

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2.5 GLAUCOMA Y LA PRESIÓN INTRAOCULAR (PIO)

Todos los tejidos intraoculares soportan una fuerza hidrostática denominada presión intraocular (PIO). La PIO está determinada por el volumen de HA existente en las cámaras anterior y posterior, y ayuda al mantenimiento de la forma esférica del globo ocular.

Existe un sistema complejo de equilibrio dinámico que mantiene la PIO dentro de unos niveles estables. Este equilibrio está basado en la formación constante del HA y su drenaje por las vías de evacuación. Cualquier cambio en estos dos factores tiene como resultado la variación de la PIO. En condiciones de equilibrio la PIO permanece constante, y el volumen de HA producido equivale al drenado. En humanos, aunque puede fluctuar ligeramente, este equilibrio se establece alrededor de los 16 mm de Hg, lo que es considerado un valor de PIO normal [76]. Cuando este valor supera los 21 mm de Hg se considera que estamos en situación de hipertensión ocular [77]. La alta y persistente hipertensión, resulta en daños en el disco óptico, en la unión del nervio óptico y la retina, causando la degeneración de las células ganglionares (Fig. 1). La degeneración de los axones de las células ganglionares, conlleva su muerte por procesos apoptóticos. La hipertensión ocular es una situación fisiológica anómala relacionada con la aparición de patologías oculares como el glaucoma. La relación existente entre la hipertensión ocular y la patología glaucomatosa es bien conocida desde hace muchos años [78]. Incluso hoy en día se sigue considerando la hipertensión ocular como el factor de riesgo más importante para el desarrollo de esta enfermedad [79] [80] [81] [82]. El glaucoma es una patología caracterizada por la pérdida del campo visual asociada al daño en el nervio óptico y la retina, que se puede llegar hasta de una pérdida total de la visión [83]. Es una enfermedad progresiva que provoca daños permanentes a la papila, el nervio óptico y el campo visual (Fig. 3). Los principales factores de riesgo son el aumento de la PIO, la edad, los antecedentes familiares el síndrome de exfoliación y la miopía. Sin tratamiento se puede conducir a la ceguera, siendo la segunda causa principal de ceguera en el mundo [84]. Se ha estimado que cerca de 61 millones de personas en el mundo padecen glaucoma, y esta cifra se cree que aumentará a alrededor de 80 millones hasta el año 2020 [85].

La enfermedad se clasifica principalmente como de ángulo cerrado o de ángulo abierto, y asimismo está categorizado como glaucoma primario o secundario [86]. En el glaucoma de ángulo cerrado, el iris periférico bloquea el ángulo de la cámara anterior por aposición o sinequias. Por consiguiente, el drenaje del humor acuoso está inhabilitado y la PIO aumenta. Por otro lado, en el glaucoma de ángulo abierto, el ángulo anterior permanece abierto, pero el drenaje del humor acuoso está impedido en la malla trabecular y especialmente en las

regiones de tejido yuxtacanalicular o más allá [83]. En el glaucoma de ángulo abierto, el más prevalente, la hipertensión ocular es la causa directa de la aparición de la patología, por lo que la estrategia terapéutica más común consiste en la reducción de la PIO (Fig. 4) [87].

Además de estas clasificaciones el glaucoma se puede describir como primario y secundario. El primario no está asociado con enfermedades pre-existentes mientras el secundario es el resultado de otras enfermedades oculares o sistémicas, traumatismos, o debido a efectos de los medicamentos que está sometido el paciente [86, 88-90]

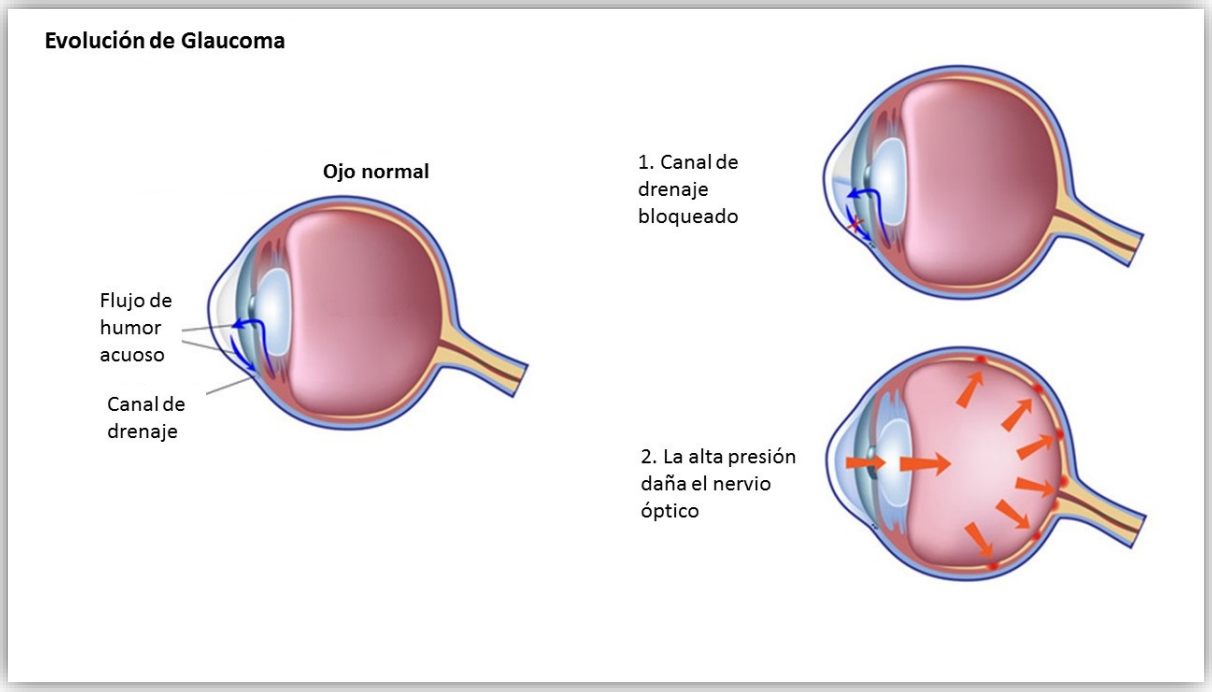

Fig. 4 Presión Intraocular sobre el nervio óptico (Imagen modificada de http://www.lookfordiagnosis.com)

2.5.1 MÉTODOS PARA EL DIAGNÓSTICO DE GLAUCOMA

El glaucoma puede ser controlado con un diagnóstico y tratamiento temprano. Lamentablemente el paciente no nota ningún síntoma o dolor a principios del aumento de la presión intraocular, por lo que el diagnóstico precoz es un problema [91]. Más de la mitad de los pacientes con glaucoma no saben que padecen esta enfermedad y en el momento en que se diagnostica, ya han perdido irreversiblemente una media de 30-50% de sus células ganglionares [92].

El glaucoma se puede diagnosticar mediante el control de los campos visuales, cambios en el nervio óptico y midiendo la presión intraocular. Los métodos actuales para el diagnóstico del glaucoma son los siguientes:

- Tonometría. Se mide la PIO con un tonómetro. Por lo general, es la prueba de detección inicial y es una parte importante de la evaluación de glaucoma. La PIO normal generalmente oscila entre 10 mm Hg y 21 mm Hg. Sin embargo, los pacientes con glaucoma de tensión normal (GTN) pueden padecer daños en su nervio óptico y pérdida del campo visual, aunque su PIO sigue siendo sistemáticamente inferior de los 21 mm Hg [93].

- Oftalmoscopia. Se trata de una inspección del nervio óptico, visualizando el fondo de ojo, para detectar posibles daños. Se realiza directamente a través de la pupila con un oftalmoscopio utilizando un examen con lámpara de hendidura, que puede revelar cambios leves que indican el inicio de la enfermedad. Las pupilas están dilatadas antes del examen con tropicamida 1 % y fenilefrina al 2%. El nervio óptico normal se compone de más de un millón de células nerviosas y como el glaucoma daña el nervio óptico, se provoca la muerte gradual de un número de estas células [94]. Así se observa un adelgazamiento de la capa de fibras nerviosas, debido a la pérdida de células ganglionares. A medida que aumenta la pérdida de células ganglionares, se produce una pérdida del campo visual. Como resultado aparecen manchas negras en el campo de la visión del paciente [95].

- Gonioscopía. Con la gonioscopía se permite obtener un panorama claro sobre el ángulo del drenaje, con el fin de determinar el tipo de glaucoma. Se lleva a cabo mediante el uso de una lente de espejo y examinando el ángulo de drenaje para determinar si el paciente sufre de glaucoma de ángulo abierto, donde el ángulo de drenaje no está funcionando suficientemente o glaucoma de ángulo cerrado, donde el

ángulo de drenaje se bloquea parcialmente o un ángulo estrecho en el que el iris está bloqueando el sistema de drenaje del ojo [96].

- Paquimetría. Implica la medición del espesor de la córnea con un paquímetro. El espesor de la córnea es un factor importante para el diagnóstico de glaucoma. Córneas gruesas pueden provocar presión más alta que corneas con espesor normal. Del mismo modo, las personas con córneas delgadas pueden tener lecturas de presión normales, aunque sufran de glaucoma [97].

- Prueba de campo visual. Con esta prueba es posible determinar si el campo visual se ha visto afectado por el glaucoma utilizando un perímetro. Durante esta prueba, el médico busca áreas de visión disminuida que corresponden a una pérdida de la capa de fibras nerviosas. Las pruebas de campo visual se realizan generalmente cada 6 a 12 meses para monitorizar posibles cambios. El inconveniente de esta prueba es que es subjetiva y depende de la colaboración del paciente [98].

- Fotografías estereoscópicas del nervio óptico. Es otro método para detectar la progresión del glaucoma mediante el estudio de los cambios en el nervio óptico en sesiones repetidas. Las fotografías del nervio óptico generalmente se toman cada año y se comparan para seguir la progresión de la enfermedad [99, 100].

- Imágenes de las fibras nerviosas. Esta prueba se trata de medir el espesor físico de la capa de fibras nerviosas en la retina. En el glaucoma las fibras nerviosas están disminuyendo y los pacientes son propensos a tener una capa más delgada de lo normal. El analizador de fibras nerviosas es un instrumento capaz de medir el espesor real de esta capa, utilizando el principio de desplazamiento de la polarización de la luz láser. El glaucoma-scope es otro instrumento utilizado para medir el espesor de la capa de las fibras nerviosas. Este tipo de prueba es bastante nuevo y en la actualidad sólo se considera como prueba complementaria [101].

- Tomografía de Coherencia Óptica. Es un método indoloro, de no-contacto, de una técnica de imagen no invasiva, que se utiliza para obtener imágenes en tres dimensiones de la cámara de la retina, córnea y anterior del ojo, de alta resolución de la sección transversal. Esta técnica ofrece imágenes de mayor claridad y resolución que otros instrumentos de imagen como la resonancia magnética o la ecografía. Con la tomografía de coherencia óptica se obtienen imágenes sub-superficiales de materiales

translúcidos u opacos con una resolución equivalente a un microscopio de baja potencia [102].

Finalmente, una serie de nuevos enfoques están siendo utilizados para la prueba de daños neuronales inducidos por glaucoma, como es la medida del perímetro Dicon que se considera como una buena alternativa en la detección de glaucoma e incluso un substituto de los métodos actuales [103].

2.5.2 TRATAMIENTO ACTUAL DEL GLAUCOMA

Gran parte de los fármacos disponibles para el tratamiento de la hipertensión ocular y su reducción, tiene como dianas diferentes mecanismos relacionados con la regulación de la dinámica del HA. El método convencional para el tratamiento de glaucoma consiste en la terapia de reducción de la PIO, de modo farmacológico, con terapia láser, o a través de intervención quirúrgica [86, 89, 90]. En cualquier caso, el objetivo del tratamiento es la reducción de la presión antes de que cause la pérdida progresiva de la visión. Un número importante de estudios de los últimos años ha demostrado que la disminución de la PIO es beneficiosa en el glaucoma primario de ángulo abierto [104]. Hasta el momento, los resultados muestran un riesgo del 10% en 5 años de desarrollo de glaucoma en pacientes con PIO basal de 24-31 mm Hg. Este riesgo se reduce al 5% con la terapia farmacológica. El objetivo de la terapia médica es la reducción de la presión intraocular por lo menos un 20% de la línea base media de la PIO con

Fig. 5 Timolol

Fig. 6 Brimonidina

su valor absoluto tratada de 24 mm Hg o menos. El objetivo sobre la PIO debe ajustarse de forma independiente para cada paciente, dependiendo de los factores de riesgo. Un nivel de PIO para una persona con un mínimo de factores de riesgo puede ser demasiado alto para un paciente con múltiples factores de riesgo [105].

El tratamiento inicial suele ser farmacológico. Los medicamentos, según su naturaleza, se pueden dividir en agentes simpaticomiméticos y simpaticolíticos, inhibidores de las anhidrasas carbónicas, agentes parasimpaticomiméticos y análogos de las prostaglandinas (Tabla 1). Estos enfoques farmacológicos pueden contribuir a la reducción de la PIO, ya sea inhibiendo la producción de humor acuoso por el cuerpo ciliar, con inhibidores como por ejemplo la

anhidrasa carbónica, o facilitando el flujo de la salida del humor acuoso a través de la malla trabecular, por ejemplo con agentes parasimpaticomiméticos [86, 90]. Durante la terapia, cuando el fármaco de primera elección no es eficaz (cuando tenemos una disminución de la PIO <20%) o se producen adversos efectos, se escoge otro fármaco de los de primera elección (mencionados en el párrafo superior). Sin embargo, si después de este cambio, no se alcanza la PIO deseada (nivel esperado para prevenir el avance del daño glaucomatoso), la opción siguiente es una combinación de dos o tres fármacos de los grupos mencionados. Habitualmente se utilizan combinaciones de medicamentos para obtener una disminución adicional de la PIO, sin las desventajas de un mayor coste y con menos efectos secundarios [90].

La administración del fármaco se hace normalmente de manera tópica con gotas oftálmicas. En la mayoría de los pacientes las gotas para los ojos pueden ser tan eficaces como la cirugía.

2.6 SÍNDROME DE OJO SECO

El síndrome de ojo seco es un trastorno que provoca cambios en la cantidad y composición de las lágrimas, siendo la manifestación oftálmica más común de las enfermedades inflamatorias sistémicas [106].

El ojo seco es una enfermedad multifactorial de la película lagrimal y la superficie ocular, que causa síntomas de malestar, trastornos visuales e inestabilidad de la película lagrimal con daño potencial en la superficie ocular [107].

La patología se produce cuando la superficie de la capa protectora lacrimal ocular se debilita lo que podría ser el resultado de una producción insuficiente o atípica de uno o más componentes de la lágrima [108]. No es un trastorno solamente de la película lagrimal sino de toda la LFU [66 67]. Un daño en cualquiera de los componentes de la LFU puede desestabilizar la película lagrimal y causar el ojo seco [66 67 109].

El síndrome va acompañado por un incremento en la osmolaridad de la película lagrimal e inflamación de la superficie ocular [107].

Actualmente los signos oculares más relevantes se incluyen intencionadamente en la definición de ojo seco. Los pacientes con ojo seco presentan habitualmente síntomas de malestar como disconfort, sequedad, sensación de arena, irritación, sensación de cuerpo extraño, picor o sensibilidad a la luz [110 111 112 113 114 115]. En casos severos, se producen lesiones de la superficie ocular. En cuanto a los signos más habituales, y en función del tipo de ojo seco que presenten, los pacientes padecen hiposecreción lagrimal [116], inestabilidad de la película lagrimal [117], hiperosmolaridad lagrimal [118 119], pérdida de agudeza visual [120], aumento de las aberraciones oculares [121 122] e inflamación ocular [123 124]. Los signos y síntomas de estas complicaciones pueden variar de un paciente a otro, a veces con poca o ninguna correlación entre ellos, a pesar de que generalmente están relacionadas con la composición de la película lagrimal. Los signos y síntomas pueden incluir manchas y queratitis en la conjuntiva y la córnea, enrojecimiento, visión borrosa, disminución de la película lagrimal tiempo de ruptura, disminución del volumen de la producción de lágrimas, y el caudal, mayor enrojecimiento conjuntival, el exceso de suciedad en la película lagrimal, sequedad ocular, sensación de arenilla ocular, ardor ocular, sensación de cuerpo extraño en el ojo, sensibilidad ocular, irritación ocular y escozor y fotofobia [107, 125-127].

Otras causas de la disminución de la secreción lagrimal pueden ser infiltraciones de células inflamatorias en la glándula lagrimal que ocurre en los pacientes con sarcoidosis [128] y SIDA [129], también la obstrucción de los conductos lagrimales, como ocurre en el tracoma [130] y en el eritema multiforme [131].

Algunos fármacos sistémicos provocan una disminución de la secreción lagrimal entre los que se encuentran los antihistamínicos, beta-bloqueantes, diuréticos, anticonceptivos o ansiolíticos y antidepresivos [132] [133] [134] [135]. Los conservantes también de los fármacos tópicos inducen una respuesta inflamatoria en la superficie ocular que desencadenaría sintomatología de ojo seco. Los pacientes con enfermedades autoinmunes son propensos a sufrir inflamaciones, que son factores clave por la aparición de la enfermedad del ojo seco grave [136].

El Ojo Seco está relacionado con el envejecimiento. Progresivamente está afectando a mas partes de la población. Bajo ciertas condiciones casi todos experimentan una irritación ocular o los síntomas de ojo seco, como por ejemplo durante trabajo prolongado con el ordenador o residiendo en un ambiente seco o después de utilizar ciertos tipos de fármacos [137, 138].

Los tipos de síndrome de ojo seco se clasifican de acuerdo a las perspectivas etiológicas y las influencias ambientales como: el ojo seco acuodeficiente (ADDE) y el ojo seco evaporativo (EDE) (Fig. 6) [107]. Los dos tipos no son independientes, sino que habitualmente los pacientes presentan eventos de los dos tipos.

El ojo seco acuodeficiente implica principalmente la disminución de la secreción lagrimal, que causa hiperosmolaridad y desencadena el resto de eventos que describiremos en la patogénesis del ojo seco [139] [140]. Hay estudios que sugieren un aumento de la evaporación [139] [141] y otros todo lo contrario, que se reduce la evaporación [142], probablemente porque sean etapas diferentes de la enfermedad. El ojo seco acuodeficiente se divide en dos subclases, el ojo seco asociado al Síndrome de Sjögren y el ojo seco no asociado a Síndrome de Sjögren. El síndrome Sjögren se define actualmente como una patología autoinmune en la cual el sistema inmune del cuerpo ataca erróneamente a las glándulas exocrinas, en particular a las glándulas salivares y la glándula lagrimal principal [143]. Hay estudios que relatan una prevalencia del 0,5% de la población, afectando entre 1 millón y 4 millones de personas en el mundo, siendo más prevalente entre las mujeres con un ratio de 9 mujeres por cada hombre afectado [144] y está considerada como una enfermedad rara tanto en Europa como en Estados Unidos.

La falta de secreción acuosa en el ojo seco no asociado a Síndrome de Sjögren más común es el relacionado con la edad [145], al que en el pasado se denominaba keratoconjunctivitis sicca (denominación que actualmente se utiliza para cualquier tipo de ojo seco) [146].

El otro tipo principal de ojo seco es el evaporativo, en el cual hay una pérdida de lágrima exagerada dejando la superficie ocular más expuesta a pesar de una secreción lagrimal normal. Este tipo de ojo seco se divide a su vez en intrínseco, cuando la causa es una patología o una disfunción propia del paciente, y en extrínseco, cuando algún factor externo produce la evaporación excesiva [107]. La diferenciación entre estos dos subtipos de ojo seco evaporativo es muy difusa, al igual que entre los dos tipos principales.

Entre las causas intrínsecas de ojo seco evaporativo nos encontramos con disfunciones en las glándulas de Meibomio, que afectan a la formación de la capa lipídica uniforme y estable, la cual es necesaria para mantener la estabilidad de la película lagrimal [147].

La causa extrínseca más relevante es el uso de lentes de contacto. La prevalencia del ojo seco en usuarios de lentes de contacto está entre el 20 y el 70% de los usuarios [148], siendo el ojo seco la principal causa de abandono de las lentes de contacto [149]. La lente de contacto rompe la película lagrimal, dejando una capa pre lente muy fina, con una capa lipídica irregular que provoca un aumento de la evaporación durante su uso [150].

Existen otras condiciones que se consideran de riesgo para padecer ojo seco. Diversos estudios evidencian el aumento de ojo seco en mujeres, principalmente en el periodo post-menopaúsico [151], y en sujetos que utilizan ordenadores o están expuestos a ambientes con una baja humedad relativa [152].

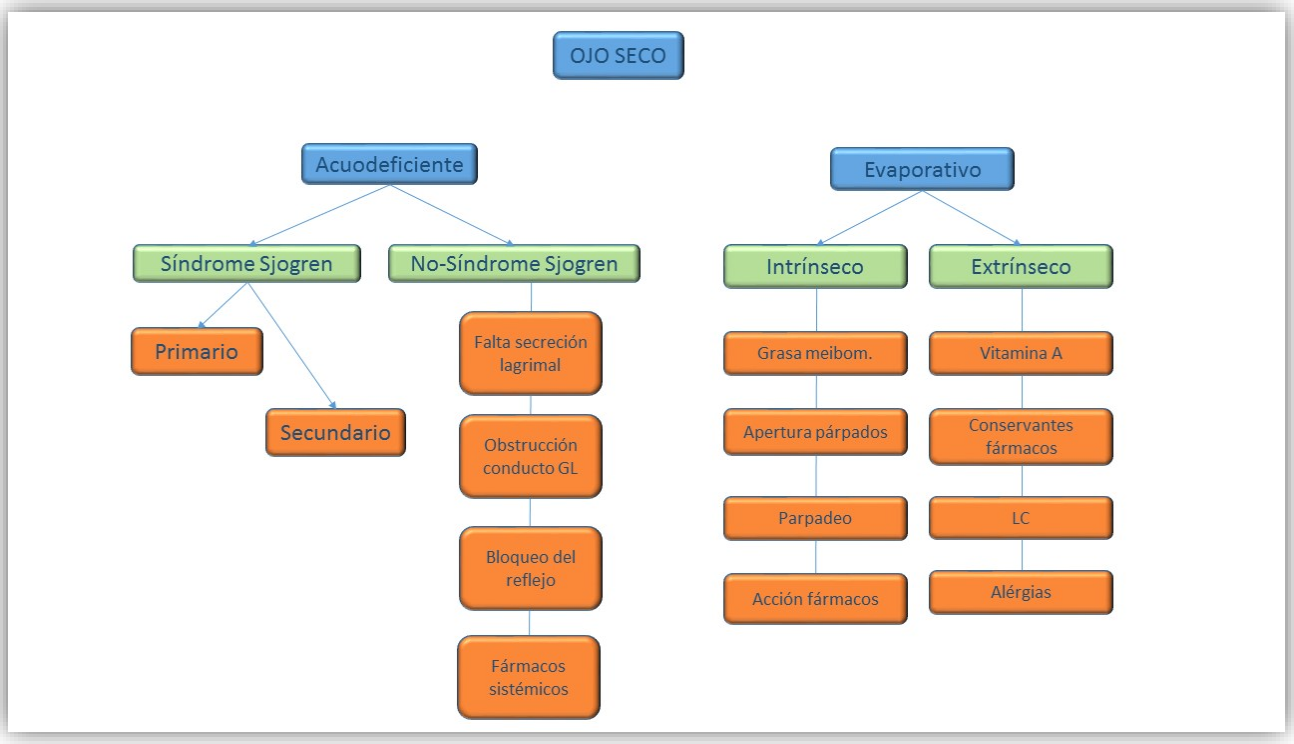

Fig. 7 Tipos de Ojo Seco

La hiperosmolaridad de la lágrima provoca daño en el epitelio de la superficie ocular al activar una cascada de eventos inflamatorios en la superficie, así como la liberación de mediadores inflamatorios a la lágrima como son las citoquinas, las metaloproteasas (MMP) o las interleuquinas [153]. Estos eventos llevan a la apoptosis de las células caliciformes, lo que reduce significativamente la producción de las mucinas [154].

2.6.1 MÉTODOS PARA EL DIAGNÓSTICO DE LA ENFERMEDAD DEL OJO SECO

El diagnóstico clínico de ojo seco es un reto desde que es una patología caracterizada por una extensa variedad de signos y síntomas y además no existe una correlación entre ellos. Se dan casos de ojo seco con sintomatología pero sin presencia de signos oculares y viceversa [155] [156].

La ambigüedad en la etiología y fisiopatología de la enfermedad está contribuyendo a la dificultad de un diagnóstico preciso [126, 156-158]. Las pruebas que se utilizan convencionalmente incluyen el uso de tiras de Schirmer, el tiempo de ruptura de la película lagrimal o BUT y la tinción de la superficie ocular. Estos diagnósticos se podrían considerar que son invasivos y que ofrecen un bajo nivel de estandarización.

Otro factor negativo es la falta de conocimiento sobre la fisiopatología de la enfermedad y los síntomas confusos que pueden confundirse con los síntomas de otras enfermedades, como la conjuntivocalasia (que puede inducir fácilmente una película lagrimal inestable) o aclaramiento lagrimal retardado (que es un causa frecuente de irritación ocular) [159]. Sin embargo, debido que la película lagrimal es un proceso dinámico y abierto y el sistema lagrimal es objeto de varias modificaciones internas y ambientales, se produce con frecuencia a interpretaciones erróneas de los resultados obtenidos [160].

Al no existir un prueba “Gold standard” para el diagnóstico del ojo seco, el subcomité de metodologías para el diagnóstico del DEWS recomienda la combinación de algunas de las siguientes pruebas [161]:

1.- Cuestionarios de síntomas. - Son tests diseñados para conocer la sintomatología relacionada con el ojo seco que presenta el paciente, así como los factores ambientales e historia médica que supongan un factor de riesgo para el ojo seco.

Existen varios tipos de cuestionarios de ojo seco siendo el cuestionario de McMonnies [162], el cuestionario de ojo seco (DEQ, en inglés) con su versión para lentes de contacto (CLDEQ, en inglés) [114] [148], el índice de enfermedad de la superficie ocular (OSDI, en inglés) [112] y el cuestionario de Schein los más utilizados [163]. En España, contamos con un cuestionario validado y desarrollado por Donate y colaboradores [164].

2.- Test para evaluar la tinción corneal y conjuntival. - Para determinar defectos o erosiones en la superficie ocular se utilizan colorantes como la fluoresceína para la córnea, la cual se observa mejor con un filtro amarillo, o verde de lisamina o rosa de bengala para evaluar la

conjuntiva. Los sistemas más habituales para cuantificar la tinción de la superficie ocular son el sistema de Van Bijsterveld [165], el sistema Oxford [166] y el sistema CLEK [146].

3.- Estabilidad de la película lagrimal. - Para la evaluación de la inestabilidad lagrimal el test más utilizado es el tiempo de rotura de la película lagrimal (TFBUT, en inglés). Para realizar esta prueba hay que instilar fluoresceína sódica en la superficie ocular. El valor de corte habitual es 10 segundos [167], aunque últimamente Abelson y colaboradores sugirieron que cuando se instilan volúmenes pequeños de fluoresceína (en su estudio se instilaban 2 µl con una pipeta) el valor diagnostico debía ser 5 segundos [168].

Para evaluar la estabilidad lagrimal también existen pruebas no invasivas como la observación de las glándulas de Meibomio o el tiempo de rotura no invasivo (NIBUT, en inglés) [169].

4.- Test para evaluar el volumen lagrimal. - Con este tipo de pruebas se determina la secreción lagrimal, tanto refleja como basal. El más común es el test de Schirmer, que se realiza con un papel Wathman Nº 1. El valor de corte para esta prueba es de 5,5 mm en 5 minutos [165]. Estudios recientes plantean la reducción del tiempo de la tira en el ojo a 1 o 2 minutos, reduciendo así la incomodidad al paciente [170] [171].

Otros tests considerados como menos invasivos para la evaluación del volumen lagrimal son el hilo de rojo fenol, el índice de producción lagrimal (TTR, en inglés) y la cuantificación del menisco lagrimal [172].

5.- Test para la evaluación de la osmolaridad. - Siendo la hiperosmolaridad uno de los mecanismos centrales en la patogénesis del ojo seco, poder obtener una medida de la osmolaridad lagrimal es una herramienta relevante para el diagnóstico del ojo seco. Actualmente se dispone de un osmómetro compacto que necesita muy poca cantidad de lágrima para medir la osmolaridad al instante. Este instrumento, denominado Tearlab osmolarity, es el primero de uso completamente clínico que evalúa la osmolaridad lagrimal [173].

2.6.2 TRATAMIENTO ACTUAL DEL OJO SECO

El ojo seco no tratado podría causar un aumento de riesgo de infección ocular, úlceras de la córnea y en casos extremos ceguera [70].

Los tratamientos más eficaces hasta ahora están destinados hacia la aplicación de productos lubricantes como son las lágrimas artificiales, la estimulación de la secreción lagrimal, o el control de la inflamación. Actualmente sólo unos pocos fármacos están autorizados para el tratamiento de la esta enfermedad y las posibilidades de evolución de este sector son inmensas. En consecuencia, un número significativo de nuevas soluciones potenciales están en desarrollo en los laboratorios farmacéuticos, dando mejores resultados y menos efectos secundarios [107].

Hay muy pocos tratamientos farmacéuticos para el ojo seco en el mercado. En algunos países como Japón, los fármacos se comercializan más fácilmente que en EEUU ya que las normas de aprobación no son tan rigurosas.

Un factor importante, sobre el tratamiento de ojo seco es que, en la mayoría de los casos, las empresas de biotecnología están tratando de resolver el problema sobre la base de estrategias "over the counter", tratando de aliviar los síntomas en lugar de llegar hasta la causa de la enfermedad.

La primera opción terapéutica ante un ojo seco leve o moderado son los lubricantes, conocidos como lágrimas artificiales a pesar de que no mimetizan la composición de la lágrima humana. Se caracterizan por ser soluciones isotónicas o algo hipotónicas, que contienen electrolitos, surfactantes, tampones y diversos tipos de agentes viscosos y lubricantes [107, 174]. Entre sus propiedades físico-químicas más importantes está una baja tensión superficial, un pH neutro o ligeramente alcalino y una osmolaridad similar a la lágrima humana [175]. Lo ideal es que no lleven conservantes, ya que son citotóxicos [174].

Las lágrimas artificiales permiten no sólo aumentar la cantidad de lágrimas, sino también mantener la superficie ocular humedecida, ofreciendo un alivio a la molestia. Otro tipo de tratamiento son los dispositivos de retención de desagüe/implantes conocidos también como tapones lagrimales, que pueden ser absorbibles y no absorbibles [176]. Estos dispositivos mantienen las lágrimas sobre la superficie ocular para aliviar los síntomas no deseables. Se ha demostrado de que el aumento de la humedad periocular puede hacer que la capa lipídica de la película lagrimal sea más espesa y que los usuarios de gafas con ojo seco tienen un intervalo más largo entre abrir y cerrar los párpados que las personas que no usan gafas [177].

Los tratamientos farmacéuticos actuales se centran principalmente en hacer frente a la inflamación y la restauración de las lágrimas. La enfermedad del ojo seco, es el resultado de muchos factores y frecuentemente produce inflamación en la córnea y la conjuntiva. La disfunción de las glándulas secretoras, conduce a cambios en la composición de las lágrimas, llamada híper-osmolaridad, estimulando la producción de mediadores de la inflamación en la superficie ocular. Esta inflamación puede ser iniciada por el estrés crónico irritativo, como el uso de lentes de contacto o de una enfermedad autoinmune inflamatoria sistémica, como la artritis reumatoide [178, 179].

Los fármacos anti-inflamatorios son ampliamente utilizados para el tratamiento de la inflamación producida y las gotas con corticosteroides tópicos se consideran como el tratamiento más común. Los corticosteroides pueden aliviar los moderados o graves síntomas de ojo seco, con rapidez y eficacia [180].

La ciclosporina presenta un efecto de mejora en los signos y síntomas de ojo seco después de varias semanas de tratamiento, siendo el mayor efecto más allá de los 6 meses. En España no se comercializa bajo ningún nombre comercial, pero si bajo fórmula magistral al 0,05% [181].

Debido que los fármacos anti-inflamatorios no esteroideos (AINE) están acreditados como causantes de efectos secundarios menos graves, recientemente están siendo evaluados como un potencial tratamiento del ojo seco [182]. Los AINE pueden reducir la inflamación y las molestias en los ojos, debido a su efecto analgésico, pero al otro lado podrían inducir a una sensibilidad decreciente. En 2002, la FDA aprobó el fármaco Restasis® de la empresa Allergan Inc., como el primer medicamento recetado, capaz de aumentar la producción lagrimal. Restasis® es una emulsión oftálmica de aplicación tópica, que contiene ciclosporina al 0,05 % [183].

Los esteroides por otra parte, pueden causar efectos secundarios graves después del uso prolongado. Los efectos varían de una infección bacteriana o fúngica, a una presión intraocular elevada hasta llegar a la formación de cataratas. Otro problema es que los esteroides suprimen

localmente la respuesta inmune en pacientes con la superficie ocular ya comprometida. Por lo tanto, los esteroides se suelen utilizar sólo durante un período de tiempo limitado [184].

Otro tipo de fármaco utilizado globalmente son los antibióticos, tales como la azitromicina, y la tetraciclina. Las tetraciclinas y sus análogos, minociclina y doxyciclina, son usadas para el tratamiento de patologías que causan ojo seco como el acné rosáceo, la blefaritis o la

Fig. 8 Restasis

meibomianitis [185]. Las propiedades de estos compuestos que mejoran los signos clínicos del ojo seco son su capacidad anti-bacteriana, anti-inflamatoria y anti-angiogénica [186].

La aplicación tópica de sueros biológicos como el suero sanguíneo o el suero amniótico puede mejorar lo signos clínicos del ojo seco. Estos fluidos tienen una composición con ciertas similitudes a la lágrima natural y además son ricos en factores de crecimiento, vitaminas, inmunoglobulinas y otras proteínas necesarias para mantener sana la superficie ocular. El más utilizado es el suero sanguíneo del propio paciente (suero autólogo) [187].

Otro posible tratamiento son los ácidos grasos esenciales como el Omega-3 [126]. Se ha demostrado que el ácido linoleico y el ácido γ-linoleico administrado oralmente dos veces al día provoca una mejora significativa en los síntomas de irritación ocular [188].

Por otra parte, algunos grupos de investigación están estudiando como tratamientos potenciales el uso de lágrimas en suero y la luz pulsada intensa [189].

El único fármaco aprobado que realmente aborda el problema, es el DIQUAS de Santen Pharmaceuticals, que se comercializado solamente en Japón. Es un secretagogo análogo del diadenosina polifosfato (Ap_4A), el cual tiene la característica de estimular la secreción de los tres componentes principales de la lágrima, el agua, las mucinas y probablemente a los lípidos. Los ensayos clínicos también han demostrado que reducen significativamente la tinción corneal [190].

Fig. 9 Diquas

3. HIPOTESIS Y OBJETIVOS

Durante muchos años, la principal tendencia de las empresas farmacéuticas ha sido concentrar la mayor parte de sus recursos humanos y económicos en unos pocos principios activos o moléculas, promocionándolas en gran medida y convirtiéndolas en éxitos. En las últimas décadas su productividad en I+D ha caído en picado y se han producido cambios en las políticas empresariales del sector. Estos cambios responden a la aparición de nuevas tendencias que están transformando el mercado, como es la preferencia de los organismos responsables a aprobar solamente medicamentos verdaderamente innovadores y el reciente refuerzo de los gobiernos a centrarse en la prevención más que en el tratamiento. Otras nuevas incógnitas que influyen en el mercado, es la cada vez más dinámica demanda de las economías emergentes para nuevos medicamentos superando a los países industrializados y el número cada vez mayor de los servicios nacionales de salud que están analizando el rendimiento farmacoeconómico de un mayor número de diferentes medicamentos.

La realización de esta tesis doctoral se centra en el análisis de las nuevas tendencias del mercado y la industria farmacéutica, buscado nuevas vías de investigación, que podrían ayudar a la industria farmacéutica-oftalmológica a organizar su estrategia a largo plazo, adaptándose a la evolución de las tendencias en I+D y acomodándose a las nuevas expectativas del mercado.

Los objetivos de la tesis doctoral son los siguientes:

- Investigación del desarrollo de licencias de patentes internacionales, analizando y estudiando las estrategias de la industria farmacéutica y biotecnológica. Análisis del mercado farmacéutico a través de las patentes existentes, investigando las presentes y futuras tendencias sobre la evolución de nuevos tratamientos para las enfermedades de glaucoma y ojo seco.

- Análisis de los ensayos clínicos en curso, sobre la validez de las estrategias de las empresas farmacéuticas. Análisis del mercado farmacéutico a través de los ensayos clínicos en curso investigando las presentes y futuras tendencias sobre la evolución de nuevos tratamientos para las enfermedades de glaucoma y ojo seco. Valoración de la

seguridad y eficacia de los nuevos medicamentos en su uso en los seres humanos. Estudiar si estos enfoques médicos funcionan mejor en ciertas enfermedades o ciertos grupos de personas. Análisis y estudios de las futuras tendencias de la industria farmacéutica, y valoración de sus decisiones en el desarrollo de nuevos medicamentos.

4. RECOPILACIÓN DE ARTÍCULOS

Con el fin de facilitar la lectura y comprensión de este trabajo, la información de los artículos publicados ha sido desglosada en tres apartados en función de la enfermedad estudiada:

- ***Diagnóstico de Glaucoma***

  Título: Recientes desarrollos y patentes sobre biomarcadores del diagnóstico de Glaucoma.

  Título en inglés: Recent Patents and Developments in Glaucoma Biomarkers.

  Año: 2012 **Volumen y Páginas:** 6 (3):224-34.

  Autores: Colligris B, Crooke A, Gasull X, Escribano J, Herrero-Vanrell R, Benitez-del-Castillo JM, García-Feijoo J, Pintor J.

  Revista: Recent Patents on Endocrine Metabolic & Immune Drug Discovery

- ***Tratamiento de Glaucoma***

  Título: Actualización sobre nuevos medicamentos de glaucoma en química medicinal: nuevas pruebas de la importancia de los análogos de melatonina.

  Título en inglés: Update in Glaucoma Medicinal Chemistry: Emerging Evidence for the Importance of Melatonin Analogues.

  Año: 2012 **Volumen y Páginas:** 19 (21):3508-22.

  Autores: Crooke A, Colligris B, (los dos autores contribuyeron de igual forma) Pintor J.

  Revista: Current Medicinal Chemistry

  Título: El papel potencial de inhibidores de las proteínas Rho-quinasas para el tratamiento del Glaucoma.

  Título en inglés: Potential Role of Rho-Associated Protein Kinase Inhibitors for Glaucoma Treatment.

Año: 2012 **Volumen y Páginas:** 6(2):89-98

Autores: Colligris B, Crooke A, Huete F, Pintor J.

Revista: Recent Patents on Endocrine Metabolic & Immune Drug Discovery

- ***Tratamiento de Ojo Seco***

Título: El estado actual del tratamiento de la enfermedad del ojo seco. Los avances en los ensayos clínicos.

Título en inglés: An update on dry eye disease molecular treatment. Advances in drug pipelines.

Año: 2014 **Volumen y Páginas:** 15 (10):1371-90

Autores: Colligris B, Crooke A, Huete-Toral F, Pintor J.

Revista: Expert Opinion on Pharmacotherapy

Título: Dry eye disease compounds currently under evaluation in clinical trials.

Título en inglés: Compuestos para el tratamiento de Ojo Seco que están en ensayos clínicos.

Año: 2012 **Volumen y Páginas:** 80(1) 151-178

Autores: Colligris B, Pintor J

Revista: Anales de la Real Academia de Farmácia

Artículo sobre Biomarcadores de Glaucoma

Recent Patents and Developments in Glaucoma Biomarkers

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Abstract: Glaucoma is an eye condition mainly developed from an excessive intraocular pressure. The condition tends to be inherited and may not show up until later in life. The increased pressure, can damage the optic nerve, provoking loss of vision. Without treatment, glaucoma can cause blindness within a few years; consequently glaucoma has to be diagnosed before long-term visual loss occurs. If it is diagnosed and treated early, the disease can be controlled. Usually, the patient does not notice any early symptoms or pain from this increased pressure, so the early diagnosis is problematic. Over half of the patients with glaucoma are unaware they have this blinding disease and by the time they are diagnosed, they already have irreversibly lost approximately 30-50% of their retinal ganglion cells. Glaucoma diagnosis is currently based on specific signs of the disease, characteristic optic nerve head changes and visual field loss. Thus, improved methods for early diagnosis of glaucoma are needed. Molecular genetics are valuable for the understanding the pathophysiology and cure of glaucoma, but still are not widely used for its diagnosis. Genetic studies on glaucoma have revealed many genes and chromosomal loci associated to glaucoma. Consequently, a better understanding of the molecular mechanisms underlying glaucoma is required to obtain early diagnosis and avoid potential disease progression. In this article, we revise the patents and the corresponding literature on the latest developments and approaches in glaucoma diagnosis, using mainly molecular genetics.

Keywords: Biomarkers, diagnosis, glaucoma, intraocular pressure, trabecular meshwork.

INTRODUCTION

Glaucoma is a group of diseases characterized by a retinal and optic neuropathy, where progressive visual field loss is very often associated with chronic elevation of intraocular pressure (IOP). Sufficiently high and persistent IOP elevation is believed to result in damage to the optic disc at the juncture of the optic nerve and retina, resulting in degeneration of retinal ganglion cells and blindness [1].

There are several types of glaucoma. The two main types are open-angle and closed-angle glaucomas. In closed-angle glaucoma, the peripheral iris blocks the anterior chamber angle by apposition or synechiae, preventing the drainage of the aqueous humor. In the case of primary open-angle glaucoma (POAG), the anterior chamber angle is open but the aqueous humor drainage through trabecular meshwork is almost shut (especially in the juxtacanalicular tissue regions) [2]. Primary glaucoma is not associated with pre-existing diseases whereas secondary glaucoma is a consequence of another ocular or systemic disease, trauma, or from drug adverse effects [3, 4].

Glaucoma is currently treated by lowering the elevated intraocular pressure associated with the disease, using three methods: with medical management (with drugs such as beta-blockers, carbonic anhydrase inhibitors, miotics or prostaglandins) [5], with laser therapy [6]and surgical management [7]. All of these therapies indirectly lower intraocular pressure but does not address the underlying disease process. It would be advantageous to be able to diagnose glaucoma before the patient begins experiencing a loss in visual field and deterioration of the optic disc.

CURRENT METHODS USED FOR THE DIAGNOSIS AND MANAGEMENT OF GLAUCOMA

Glaucoma is usually diagnosed by monitoring visual fields, optic nerve, and intraocular pressure. The methods in use for the diagnosis of glaucoma are the following:

Tonometry. With a tonometer intraocular pressure is measured. It is usually the initial screening test and an important part of glaucoma evaluation. High pressure readings are often the first suggestion for glaucoma. Normal eye pressure generally ranges between 10 and 21 mm Hg. However,

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patients with normal-tension glaucoma (NTG) can have damage in their optic nerve and visual field loss even though their eye pressure remains consistently lower than 21 mm Hg [8].

Ophthalmoscopy. It is an inspection of the optic nerve for possible damages. It is performed directly through the pupil using an ophthalmoscope using a slit lamp exam with a 70D lens that can reveal slight changes indicating the beginnings of glaucoma. The pupils are dilated prior to examination with tropic amide 1% and phenylephrine 2%. The normal optic nerve is made up of more than one million nerve cells and as glaucoma damages the optic nerve, it causes the death of a number of these cells. Cupping is a result of thinning of the NFL due to ganglion cell loss. As ganglion cell loss increases, there is loss to the visual field. As a result, the appearance of the optic nerve changes in a procedure referred as cupping. As the cupping increases, blank spots begin to develop in the patient's vision field [9].

Gonioscopy. Gonioscopy allows getting a clear look at the drainage angle in order to determine the glaucoma type. It is conducted by using a mirrored lens and examining the drainage angle to determine if the patient suffers from open-angle glaucoma where the drainage angle is not working efficiently enough, closed-angleglaucoma where the drainage angle is at least partially blocked, or a narrow angle in which the iris is blocking the eye's drainage system [10].

Pachymetry. It implicates the measuring of cornea thickness with a pachymeter. Cornea thickness is an important factor in diagnosing glaucoma. Thick corneas may provoke higher than normal eye-pressure readings. Similarly, people with thin corneas can have normal pressure readings but still suffer from glaucoma [11].

Visual field test. With this vision test, it is possible to determine if the visual field has been affected by glaucoma. During this test the clinician looks for vision areas of decreased vision that correspond to loss in the nerve fiber layer (NFL). The test is performed using a perimeter. Visual field testing is usually performed every 6 to 12 months to monitor for possible changes. The drawback of the visual field test is that it is a subjective andtherefore it depends on the patient collaboration [12].

Tonography. This is a test measuring how quickly fluid drains from the eye [13].

Stereoscopic optic nerve photographs. It is another method to detect glaucoma progression by studying the changes in the optic nerve in repeated sessions. The optic nerve photographs are usually taken every year and the photographs are compared to follow glaucoma progression concerning features as disc cupping [14, 15].

Nerve Fiber Imaging. These tests, measure the physical thickness of the NFL in the retina. In glaucoma the nerve fibers are diminishing and the patients are likely to have a thinner nerve fiber layer than normal. The nerve fiber analyzer is an instrument capable of measuring the actual thickness of the NFL, by using the principle of polarization shifting of laser light. The glaucoma-scopeis another instrument used to measure the NFL thickness. Its imaging system, generates standard disc parameters such as cup/disc ratio and neural rim area, as well as more recently developed algorithms for monitoring change in disc structure. Another instrument used is the HRT (Heidelberg Retina Tomograph). These types of tests are quite new and at present they are only considered supplemental tests [16].

Optical Coherence Tomography (OCT) is a painless, non-contact, non-invasive imaging technique used to obtain high resolution cross-sectional and three dimensional images of the retina, cornea and anterior chamber of the eye. OCT provides images at much higher clarity and resolution than other imaging instruments such as MRI or ultrasound. Optical Coherence Tomography obtains sub-surface images of translucent or opaque materials at a resolution equivalent to a low-power microscope [17].

Finally, a number of new approaches are being used to test for functional glaucoma damage. A whole-field test of scotopic function to detect injury is considered faster than other types of tests [18]. Furthermore the Dicon perimeter is being measured as an alternative in glaucoma screening and even as a replacement for present threshold test methods, because in patients with glaucoma, the frequency of fixation errors was significantly greater for kinetic fixation than for static fixation, forming a measurable difference to evaluate [19].

The drawback of all the above methods is that patient's collaboration is needed and almost all are subjective. Furthermore, none is definitive in the prediction of the progression of glaucoma to blindness.

MOLECULAR GENETICS USED AS DIAGNOSIS OF GLAUCOMA

Molecular genetics area valuable tool for the understanding of the pathophysiology and for the cure of glaucoma, but still they are not widely used for its diagnosis. This could be explained by the fact that the majority of the molecular mechanisms leading to glaucoma development are generally still unknown. In various studies it has been identified the association of the genetic loci and the responsible genes with various types of glaucoma. The high ocular tension was considered as a major risk factor for glaucoma, and the principal cause of optic nerve damage. In fact at the moment, the existing therapeutic procedure is to diminish the IOP with drugs or through surgical operations [5, 20, 21]. In fact at the moment, the existing therapeutic procedure is to diminish the IOP with drugs or through surgical operations [4, 19, 20]. Recently the increase in biomarkers has been correlated to glaucoma. Specifically, the increase in nitric oxide NO [22], bone morphogenetic proteins (BMP) [23], cytotoxicity [24] and the absence of the vital nutrient brain derived neurotrophic factor (BDNF) [25].

In glaucoma because due to the elevated intraocular pressure and the above mentioned factors, retinal ganglion cell (RGC) death occurs causing loss of vision [26]. The RGC death is due to programmed cell death in a process called apoptosis [27]. In the near future, the molecular genetics of glaucoma will define the exact mechanism of cell death [28], and explain the different therapeutic approach for the management of each glaucoma type [29, 30].

Several efforts were made towards the identification of the genetic factors which are associated to glaucoma. Adult-

primary open angle glaucoma (POAG), the most common type of glaucoma, has been reported to show strong evidence for genetic heterogeneity [31]. Recent genetic studies have identified gene mutations in various populations and a genetic basis for glaucoma pathogenesis has been established [32]. Linkage analysis, association studies and genome-wide association studies were used for the investigation of the genetic basis of POAG which is linked to at least 20 genetic loci [33]. About 5% of POAG is attributed to single-gene or Mendelian forms of glaucoma [33]. The myocilin gene (MYOC) mutation, is reported as a common cause of primary juvenile open-angle glaucoma (JOAG) and infrequently can be implicated in congenital/infantile or adult-onset forms [34]. The cytochrome P4501B1 (CYP1B1) gene in chromosome 2p21 is connected with primary congenital glaucoma [35]. Mutations in CYP1B1, in addition to being the most common identifiable cause of autosomal recessive primary congenital/infantile glaucoma, can infrequently underlie JOAG and even POAG [36]. It is reported that seven different heterozygous mutations in the gene are accounting for about 1.7% of patients of European origin. Another candidate mutation is located on the Neurotrophin-4 (NTF4) gene. The gene is associated to the neurotrophin 4 protein (NT-4) and its specific tyrosine kinase receptor B (TrkB). Expression of recombinant NT-4 carrying the most frequent mutation was demonstrated to lead to decreased activation of TrkB [37]. The optineurin (OPTN) gene codes for the 66-kDa, Ras-associated protein RAB8, and transcription factor IIIA. It is expressed in trabecular meshwork, non-pigmented ciliary epithelium, retina, and brain [38]. Monemi *et al.* described WDR36 at the GLC1G locus as a causative gene for adult-onset POAG [39]. There are reports for other loci genes and variants to have a role in the development of POAG but their participation is uncertain [40, 41]. POAG rarely follows simple Mendelian genetics, but genomic studies in different populations are revealing potential genetic risk factors for the phenotype [42].

The mutations mentioned above most likely are leading to glaucoma and usually they are not found in normal subjects [33] but there is still a lot of ground to cover before we have a complete picture of the many different genes that cause the disease. At the moment only 2 genes, MYOC and OPTN are considered definitively as glaucoma causing genes [43]. In any case, the already known pathogenetic mechanisms of glaucoma could be used to screen individuals routinely for disease susceptibility and generate methods for objective diagnosis. This is depending on the future increase in the sensitivity and specificity of genotyping. Myocilin GLC1A, LOXL1 gene on chromosome 15q24, 27 Glu in the ADRB2 gene among others are the predominant gene mutations used for the glaucoma diagnosis, management and in methods to follow the progression of the disease.

MYOCILIN GLC1A GENE MUTATIONS

MYOC gene encodes a secreted glycoprotein of 504 amino acids called myocilin which is highly expressed in eye drainage structures. It was identified as an up-regulated molecule in cultured trabecular meshwork cells after treatment with dexamethasone and for some time it was named trabecular meshwork-inducible glucocorticoid response (TIGR) [44]. MYOC gene is situated in chromosome 1q23-25 and its mutations may lead to JOAG and POAG. It was the first gene to be linked to JOAG discovered in 1995 [45]. It promotes cell migration through the activation of the integrin-focal adhesion kinase-serine/threonine kinase signaling pathway [46]. Functions of wild-type myocilin are still unclear. Polymorphisms in an intron region of the myocilin gene are associated with some forms of POAG and they have a possible role for alternate splicing [47]. Nguyen *et al.* demonstrated that characterization of the gene and its products has provided evidence as to the mechanisms by which the MYOC could produce biochemical changes resulting in physiologic and pathogenic effects obstructing the aqueous humor outflow and participating in the pathogenesis of glaucoma [48].

Kwon *et al.* has demonstrated that myocilin is a modulator of Wnt signaling and may affect actin cytoskeleton organization [49]. Myocilin and its naturally occurring proteolytic fragments, similar to Wnt3a, are able to stimulate trabecular meshwork, NIH3T3, and FHL124 cell migration, with the N-terminal proteolytic fragment of myocilin lacking the olfactomedin domain producing the highest stimulatory effect [50]. Stimulation of cell migration occurs through activation of the integrin-focal adhesion kinase (FAK)-serine/threonine kinase (AKT) signaling pathway [51]. Activation of several components of this signaling pathway was also demonstrated in the eyes of transgenic mice expressing elevated levels of myocilin in the eye drainage structures. These data extend the similarities between actions of myocilin and Wnt proteins acting through a β-catenin-independent mechanism [49]. The modification of the migratory ability of cells by myocilin may play a role in normal functioning of the eye anterior segment and its pathology including glaucoma [50]. Pandaranayaka *et al.* suggested that polymorphisms in the myocilin genomic region that cause synonymous codon changes or those that occur in the intron regions can possibly lead to altered myocilin protein products through altered intron-exon splicing [52]. Menaa *et al.* suggested that the modulation wild-type (wt) myocilin protein expression is not causative of glaucoma but some misfolded and self-assembly aggregates of mutated myocilin may be associated with POAG in related or unrelated populations [53].

The above findings result in innovative prospects for the presymptomatic molecular diagnosis of POAG. Errors in Myocilin account for 4% of individuals affected with glaucoma worldwide, but given the prevalence of glaucoma, this is still a large number. Myocilin mutations account for 10% of disease in families known to have JOAG [54].

The role of the MYOC gene mutation, in the development of glaucoma, directed Nguyen *et al.* to fill the patent US20026475724 [55], (Table **1**) about using the upstream sequences of the MYOC/TIGR protein encoding sequence as a diagnosis tool for glaucoma and ocular hypertensive disorders. The method is related to nucleic acids sequences comprising non-coding regions or promoter regions associated with the MYOC. The nucleotide sequence includes a functional regulatory region. These nucleic acids can be used in identifying polymorphisms in the genomes of patients predicting steroid sensitivity or a susceptibility to glaucoma or diseases related to alterations in IOP. For polymorphisms

Table 1. Patents Reviewed in This Review Article.

Patent Number	Authors	Title
US7357931 (2008), US0064532 (2012)	Clark, A.F., Wang, W.H., Mcnatt, L.	Use of serum amyloid a gene in diagnosis and treatment of glaucoma and identification of anti-glaucoma agents
US7220546 (2007)	Clark, A.F., Wordinger, R.J.	Methods for diagnosing glaucoma and discovering anti-glaucoma drugs
US0278747 (2010)	Cordeiro, F., Moss, S.	Carrier
US0284922 (2010)	Cordeiro, F., Moss, S., Fitzke, F.	Marker
US0166268 (2006)	Deelman, B.J., De Pater, J.J.M., Saman, E.J., Tartarin, I.	High purity monoalkyltin compounds and uses thereof
US7718697 (2010)	Drace, C.D., Williams, G.W., Kelly, C.R., Sharif, N.A.	Method for treating glaucoma comprising administering a-lipoic acid
US0069893 (2005)	Flammer, J., Golubnitschaja, O.	Diagnostic method for glaucoma
US0166268 (2006)	Grus, F., Joachim, S., Pfeiffer, N.	Diagnosis of glaucoma by complex autoantibody repertoires in body fluids
US0196895 (2010), US0207122 (2011)	Kinoshita, S., Tashiro, K., Nakano, M., Yagi, T., Mori, K., Ikeda, Y., Taniguchi, T., Kageyama, M.	Method for determining of progression risk of glaucoma
US0298839 (2009)	Linz, G., Sieger, P., Kraemer, G.F., Herter, R., Hoffmann, M., Rall, W., Schmid, R.	ADRB2 gene polymorphism associated with intraocular pressure response to topical beta-blockers
US6475724 (2002)	Nguyen, T.D., Polansky, J.R., Chen, P., Chen, H.	Nucleic acids, kits, and methods for the diagnosis, prognosis and treatment of glaucoma and related disorders
US6150161 (2006)	Nguyen, T.D., Polansky, J.R., Huang, W.	Methods for the diagnosis of glaucoma
US6956103 (2005)	Stone, E.M., Sheffield, V.C., Alward, W.L.M., Fingert, J.	Methods and compositions for preventing and treating glaucoma; and glaucoma diagnostics are disclosed
US0035279 (2009)	Thorleifsson, G., Magnusson, K.P.	Genetic variants on chr 15q24 as markers for use in diagnosis, prognosis and treatment of exfoliation syndrome and glaucoma
US0124009 (2011)	Iwata, K., Matsuno, K., Tanahashi, K.	Composition, kit and method for assaying neuropathy

detection, it is possible to use hybridization, hybridization to microarrays containing immobilized nucleic acids or other immobilized nucleic acids, amplification based methods such as PCR and an appropriate biosensor apparatus comprising a nucleic acid or nucleic acid binding reagent. Nguyen *et al*. filled a continuation patent, the US20066150161 [56] (Table **1**). The invention concerns a novel peptide sequence which is encoding a substantially purified MYOC protein and was demonstrated to be highly induced by glucocorticoids in the endothelial lining cells of the human trabecular meshwork (HTM). The cDNA for this protein, the protein itself, molecules that bind to it and nucleic acid molecules that encode it, could provide a method for diagnosing glaucoma and related disorders connected to elevated IOP. The method proposed is about determining the amount of MYOC/TIGR protein present in the trabecular meshwork of an eye of a patient and determining whether that amount exceeds the normal figures.

The University of Iowa, working on mutations of the same locus, filled the patent US20056956103 [57] (Table **1**), for a molecular diagnostic of glaucoma. In the invention features the use of isolated GLC1A nucleic acid molecules as diagnostic tool. There are two possibilities in the use of these molecules. The first possibility is indirect action including non-coding means probe or an antisense or ribozyme molecule. Ribozymes are antisense RNA molecules that have catalytic activity. They function by binding to the target RNA moiety through Watson-Crick base pairing and inactivate it by cleaving the phosphodiester backbone at a specific cutting site [58]. The second possibility is a direct action including a functional polypeptide. For example, they can encode a polypeptide which specifically modulates, or by acting as either an agonist or antagonist to at least one bioactivity of a myocilin polypeptide [59].

The subject GLC1A nucleic acids can include a transcriptional regulatory sequence, which is operably linked to the GLC1A gene sequence. Such regulatory sequences in conjunction with a GLC1A nucleic acid molecule can provide a useful vector for gene expression. Host cells transfected with this expression vector whether prokaryotic or eukaryotic and *in vitro* and *in vivo* could be used for producing GLC1A proteins by employing these expression vectors.

The invention also provides probes and primers comprising substantially purified oligonucleotides, which include an attached label group, capable of being detected.

Iwata *et al.* presented the patent US20110124009 [60] Table **1**, related to the above method for detecting glaucoma. The method comprises of measuring and detecting one or more specific polypeptides. The proteins used are the following: tubulin alpha-1A chain, SAPS domain family member 1, lim and SH3 domain protein 1, synaptosomal-associated protein 23, latent-transforming growth factor beta-binding protein, isoform lL, drebrin, proto-oncogene tyrosine-protein kinase Src, thymosin beta-10, zinc finger protein 185, dynamin-1-like protein, protein phosphatase 1 regulatory subunit 12A, platelet endothelial cell adhesion molecule, transgelin-2, AP-2 complex subunit sigma-1 and exportin-7.

A biological sample from a patient is tested for mutants or fragments carried out by mass spectrometry. The inventors proposed blood protein markers specifically detected in glaucoma patients by subjecting blood specimens of patients with glaucoma and samples from patients with other ocular diseases to proteome analysis. This finding led to the completion of a method for determining glaucoma using protein markers. The marker comprises of a substance capable of binding to a polypeptide. The substance capable of binding is an antibody or an antigen-binding fragment. The antibody is labeled with an enzyme, a fluorophor, a dye, a radioisotope, or a biotin. The antibody is a monoclonal or a polyclonal antibody or an antigen-binding fragment. According to the inventors the principal advantage is that the described markers are found in a biological sample of a patient with glaucoma, but not in the same sample of a patient with a different ocular disease such as cataract or age-related macular degeneration. The simple use of such markers as an indicator provides a significant advantage that glaucoma can be easily detected using as sample blood or serum.

Thorleifsson *et al.* presented the patent US20090035279 [61] (Table **1**) related to a method of diagnosing a susceptibility to an ocular disorder, including glaucoma and exfoliation syndrome (XFS). The method is based to the evaluation of certain markers that have been found to be associated with increased and decreased susceptibility of glaucoma and XFS. The inventors have discovered that certain variants associated with the LOXL1 gene on chromosome 15q24 are associated with XFS and glaucoma. Amino acid changes at polymorphic positions 141 and 153 in the LOXL1 amino acid sequence are associated with glaucoma and XFS. This was later verified by Sethi *et al*. who demonstrated that the five LOX genes (LOX, LOXL1 to 4) are expressed in cultured human TM cells [62]. Certain alleles at polymorphic sites are more frequently present in individuals diagnosed with XFS and/or glaucoma than in the general population. The invention also pertains to determination of the amino acid sequence of LOXL1 protein. The method for identifying particular alleles of such markers, for use in diagnostic applications is through obtaining nucleic acid sequence data from a patients and identifying at least one allele of at least one polymorphic marker associated with the LOXL1 gene. Different alleles of the at least one polymorphic marker are associated with different susceptibilities to the at least one condition in humans, and determining a susceptibility to at least one condition selected from XFS and glaucoma from the nucleic acid sequence data.

OTHER SINGLE NUCLEOTIDE POLYMORPHISMS (SNP)

Based on a single nucleotide polymorphism (SNP) Linz *et al.* presented the invention US20090298839 [63] (Table **1**), for a method related to the SNP-rs1042714 in the human ADRB2 gene (Gln27Glu). The gene is associated with a clinically reduction in IOP in patients following treatment with a topical beta-blocker. It is already shown that the IOP is significantly higher in POAG patients carrying 27Glu in the ADRB2 gene than in patients without this allele [64, 65]. In the mentioned invention, the nucleic acids comprising the SNP are used to screen glaucoma patients and to provide an improved method for genotype-based prescribing of beta-blockers in glaucoma management. The method is useful to interpret the results of treatment with a beta-blocker and predict the lowering of the IOP due to the drug. Usually, allele-specific hybridization (ASH) to DNA microarrays is used for SNP genotyping and mutation analysis [66]. Another method to assay the SNPs is with allele specific primer extension (ASPE) which is a solution based, sequence specific enzymatic reaction technology that can be used to assay multiple SNPs in a single tube [67]. It could be used as well, allele-specific amplification, sequencing, 5' nuclease digestion, molecular beacon assay, oligonucleotide ligation assay, restriction fragment size analysis, invasive cleavage assay, branch migration assay, denaturing gradient gel electrophoresis, immunoassay, or single stranded conformation polymorphism analysis. The method is working by identifying patients who will experience a clinically significant reduction in IOP after the treatment with a topical beta-blocker. It comprises of a detection probe for detecting a CC genotype at coding single nucleotide polymorphism SNP rs1042714 in the ADRB2 gene (Gln27Glu) in a nucleic acid sample from a patient. Suitable biological samples include, blood, buccal swabs, hair, bone, and tissue samples, such as skin or biopsy samples. The presence of the CC genotype indicates that the patient will experience a reduction in IOP after treatment with a topical beta-blocker as compared to a patient lacking the CC genotype and undergoing the same treatment.

Kinoshita *et al*. presented the series of patents US20100196895, US20110207122 [68] (Table **1**), about a method of determining the presence or the absence of glaucoma risk, by detecting *in vitro* an allele and/or a genotype of a SNP which is located on a 31st base of a base sequence. A patient sample is taken and compared to the allele and the genotype detected with one of an allele and a genotype, containing a high-risk allele. The method according to the inventors is working for both POAG and normal tension glaucoma (NTG). According to the inventors, the method is useful because the period of a clinical trial for a glaucoma therapeutic drug can be shortened by selecting patients with a high progressive risk of glaucoma using a single nucleotide polymorphism associated with the progression of glaucoma and performing a clinical trial for a glaucoma therapeutic drug.

SERUM AMYLOID A AS A DIAGNOSIS MARKER

In the year 2008, Alcon and Novartis filled a series of patents US20087357931, US20120064532 [69] (Table **1**), providing a method for diagnosing glaucoma modulating the expression of Serum Amyloid A (SAA). SAA, which is an acute-phase apolipoprotein, is playing an important role in infection, inflammation, and tissue repair and may contribute to the pathogenic changes to the TM in glaucoma.

The invention is based on the discovery that the expression of SAA mRNA and protein are significantly unregulated in glaucomatous TM tissues and cells. It is verified the differential mRNA expression seen using Affymetrix gene chips, by real time quantitative polymerase chain reaction (qPCR) and by increased SAA protein levels detected using SAA ELISA. It is demonstrated that elevated SAA expression in glaucomatous TM tissues and cells causes the glaucoma phenotype of elevated IOP. From these results, the authors believe that SAA is involved in the development or the pathogenesis of glaucoma [70].

Based on the inventors' finding that certain patients with glaucoma have increased levels of SAA expression, the invention is providing a method for glaucoma diagnosis. With the invention can be detected mutations in nucleic acid sequences resulting in high levels of SAA protein. The diagnosis is based on the known nucleic acid sequence of human SAA, or the encoded amino acid sequence. Other options of diagnosis could use the genomic sequence of human SAA, the sequence of genes that regulate expression of SAA or the change in the level of SAA gene expression at the mRNA level [71].

For the diagnosis of glaucoma, a biological sample is obtained from a patient like ocular tissue, tears, aqueous humor, cerebrospinal fluid, nasal or cheek swab, trabecular meshwork cells or serum. The sample is analyzed for an abnormal level, aberrant bioactivity or mutations of the gene encoding SAA, its promoter region or its gene products. Another method presented is by collecting cells from a patient and isolate nucleic acid from the cells. The sample has to be conducted by one or more primers which specifically hybridize 5' and 3' to at least one allele under conditions such that hybridization and amplification of the allele occurs. From this procedure has to be detected an aberrant level or existing mutations.

SERUM CITRATE IN DIAGNOSIS AND FOLLOW-UP FOR GLAUCOMA

Glaucoma might be mitochondria associated disease. Fraenkl *et al*. demonstrated that since citrate is a major component in mitochondrial metabolism its determination in blood might serve as a biomarker. In their study they found that plasma citrate levels were significantly decreased in Caucasian patients with glaucoma driving them to the conclusion that citrate could be used as a biomarker [72]. According to the abstract presented at the Annual Meeting of the Association for Research in Vision and Ophthalmology, 2010, Ft Lauderdale, FL. USA, they started clinical trials (ClinicalTrials.gov Identifier: NCT00804063) in 2008 and the estimated study completion date is July 2013. The primary outcome measures are the serum citrate levels and the secondary outcome measures are the urine citrate levels.

GLUCOCORTICOID RECEPTOR GR®

Glucocorticoid administration can lead to increased intraocular pressure in more than 90% of patients with POAG, compared with 30% to 40% of the general population. The molecular mechanisms for increased steroid responsiveness among patients with glaucoma are unknown. An alternative splicing variant of the human glucocorticoid receptor GRβ has dominant negative activity and has been implicated in a variety of steroid-resistant diseases. GRβ also may play a role in glucocorticoid hyper-responsiveness in glaucoma [73].The human glucocorticoid receptor hGR has two isoforms, hGRα and hGRβ [74]. The hGRβ is expressed in an alternatively spliced form of non-ocular tissues and cells [75]. This alternatively spliced form of hGRβ is expressed as a protein. This protein no longer binds glucocorticoids, but is interfering to the activated form of the normal glucocorticoid receptor and blocks or alters its physiological functions [76].

Trabecular meshwork (TM) cell lines derived from normal individuals expressed higher levels of GRβ than did glaucomatous TM cells. Glaucomatous TM cells were more susceptible to dexamethasone induction of a luciferase reporter gene than were TM cells derived from normal donors. Overexpression of GRβ in glaucomatous TM cells inhibited dexamethasone induction of a luciferase reporter gene as well as the endogenous genes MYOC and fibronectin [77, 78]. Alcon filled the patent US20077220546 [79] (Table **1**), about a method for diagnosis of glaucoma by measuring the amount of glucocorticoid receptor GRβ present in the trabecular meshwork of a patient's eye. The method consists on obtaining a biological sample from the TM of a patient and analyzes it for the expression of GRβ. According to the company, a decrease in expression of GRβ compared to the normal expression is an indication of glaucoma. This indication is based on the assumption that glucocorticoids are involved in the generation of elevated IOP and glaucoma and a decreased amount of GRβ in glaucomatous TM cells could result in enhanced glucocorticoid responsiveness and ocular hypertension [80].

APOPTOSING RETINAL CELLS (DARC)

Recently in the University College of London, it was developed a new, noninvasive real-time imaging method, named DARC (detection of apoptosing retinal cells) to make early diagnosis and management of age-related neurodegenerative processes. It is not considered a direct molecular genetics diagnostic method since it is using imaging technology for the detection of glaucoma. The conventional clinical tests cannot detect abnormalities until extensive retinal ganglion cells (RGC) death and significant vision loss has already occurred. The RGC neuronal apoptosis is considered the earliest sign of glaucoma [26]. The purpose of this study was to develop an automated method of counting the number of RGCs. The method, according to the inventors, is accurate, time-efficient and non-operator dependent. An algorithm was developed on Matlab software, which allows identification of apoptosing RGCs labeled with fluorescent annexin-5. Processing included a local-luminance and local-

contrast "gain control", a “blob analysis” to differentiate between cells, vessels and noise, and the exclusion of non-cell structures using combined size and aspect ratio criteria. The algorithm then generates an automated count of the total number of accurately positively identified apoptosing RGCs. The manual count of apoptosing RGCs was used as the gold standard comparator in the analysis. 100 rat eyes imaged with DARC underwent assessment using manual counting by a blinded individual and automated counting using our newly developed technique. To test statistical correlation, Pearson’s R, Intraclass Correlation Coefficient (ICC) and Cronbach’s Alpha Reliability Coefficient were used [81]. It was filled the patent US20100284922 [82] (Table **1**), about the marker related to the use of a cell death marker labeled with a wavelength-optimized label for identifying cell death in the eye. The marker is used in the direct visualization of cell death, particularly apoptosis in the eye, especially single cell death of retinal nerve cells.

As cell death markers, Annexins fragments and derivatives were used. Annexins (natural or recombinant) are proteins that bind reversibly to cellular membranes in the presence of cations. Fluorescent substances such as IRDye800, D-776 and D-781 (see Fig. **1**) were used as wavelength-optimized labels. In order to generate an image of cell death, the labeled marker is administered to the subject by, for example, intravenous injection. The area of the subject to be imaged, the eye, is placed within the detection field of a medical imaging device. Emission wavelengths from the labeled marker are then imaged and an image constructed so that a map of areas of cell death is provided. Generation of the image may be repeated to allow cell death to be monitored over a period of time and in real time. It is particularly preferred to monitor the death of retinal cells, especially retinal nerve cells.

The same inventors filled the patent US20100278747 [83] (Table **1**), about a carrier for delivering the above mentioned agent to the posterior region of the eye. The carrier comprises of a Vitamin E derivative, especially tocopherol. Vitamin E is used as the generic description for all tocol and tocotrienol derivatives, with similar biological activity as a-tocopherol (see Fig. **2**). The combination of tocopherol/tocotrienol with Annexin mediates trans-scleral delivery of the annexin to the retina following application as an eyedrop in the eye.

In ARVO 2011, there were presented the results of these studies. During the experiments there was an agreement between the apoptosing RGC counts obtained through manual and these using the new method. The novel automated image analysis algorithm enabled an accurate calculation of the total number of apoptosing RGCs and it was highly comparable to the manual count. The new method has the advantages of being reproducible, fast, non-labor intensive and cost-effective. It could be used in the early diagnosis, monitoring and assessment of glaucoma and retinal neurodegeneration [84]. Clinical trials of DARC in glaucoma patients (ISRCTN-59484478) lasted from 2008 to 2011. They were executed in three stages. In the first stage they did a cross-sectional pilot study to evaluate DARC counts in age-matched groups of "normals" to non-progressing and progressing glaucoma patients, and ocular hypertensive and normal tension glaucoma patients. In the second stage they did a prospective investigator masked randomized active placebo-controlled pilot study comparing age-matched groups of “normals” to non-progressing and progressing glaucoma patients to establish baseline DARC counts before and after treatment with brimonidine or brimonidine-containing formulation. In the third stage, it was conducted a longitudinal pilot study to correlate DARC counts to visual field assessment and analysis of optic disc cupping in age-matched groups of "normals" and ocular hypertensive and normal tension glaucoma patients. The results of the trials have been not published yet.

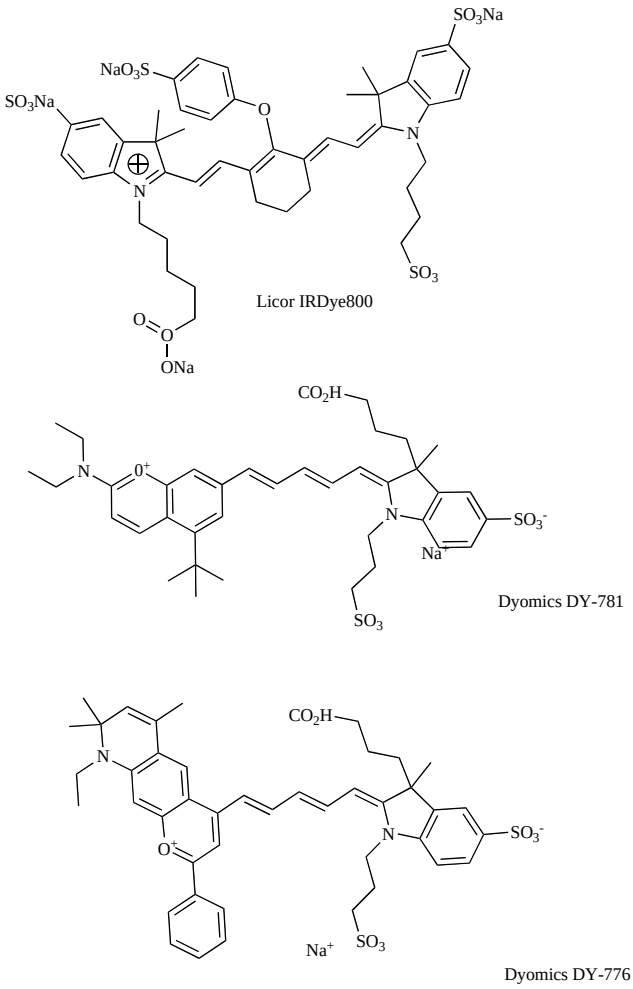

Fig. (1). The molecular structure of three of the infrared dyes used.

ANTIOXIDANT TREATMENT

Drace *et al.* filled the patent US20107718697 [85] (Table **1**), about a method for treatment and diagnosis of glaucoma by administering α-lipoic acid. The method is describing the use of agents that down-regulate expression of tanis and/or p21Waf1/Cip1/Sd1 genes to treat glaucoma. Karlsson *et al*. described a relationship between serum amyloid A level and Tanis/SelS mRNA expression [86]. The diagnosis proposed from the inventors of the patent, is realized by comparing the

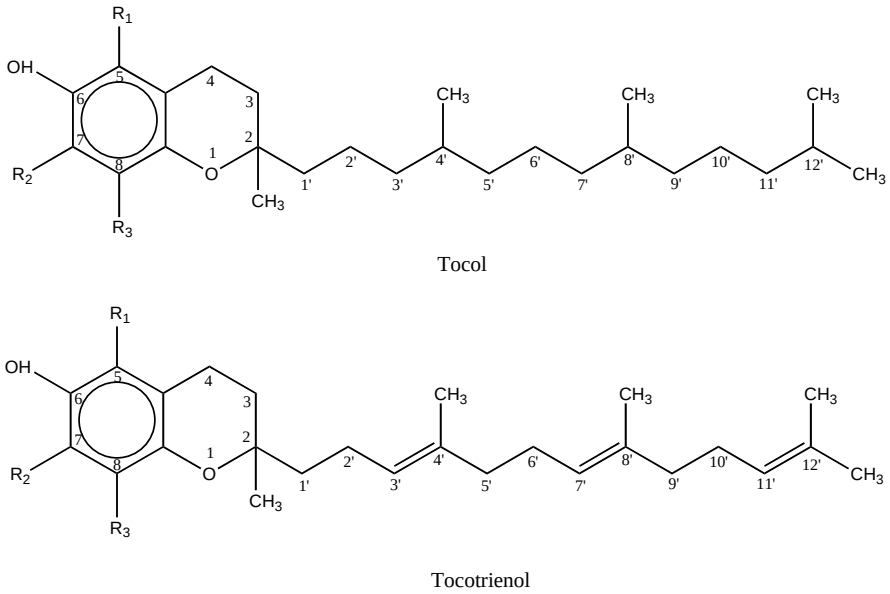

Fig. (2). The carrier used tocol and tocotrienol.

tanis gene product protein (TGPP) in patient samples to normal biological samples. Filina *et al*. described the effect of α-lipoic acid on tyrosine metabolism [87]. The effect of lipoic acid may be explained by its antioxidant properties and direct influence on ocular tissue metabolism [88].

OTHER METHODS

Mainz *et al*. presented a patent US20060166268 [89] (Table **1**), related to a method for the diagnosis of glaucoma based on the composition of auto antibodies against ocular antigens in body fluids. The method uses auto antibodies against retinal and/or optic nerve head antigens. The method is related to analysis of the autoantibody repertoire, in the patient's body fluids, against ocular antigens as biomarkers for the diagnosis of glaucoma and compared to the autoantibody pattern of healthy individuals. It is of no relevance whether the particular antibody or the antigens are properly characterized, since the procedure relies only on a molecular mass comparison. Among the antibodies used are the following: antibodies against human vimentin and humanglial fibrillary acid protein (GFAP), anti-Ro/SS-A (Sjogren syndrome A; also commonly called Ro antigens), serum antibodies that bind to human optic nerve head proteoglycans, including chondroitin sulfate and heparin, serum autoantibodies against gamma-enolase(y-enolase) in retinal ganglion cells of glaucomatous patients and glutathione S-transferase (GST) antibodies.

Finally, Flammer *et al*. filled the patent US20050069893 [90] (Table **1**), related to an *ex vivo* method for the diagnosis and/or prediction of glaucoma. The method comprises of detecting in a tissue and/or blood sample of a patient, an altered gene expression pattern of genes selected from the group of genes related to tissue remodeling. The screened genes are the following: matrixmetalloproteinase 9 (MMP-9; gelatinase B; 92-kDa type IV; collagenaseprecursor), membrane-type matrix metalloproteinase1 (MT1-MMP, MMP-14 precursor) and metalloproteinase inhibitor1 precursor 1 (TIMP-1).

Furthermore, the invention relates to a DNA microarray comprising nucleic acid probes of genes of the specified gene group. The determination of expression patterns of specific genes according to the method of the present invention allows an exact diagnosis of patients with glaucoma as well as an exact identification of patients with a predisposition for chronic glaucoma development. Additionally, the progression of the disease is monitored. According to the inventors, the method offers a number of diagnostic advantages. It is highly sensitive and minimal-invasive. It is sufficient only the collection of a small tissue sample and/or a small amount of a body fluid, in particular blood, from a patient. Then are employed gene expression detection methods such as northern blot analysis, RT-PCR, real time quantitative PCR, immunohistochemical methods, ELISA or Dot blot analysis.

CURRENT & FUTURE DEVELOPMENTS

The currently used methods for glaucoma diagnosis suffer from inaccuracy and require multiple examinations. The effectiveness of the novel drugs is carried out by monitoring the visual field changes and using conventional perimetry but the damage resulting from glaucoma is not easily detected. Most of the times it is detected when vital cells already have been lost and is impossible to replaced. It is evident that new diagnostic methods are needed, more accurate and more objective. The solution is the discovery of efficient biomarkers. A biomarker can objectively be measured and evaluated. It could indicate normal and pathologic situations, or the efficacy of a novel medication. The measurement in biomarkers is qualitative for mutation or quantitative for expression level. Molecular diagnostics and biomarkers area future diagnostic tool, but although mutation research in glaucoma detected some potential targets, the mutated genes found are playing a limited role in the pathogenesis of glaucoma and there are involved in a minority of glaucoma pa-

tients and therefore is problematic to be used for diagnosis. Furthermore the mentioned markers in this article, it seems that they lack enough specificity and sensitivity to be detected in the biological samples with the existing technology. The solution may lie in the discovery of novel efficient biomarkers. For these reasons only two of the specified methods reached clinical trials so far, the method of measuring serum citrate and urine citrate levels and the DARC non invasive real-time imaging method. For all the patents reviewed in this article (see Table **1**).

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CONFLICT OF INTEREST

It is certified that there is no actual or potential conflict of interest in relation to this article and the authors are unrelated to the companies mentioned in the patents.

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Artículo sobre los fármacos melatoninérgicos

Update in Glaucoma Medicinal Chemistry: Emerging Evidence for the Importance of Melatonin Analogues: Review on Glaucoma Drugs Focusing on Melatonin

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Abstract: Glaucoma is a chronic progressive optic neuropathy, which can result in visual impairment and blindness. Elevated intraocular pressure (IOP) is currently the only modifiable risk factor. Several recent studies have shown the benefits of IOP reduction in open-angle glaucoma . Therefore, current glaucoma drugs are IOP-lowering substances such as α_2-adrenergic agonists, β_2-adrenergic antagonists, carbonic anhydrase inhibitors and hypotensive lipids, which are used separately or in combination. In spite of the wide variety of anti-glaucoma medicines, all therapies have several undesirable side effects. As a consequence, there are constant research attempts on the discovery of novel ocular hypotensive drugs. In the current paper, we review the latest available patents and literature for the pharmacological treatment of glaucoma, focusing on their molecular targets and/or their chemical characteristics and especially directed to melatoninergic drugs. Melatonin is a hormone secreted into the blood mainly from the pineal gland allowing the entrainment of the circadian rhythms of several biological functions. Melatonin and its analogues potently reduce IOP in rabbits, monkeys and humans. In addition, there are indications of long-term hypotensive effects and a proven neuroprotective role of melatoninergic substances. Furthermore, antidepressant and normalizing circadian rhythm actions of melatonin analogues might be beneficial for glaucoma patients. All the above mentioned facts suggest these agents as proper candidates for the glaucoma treatment . Consequently, the scientific research has given new and significant progress on the development of new, potent and selective melatonin ligands.

Keywords: Glaucoma, indoles, intraocular pressure, melatonin, melatonin analogues, melatonin regulation, ocular hypertension, neuroprotection, oxidative stress, nitrosative stress.

INTRODUCTION

Glaucoma is a group of diseases characterized by retinal and optic neuropathy which progressive visual field loss is mainly associated with chronic elevation of intraocular pressure (IOP). Sufficiently high and persistent IOP is believed to result in damage of the optic disc at the juncture of the optic nerve and retina, resulting in degeneration of retinal ganglion cells leading to blindness [1]. It has been estimated that about 61 million people suffer glaucoma, and this figure is expected to rise up to around 80 million by 2020. Thus, glaucoma is the second leading cause of blindness in the world. There are many risk factors for glaucoma but the most important is high IOP (> 21 mmHg) as previously mentioned. As a result, the most common form of glaucoma classification is based on the mechanism leading to an increase of IOP. In the healthy eye, the IOP is determined by the balance between aqueous humor formation and its drainage through the major outflow pathway (trabecular meshwork and Schlemm´s canal).

Glaucoma is primarily classified as angle-closure or open-angle glaucoma, and is further categorized as primary or secondary glaucoma. In angle-closure glaucoma, the peripheral iris blocks the anterior chamber angle by apposition or synechiae. Therefore, the drainage of the aqueous humor is prevented and the IOP is increased. On the other hand, in open–angle glaucoma (POAG), the anterior angle remains open, but aqueous humor outflow is impeded in the trabecular meshwork (especially in the juxtacanalicular tissue regions) or beyond. In addition, primary glaucoma is not associated with pre-existing disease while secondary glaucoma results from other ocular or systemic diseases, trauma, or drug effects [2-5].

CURRENT GLAUCOMA TREATMENTS

The conventional method for treating glaucoma consists of an IOP reduction therapy, through pharmacology, laser therapy or surgical operation. Even to patients suffering from glaucomatous vision loss and having low IOP (known as low-tension or normal-tension glaucoma), therapies controlling IOP are recommended [2, 4, 5].

Initial treatment is generally pharmacological. The drugs, according to their nature, can be divided into (in alphabetical order, see Table **1**) α2-adrenergic agonists such as brimonidine, β2-adrenergic antagonists such as timolol, carbonic anhydrase inhibitors for instance dorzolamide, parasympathomimetics agents for example pilocarpine and hypotensive lipids like latanoprost. These pharmacological approaches contribute to the reduction of IOP either by inhibiting the production of aqueous humor by the ciliary body such as carbonic anhydrase inhibitors, or facilitating trabecular aqueous humor outflow for example parasympathomimetics agents [2, 5]. Currently used drugs for the treatment of glaucoma and their main characteristics are shown in (Table **1**).

During therapy, when the first-choice drug is not effective (IOP decrease of < 20%) or produces adverse effects, is common procedure the selection of another drug of the same group see (Table **1**). However, if after this change the target IOP (level that is expected to prevent further glaucomatous damage) is not reached, the next option is a combination of two or three drugs of the mentioned groups. Fixed drug combinations are used to obtain an additional IOP decrease without the disadvantages of higher cost and adverse effects. Normally, a β-blocker is combined with a drug of the other groups. Combination of drugs comes with drawbacks, as the mixture of compounds multiplies the probability of the development of side effects. For a more detailed review of possible anti-glaucoma medicinal combination and their effects on IOP see [5].

When medical intervention is ineffective or not tolerated, glaucoma surgery is performed but a great variety of complications have been reported [2]. In other cases as acute narrow-angle glaucoma, the use of drugs is essential to deal with a sharp attack, but long-term management is performed by surgery.

The current strategies to minimize the disadvantages of existing glaucoma pharmacological therapy are: improving existing medicines, developing new drug combinations and designing new drugs of novel action mechanism.

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Table 1. Features of Current Glaucoma Drugs

β-Adrenergic Antagonists (Non-Selective)-Decrease in Aqueous Humor Production
Carteolol (Dosis twice daily) Levobunolol (Dosis once or twice daily) Metipranolol (Dosis twice daily) Timolol (Dosis once or twice daily) Contraindications: Asthma, obstructive pulmonary disease, sinus bradycardia, heart block. Adverse effects: Allergy, irritation, blurring, corneal anaesthesia, keratitis, allergy, bradycardia, heart block, bronchospasm, decreased libido, CNS depression.
β-Adrenergic Antagonists (Selective)-Decrease in Aqueous Humor Production
Betaxolol (Dosis once or twice daily) Contraindications: Sinus bradycardia, heart block or cardiac failure asthma, (relative contraindication), obstructive pulmonary disease. Adverse effects: Blurring, irritation, corneal anaesthesia, punctate keratitis, allergy, bradycardia, heart block, bronchospasm, decreased libido, CNS depression.
Carbonic Anhydrase Inhibitors-Decrease in Aqueous Humor Production
Brinzolamide (Dosis twice or three times daily) Dorzolamide (Dosis twice or three times daily) Contraindications: Severe renal disease and sulfonamide allergy; caution must be exercised in patients with compromised corneal endothelium Adverse effects: Irritation, induced myopia, blurring, keratitis, stinging, burning, conjunctivitis, dermatitis, headache, nausea, fatigue, bitter taste.
Adrenergic Agonists (Non-Selective)-Increase in Aqueous Humor Outflow
Epinephrine (Adrenaline) (dosis twice daily) Dipivefrine (Dosis twice daily) Contraindications: Narrow anterior chamber angle and aphakia Adverse effects: Irritation, allergy, conjunctival hyperaemia, hypertension, headache, a tachycardia (less likely for prodrug).
Adrenergic Agonists (α2-Selective)-Decrease in Aqueous Humor Production, Increase in Aqueous Humor Outflow
Apraclonidine (dosis twice or three times daily) Brimonidine (dosis twice or three times daily) Contraindications: Paediatric age, monoamine oxidase inhibitor usage, antidepressant medication use, narrow anterior chamber angle and aphakia. Adverse effects: Irritation, allergy, blurring, foreign body sensation, eyelid oedema, dryness, headache, fatigue, dizziness, tachycardia, depression, dry mouth.
Hypotensive Lipids (Prostaglandin Analogues)-Increase in Uveoscleral Outflow of Aqueous Humor
Latanoprost (Dosis once daily) Travoprost (Dosis once daily) Contraindications: History or risk of cystoid macular oedema or uveitis (relative contraindications). Adverse effects: Increased iris and lash pigmentation, hypertrichiasis, conjunctival hyperaemia, blepharitis, influenza-like symptoms, joint/muscle pain, headache, increased blood pressure.
Hypotensive Lipids (Prostamides)-Increase in Uveoscleral Outflow of Aqueous Humor
Bimatoprost (Dosis once daily) Contraindications: History or risk of cystoid macular oedema or uveitis (relative contraindications). Adverse effects: Increased iris and lash pigmentation, hypertrichiasis, conjunctival hyperaemia, blepharitis, influenza-like symptoms, joint/muscle pain, headache, increased blood pressure.
Hypotensive Lipids (Decosanoids)-Increase in Uveoscleral Outflow of Aqueous Humor
Unoprostone (Dosis twice daily) Contraindications: History or risk of cystoid macular oedema or uveitis (relative contraindications). Adverse effects: Increased iris and lash pigmentation, hypertrichiasis, conjunctival hyperaemia, blepharitis, influenza-like symptoms, joint/muscle pain, headache, increased blood pressure.

REFINEMENTS OF EXISTING TREATMENTS

In this section, we review the latest available patents and literature for structural modifications and whenever possible, improvements in delivery systems.

α/β-Adrenergic Receptors Agonists/Antagonists

α_2-adrenergic agonists such as brimonidine Fig. (**1. 1**) and β_2-adrenergic antagonists such as timolol Fig. (**1. 2**) are commonly used as anti-glaucoma drugs and for a long time period. The reason is that in the eye, the adrenergic receptors present in the ciliary epithelium (α_2- and β_2-adrenoceptors) regulate aqueous humor production [6]. This regulation appears to be contradictory because both α-adrenergic agonists and β-adrenergic antagonists can be used for IOP reduction. There is quite a lot of discussion on this issue but it seems that while β-adrenergic receptors are present in the ciliary epithelium, α-adrenoceptors are located pre-junctional, therefore regulating the release of noradrenaline from the ciliary body sympathetic nerve terminals [7].

Structural modifications to the quinoxaline nucleus of brimonidine molecule i.e. Fig. (**1. 3**) and new selective agonists of α_2-adrenoceptors i.e. Fig. (**1. 4** and **5**) have been recently claimed [8-10]. In addition, it was observed improvement of brimonidine water solubility when it was forming a complex with an acid compound such as pamoic acid [8]. It has been demonstrated the use of this complex for neuroprotection purpose, to inhibit progression of glaucoma damage to the optic nerve. However, it is not fully understood how is obtained the neuroprotective effect when these compounds are topically applied. A decent delivery mechanism and adequate solubility are necessary to reach the retina on sufficient concentrations for nerve protection.

Recently, it has been reported the ocular hypotensive effect of agmantine Fig. (**1. 6**), an endogenous polyamine with high affinity to the α_2-adrenergic receptor, acting as its agonist [11]. The topical administration of agmantine, lowered IOP and reduced the loss of retinal ganglion cells in an ocular hypertensive rat model [11].

Concerning the β-adrenergic antagonists, a new class of timolol derivatives with nitric oxide (NO) groups has been described [12].

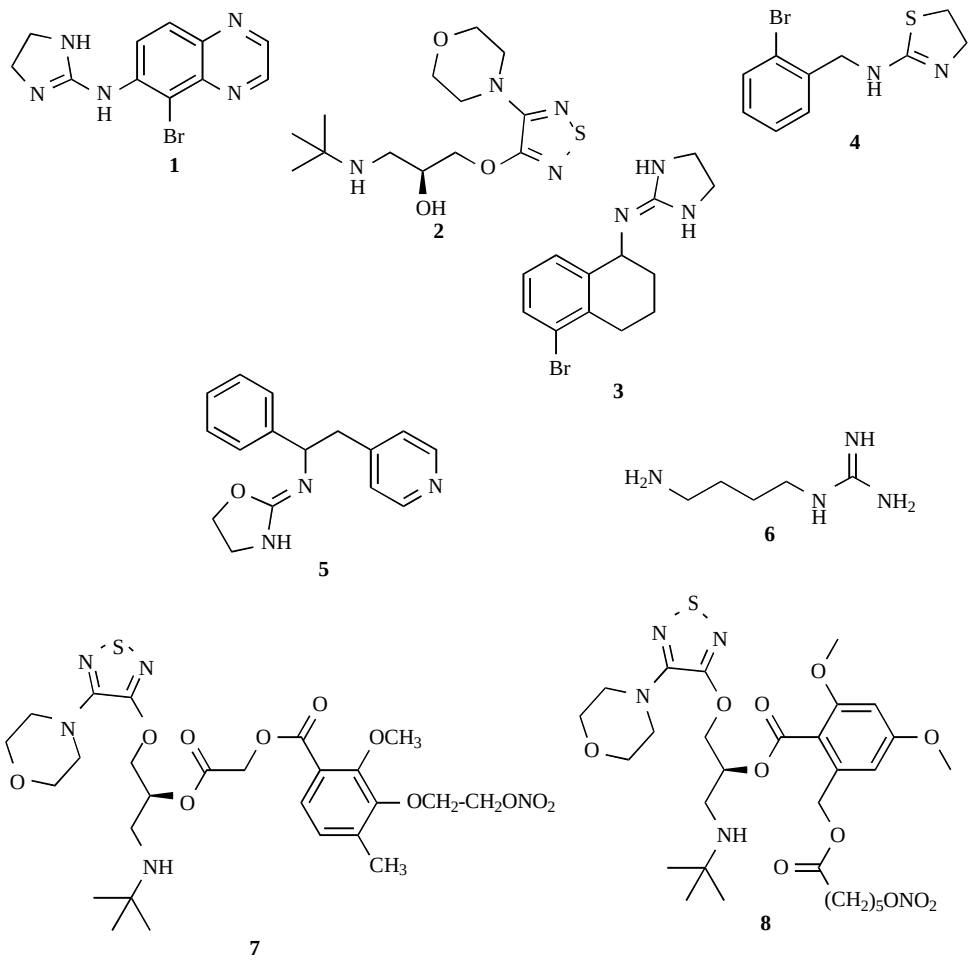


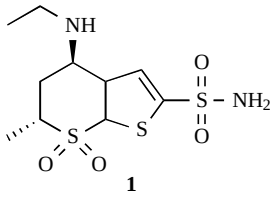
Fig. (1). Classical adrenergic agents and their derivatives.

Conjugation of these groups with classic anti-glaucoma drugs is a common strategy to enhance IOP-lowering efficacy and reduce side effects [12-16]. NO donors can effectively reduce IOP [12-15, 17] and even postpone optic nerve damage of glaucomatous patients [18]. On the contrary, there is also evidence of harmful effect of NO on glaucomatous neurodegeneration (see section New targets for glaucoma treatment: emerging role of melatonin analogues)[19-23].

Chiroli and colleagues [12], have designed and synthesized NO esters of timolol Fig. (**1. 7** and **1. 8**). Furthermore, it was evaluated their chemical stability in aqueous solution at pH 5.7 (important for eye drop formulation) and their vasodilating potency on animal tissues (to test NO-releasing). The first derivatives based on benzoates with groups in *ortho* positions i.e. Fig. (**1. 7**), were stable in aqueous solution but resistant to corneal enzymatic hydrolysis. In a second approach, the authors obtained a new type of timolol derivatives i.e. Fig. (**1. 8**), stable in solution and also cleaved by hydrolitic enzymes. This approach is innovative since it combines in the same molecule two separate activities, which may significantly reduce IOP in glaucoma patients.

A new selective β-blocker pro-drug (OT-730) has been developed by Othera Pharmaceuticals being in Phase II clinical trial (Trial ID: NCT00753168). After eye drop instillation, OT-730 is metabolized to an active β-blocker (OT-705). Afterwards is quickly broken down to an inactive product upon entry to the bloodstream. Consequently can be minimized, the side effects of β-blocker medicines, mainly the cardiovascular and respiratory ones. This is a significant improvement to the currently available β-blocker approaches, since until recently it was not possible to prescribe these types of compounds to patients with cardiovascular problems. Sylentis (ZELTIA) is also evaluating the clinical efficacy of the compound SYL040012, small interfering RNA (siRNA) targeting β_2-adrenergic receptors (Trial ID: NCT01227291). The advantage of this approach is that silencing the receptors, permitting longer-term reduction in protein expression. Therefore is extended the administration time of the hypotensive compound (is sufficient a single application every 5 days). It is important to emphasize that these results have been performed in a pre-clinical animal model hence is necessary to scale-up the administration and dosage for humans [24].

Another approach to achieving safety and efficacy is by improving the delivery systems. The most common mode to apply the glaucoma medical treatment is the eye-drops. Ocular topical medication has several disadvantages as the possible drug-washed out or dilution by tear and the blinking reflex. Another problem is the systemic absorption of these drugs due to the low permeability of corneal tissue. For these reasons, higher doses of anti-glaucoma drugs are augmenting the risk of side effects. Schultz and colleagues [25] have reported the uptake/release and the hypotensive efficacy of brimonidine and timolol onto hydrogel contact lenses. HEMA gels loaded with timolol microemulsion or nanoparticles and timolol microspheres have also been fabricated [26-28]. This could be especially indicated for those patients wearing contact lenses, with the constriction that these lenses should be replaced more frequently or the patients should be trained to re-load them with the hypotensive compound.



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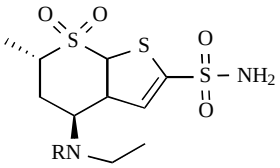
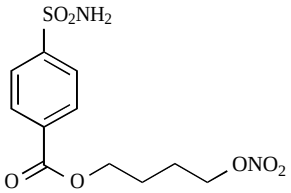


R=CO$(CH_2)_5ONO_2$ (NCX 274)
R=COO$(CH_2)_3ONO_2$ (NCX 278)

2



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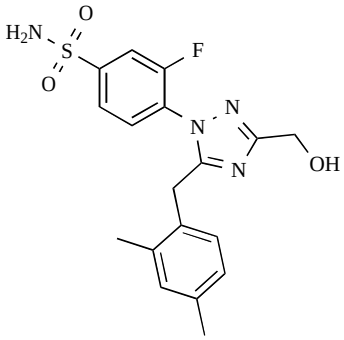

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Fig. (2). Classical carbonic anhydrase inhibitors and their derivatives.

Carbonic Anhydrase Inhibitors

Carbonic anhydrase (CA) enzymes are involved in a variety of physiological processes including the maintenance of acid-base balance and the transport and secretion of fluids [29, 30]. In the eye, these enzymes are involved in aqueous humor secretion by the ciliary epithelium [31]. Therefore, CA inhibitors (CAIs) are a common treatment for glaucoma because they produce a potent reduction in IOP by suppressing aqueous humor secretion [30, 32]. In particular, it has been suggested by many authors that CAII and CAIV are the main isozymes controlling aqueous humor production. Interestingly, it has been reported by other authors the absence of CAIV isozyme in the human ciliary epithelium [33, 34]. Conversely, an over-expression of CA12 (CAXII) isoform has been described in patients with glaucoma [34].

Systemic CAIs have been used clinically for a long time, but because they produced a wide range of side effects, they have limited use. Consequently topical CAIs such as dorzolamide (DRZ) Fig. (**2. 1**), a CAII and CAXII inhibitor, are more preferred. There is now a raised interest in developing new topical CA inhibitor more potent and selective hypotensive agents. In this sense, Steele and colleagues [15] have synthesized a large number of DRZ derivatives with NO-donating moieties at the amino group (see comments above concerning conjugation of NO groups and classical anti-glaucoma drugs). These compounds are potent inhibitors of CAII isozyme, especially NCX 274 and NCX 278 derivatives (*K*i CAII = 14 nM and 13 nM, respectively; DRZ *K*i CAII = 9 nM) Fig. (**2. 2**) and they are also present *in vitro* vascular relaxant effect (NCX 274 EC_{50} = 4.25 μM and NCX 278 2.05 μM compared to a positive reference standard which EC_{50} = 10.8 μM and DRZ (which displayed little effect) and *in vivo* hypotensive action, with similar IOP reduction than DRZ drug.

More recently, a new class of sulfonamides conjugated with NO groups has been obtained [13, 16]. These conjugated are formed by five different sulfonamide scaffolds (4-carboxy-benzenesulfonamide, 3- or 4- carboxy-benzolamides, 4-(2carboxyethyl)-benzenesulfonamide and 4-hydroxybenzenesulfonamide) attached via an ester bond with NO-containing aliphatic C3-C5 or aromatic moieties. The sulfonamides are potent inhibitors of CAII and CAXII isozymes with *K*is in the range of low nanomolar. In the case of sulfonamide derivative NCX250 Fig. (**2. 3**), a good inhibitor of CAII and CAXII (*K*i CAII = 18 nM and Ki CAXII = 30 nM) and with high water solubility (2%) which is important for topical formulation development, a potent lowering IOP effect (twice more than DRZ) was observed in hypertensive rabbits. Anti-apoptotic and anti-inflammatory effects have also been attributed to compound **3**, due to its NO moiety [35]. Accordingly, it has a double beneficial effect regarding to its anti-glaucoma potential as previously commented for other NO donors.

In another patent [36], it has been claimed the use of triazol derivatives Fig. (**2. 4**) as inhibitors of CA and consequently for glaucoma treatment but the patent does not show enzyme inhibition data or anti-glaucoma effects.

Regarding to CAIs delivery, it has been demonstrated the improved efficacy of CAIs with low water solubility when they form complexes with cyclodextrins [37]. This is a clear improvement on the final hypotensive effect not only due to the compound by itself but also in the way it is delivered. Poor soluble compounds may be better delivered with the help of carriers and other transport mechanisms.

Hypotensive Lipids (Prostaglandin Analogues and Prostamides Derivatives)

These substances are among the most powerful topical ocular hypotensive agents currently available. Prostaglandin $F_{2\alpha}$ analogues as latanoprost Fig. (**3a. 1**) bind to different prostaglandin (PG) receptors present in ciliary muscle, thereby increase biosynthesis of matrix metalloproteinases and regulate tissue inhibitors of matrix metalloproteinases. Consequently, a reduction of IOP is achieved through alteration of extracellular matrix components and resulting increase in uveoscleral (unconventional) aqueous outflow [38]. A neuroprotective effect independent of PG receptor stimulation has also been recently reported for these analogues [39].

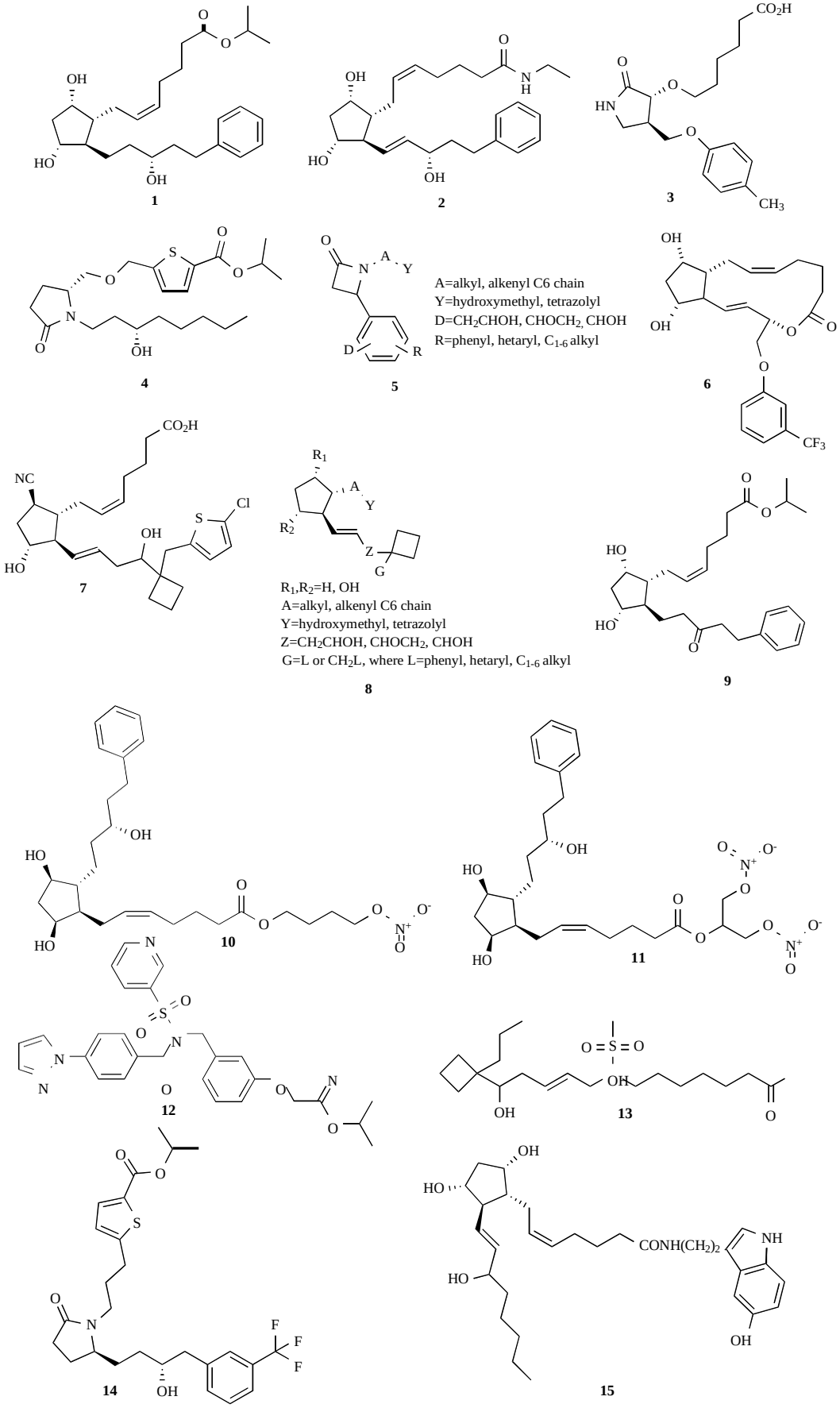


Fig. (3). Classical hypotensive lipids and derivatives.

Prostamide $F_{2\alpha}$ derivatives as bimatoprost Fig. (**3a. 2**), are acting with a slightly different mechanism than prostaglandin analogues, through a specific prostamide receptor located on trabecular meshwork cells [40, 41]. As a consequence, bimatoprost increases conventional and unconventional aqueous outflow.

Most of the last PG analogues claimed as anti-glaucoma agents are obtained from PG $F_{2\alpha}$ molecule. Thus, there have been performed structural changes in the cyclopentane scaffold of the PG $F_{2\alpha}$ such as the lactam ring present in Fig. (**3a. 3**) [42], Fig. (**3a. 4**) [43] and Fig. (**3a. 5**) [44]. In [43, 44], the affinity of the claimed compounds for PG receptors has been studied *in vitro* and the hypotensive efficacy has been proved in normotensive and hypertensive animals.

In other cases, the changes have been applied in the two lateral chains of the cyclopentane ring present in the PG $F_{2\alpha}$ backbone. Hence, there have been created compounds where lateral chains form a lactone ring as in Fig. (**3a. 6**) [45] or thiophenyl and cyclobutyl derivatives such as Fig. (**3a. 7**) [46] and Fig. (**3a. 8**) [47], respectively. Anti-glaucoma efficacy, has been only proved using thiophenyl derivatives [46]. Furthermore, the 15-ketolatanoprost analogue Fig. (**3a. 9**) has been tested in glaucomatous monkeys and its hypotensive effect was equivalent to or it was even greater than latanoprost [48]. PG analogues used in clinical prescription by ophthalmologists have normally a hydroxyl group on position 15 which is oxidized by the $NADP^+$-dependent 15-PG dehydrogenase enzyme which is highly expressed in the eye [48].

More recently, there have been reported nitric oxide releasing PG analogues as NCX116 (also called BOL-303259-X, present in Fig. (**3b. 10**) or NCX125 Fig. (**3b. 11**) [14, 49]. Regarding *in vitro* pharmacology, both nitric oxide derivatives evoke a potent and effective concentration-dependent accumulation of GMPc. NCX116 showed an EC_{50} of 1.6 μM and an E_{max} of 256% of basal, whereas NCX125 displayed an EC_{50} of 3.8 μM and an E_{max} of 795% of basal, in the same type of cells. In addition, both compounds act as potent ocular hypotensive agents in preclinical models and NCX116, is currently being evaluated in Phase II trials and other two Phase II trials have been completed but the results are not yet published (Trial ID: NCT01223378, NCT00441883 and NCT00595101) [2, 50].

Furthermore, there are other PG FP receptors agonists in phase II or III of development. This is the case of the AR-102 from Aerie Pharmaceuticals, which presents 150-fold greater selectivity and 30-fold greater potency at the FP receptor than latanoprost (Trial ID: NCT00523250). Other examples are Travoprost APS (Alcon), a solution of travoprost (PG $F_{2\alpha}$ analogue) containing an alternative preservative (Trial ID: NCT00523250 and NCT00892762) or latanoprost without benzalkonium chloride preservative from Sun Pharma Advanced Research Co (Trial ID: NCT00947661) [2].

PGE2, can induce diverse cell signaling responses by its binding to EP1-4 receptors. Vasorelaxation effect is mediated by EP2 and EP4 receptors. Thus, topical application of EP2 and EP4 agonists can be used as hypotensive agents. This is the case of compound **12** (PF-04217329, a pro-drug of CP-544326) and **13** Fig. (**3b**) which are EP2 agonists and showed a potent reduction of IOP in normotensive dogs and hypertensive monkey [51-53]. CP-544326 is a potent and selective EP2 agonist (IC_{50}=10 nM; EC_{50}=2.8 nM) whose corneal permeability and ocular bioavailability were considerably augmented when the compound was dosed as the isopropyl ester pro-drug PF-04217329. In addition, topical administration of PF-04217329 evokes an increase of AMPc in aqueous humor /iris-ciliary body, suggesting an *in vivo* activation of EP2 receptor. Pfizer has completed a Phase II clinical trial of the compound **12** (Trial ID: NCT00572455). The results showed a comparable hypotensive effect to latanoprost but with elevated adverse side effects. This is probably a sign that the results are not such promising, unless they are able to lower the side effects [50].

PF-04475270 molecule Fig. (**3b. 14**) is a pro-drug of CP-734432, a potent EP4 agonist (IC_{50}=2 nM, at the human receptor), which produces a significant IOP reduction in normotensive dogs (IC_{50} = 8 nM, at EP4 canine receptor) [54]. PF-04475270 was quickly hydrolyzed to the active acid, CP-734432 in the cornea and subsequently distributed to iris/ciliary body and aqueous humor [54]. As we comment above, pro-drug presents a higher permeability and an improved drug exposure in the anterior chamber of the eye compared to the pharmacologically active moiety. Because trabecular EP4 receptor expression is greater than its ciliary expression, the hypotensive effect of PF-04475270 seems to be owed to an increase in conventional aqueous outflow pathway [54]. To evaluate the preclinical efficacy of this compound, [55] it has been developed a population-based pharmacokinetic-pharmacodynamic model to characterize its IOP lowering profile. This model seems to be useful when a sensitization of IOP reduction occurs with multiple dose treatment and is not a pharmacokinetic time-dependent result. This phenomenon occurs after topical administration of PF-04475270 in dogs. In addition, the authors are suggesting its use in the area of ophthalmology drug development, whereas pharmacokinetic data are limited by the difficulty to obtain ocular tissue samples.

Structural modifications of the $PGF_{2\alpha}$-ethanolamide, bimatoprost, have also been performed and the derivatives obtained i.e. Fig. (**3b. 15**) have been claimed for glaucoma treatment [56]. Hypotensive effects of these derivatives have been tested in normotensive dogs, but there is a lack of data concerning human studies.

Improvement of PG receptor agonist's delivery systems have been performed as well and a punctual plug delivery system with latanoprost (L-PPDS, QLT) and bimatoprost (Vistakon Pharmaceuticals) is clinically evaluated. Four Phase II trials of L-PPDS have been completed but the results have not published yet (Trial ID: NCT00650702, NCT00967811, NCT01037036 and NCT01229982) [2, 50]. The efficacy and safety of the bimatoprost punctual plug has been studied in two completed Phase II trials (Trial ID: NCT00824720 and NCT01016691) and partial results have been published.

NEW TARGETS FOR GLAUCOMA TREATMENT: EMERGING ROLE OF MELATONIN ANALOGUES

The development of new drugs with novel actions can minimize adverse effects and provide a greater IOP reduction compared to current medications or an additive effect in combination to existing treatments. Consequently, many potential new anti-glaucoma targets have been discovered in the last years, but for the majority of them the physiological data collected are quite scarce and drugs against these targets have not been yet clinically validated. The molecules that act on these targets are quite heterogeneous, including agonists or antagonists of various receptors (such as the angiotensin II receptors [57], bradykinin receptors [58, 59], cannabinoid receptors [60, 61], corticosteroid receptors [62, 63], endothelin receptors [64-66], nucleoside/nucleotide receptors [67-70], serotonin $5HT_2$ receptors [71-76] and vanilloid receptors [77, 78]), silencer RNAs (targeting for example nucleotides receptors [79, 80], tumor necrosis factor α [81], serum amyloid A [82, 83] and the bone morphogenesis protein or its receptor or its binding proteins [84]), inhibitors of some enzymes (such as kinases [74, 85-98], caspases [99-101], superoxide dismutase [102], histone deacetylases [103-105], prenyltransferases [106], angiotensin-converting enzyme [107]), aquaporin water channels [108-114] and inhibitors of actin polymerization [115]). Several of these compounds have been clinically evaluated and for trial details see review of Chen and colleagues [50].

Perhaps some of the most promising candidates for future glaucoma treatment are the melatonin agonists. Melatonin is an indole-derived neurohormone see Fig. (**4a. 1**) secreted primarily by the pineal gland. In addition, local synthesis of melatonin occurs in several tissues including ocular structures such as retina and ciliary body [116]. Melatonin production from pineal gland follows a circadian rhythm and it is controlled by the suprachiasmatic nucleus which is regulated by light stimulation of intrinsically photosensitive retinal ganglion cells (ipRGC) [117]. This endogenous substance is the most important modulator of sleep and circadian rhythms [117] and likewise, is a potent antioxidant agent as a direct and indirect free radical scavenger [19, 20]. Melatonin actions are mainly mediated through specific melatonin receptors termed MT_1, MT_2 and the putative MT_3 [116, 118-120].

In the eye, local melatonin has a neuroprotective effect but also, is affecting directly the IOP see (Table **2**)[121-128]. The neuroprotective action is due to its antioxidant and antinitridergic properties as well as its capacity to modulate extracellular glutamate levels in the retina [19, 20, 121, 124, 129]. Melatonin inhibits retinal NO synthesis (inhibits nitric oxide synthase, NOS) and L-arginine uptake (NOS substrate) as well as the accumulation of cGMP induced by both NO donor and L-arginine. In addition, melatonin directly scavenges NO radical which in concert with oxygen free radicals is transformed in potent cytotoxic species. Therefore melatonin protect ocular tissues from oxidative and nitrosative damage [19, 20, 124, 129]. Glutamate is the main neurotransmitter in the retina. The excessive release of glutamate follows pathological situations like ocular hypertension [20], leading to an excess of free radicals (oxygen- and NO-derived) and retinal ganglion cell death [20, 130]. An increasing amount of evidence indicates that factors like elevation of extracellular glutamate levels, alteration of nitridergic pathway (increase of NO levels) and oxidative damage contribute to the glaucomatous neurodegeneration [19, 20, 130-133]. Consequently, melatonin has emerged as a promising agent for the management of glaucoma [19, 20, 117, 121]. In fact, there are experimental data demonstrating the beneficial effect of melatonin treatment in animal models and to patients suffering other neurodegenerative diseases [134-137].

Circadian fluctuation of IOP has been reported [128] and a potent reduction of IOP has been observed with exogenous melatonin [122, 123, 125-127](Table **2**). Among the melatonin analogues, 5-methoxycarbonylamino-N-acetyltryptamine (5-MCA-NAT, also known as GR 135531; Fig. **4a. 2**) is the most potent at reducing IOP when compared with other melatonin receptor agonists and also compared to other therapeutic drugs [138]. In this sense, a ciliary body MT_3 melatonin receptor and a MT_2 receptor-evoked hypotensive effect has been suggested by Alarma-Estrany and coworkers [118, 123].

Taken together, these data suggest a narrow connection between glaucoma treatment and melatonin. As we commented above, glaucoma is an optic neuropathy in which a progressive death of the retinal ganglion cells is a consequence mainly of a persistent high IOP. Because of the melatonin neuroprotective and hypotensive actions, melatoninergic drugs seem to be promising agents to counteract glaucoma damage [19, 20, 117, 121]. In addition, the loss of ipRGC characteristic of glaucoma [117, 139-141] affects pineal melatonin production [142, 143], which in turn, can affect circadian rhythm-dependent functions producing sleep disorders [144-147] or depression typical of glaucomatous patients [117, 148, 149]. Furthermore unlike other hypotensive agents, melatonin can regulate the expression of genes codifying for glaucoma-target proteins such as CAs [150] or aquaporins [151]. Moreover, the astrocytes and Müller cells swelling of hypoxic retinas mediated by aquaporins are noticeably attenuated after melatonin administration [152]. Hence, this might be another mechanism by which melatonin exerts its beneficial effect in glaucoma.

CURRENT STATUS OF MELATONIN AGONISTS AND THEIR ANTI-GLAUCOMA POTENTIAL

Although melatonin is involved in several pathophysiology processes including glaucoma, its use in clinical applications is quite limited. This compound, is characterized by a poor pharmacokinetic profile with a short half-life (15-30 min) and great interindividual variation [153]. Great effort has been paid in the design and synthesis of novel melatonin agonists/antagonists, more metabolically stable but also more potent and receptor subtype-selective [154-156].

Two melatonin receptor agonists have reached the market, ramelteon Fig. (**4a. 3**), also known as TAK-375 and agomelatine Fig. (**4a. 4**). Ramelteon is a non-selective MT_1 and MT_2 receptor agonist (*Ki* MT_1 = 0.014 nM and *Ki* MT_2 = 0.045 nM). It was developed by Takeda and approved in 2005 from FDA for the insomnia treatment. Agomelatine is a melatonin agonist (*Ki* MT_1 = 0.062 nM and *Ki* MT_2 = 0.268 nM) and selective serotonin antagonist (IC_{50} 5-HT_{2C} = 270 nM). This compound was developed by Servier and approved by the European Medicines Agency in 2009 for depression. In addition, it improves sleep quality in depressed patients and exerts neuroprotective actions [157, 158]. Recently, it has been claimed the use of agomelatine for glaucoma treatment and its hypotensive potential have been reported [159]. This compound reduces IOP similarly to melatonin and presents an EC_{50} in the picomolar range (Table **2**). The use of agomelatine to reduce IOP has the advantage of being already a medicine. Most of the preclinical features such as tolerance, lethality, toxicity etc., have already been tested when investigating this compound as an anti-depressive medicine. However, and due to the different way of administration of this compound for IOP reduction in comparison to its use as antidepressant drug additional studies such as corneal toxicity must be performed. In addition, it has been demonstrated

Table 2. ***pD₂*** **Values and Maximum Reduction in Intraocular Pressure for Melatonin and Melatonin Analogues**

Compound (Fig.)	pD_2	Max.% IOP	Reference
Melatonin (4a.1)	9.3±0.24	22.0±1.6	[126]
5-MCA-NAT (4a.2)	8.9±0.07	42.5±1.6	[126]
Agomelatine (4a.4)	9.7±0.28	20.8±1.4	[159]
IIK7 (4a.7)	7.1±0.31	38.5±3.2	[123]
INS48848 (4a.10)	5.5±0.21	36.0±2.0	[154]
INS48862 (4a.11)	5.7±0.33	26.0±1.3	[154]
INS48852 (4a.12)	5.5±0.20	33.1±1.4	[154]

The pD_2 Value was taken as the $-\log(EC_{50})$. Reduction in Intraocular Pressure was Expressed Relative to the Resting Level. Data Corresponds to the Experiments Performed in Normotensive Rabbits. Hypotensive Effect of 5-MCA-NAT Compound has also been shown in Glaucomatous Monkey (See Ref. [125]).

the abundance of serotonin receptors in human ocular tissues [160] thus caution should be taken with topical instillation of agomelatine. Nevertheless, agomelatine might be in the market faster than other melatoninergic compounds needing full pre-clinical tests.

Other two compounds being tested in clinical trials are, tasimelteon Fig. (**4a. 5**) developed by Vanda Pharmaceuticals and TIK-301 Fig. (**4a. 6**) actually developed by Tikvah Therapeutics. Tasimelteon, formerly known as VEC-162 and BMS-214778, is a MT_1/MT_2 agonist (*Ki* MT_1 = 0.35 nM and *Ki* MT_2 = 0.17 nM). Its efficacy and safety in subjects with non-24-hour sleep-wake disorder is under evaluation (Trial ID: NCT01163032). TIK-301, also known as LY-156735 and PD-6735, is also a MT_1/MT_2 agonist (*Ki* MT_1 = 0.081 nM and *Ki* MT_2 = 0.042 nM). Furthermore, is acting as an antagonist to serotonin receptors 5-HT_{2B} and 5-HT_{2C} more potent than agomelatine [155]. This ability is important for its use as antidepressant. Furthermore, presents a greater oral bioavailability than melatonin and has lower hypotensive effects [161]. This compound has been in Phase II clinical trial in the United States since 2002.

Recently a significant number of melatonin receptor ligands have been patented and published [154, 156, 162]. The ligands fulfill the structural requirements described by previous structure-activity relationship studies [161]. In this review some of the most novel compounds are analyzed and grouped in four classes of ligands by a structure-based criterion.

Indole Derivatives and their Bioisosteres

In this class, we can found compounds conserving the indole core of melatonin or its naphthalene bioisostere.

The methoxy group in position 5 is together acylaminoethyl side-chain in position 3, important position for binding to and activating melatonin receptors. Substitution of 5-methoxy group lowers binding affinity. When shifting to position 4, 6 or 7 leads to a more potent reduction of binding affinity [161]. Melatonin derivatives modified at position 5 have also been patented by Servier [163]. An example is the compound **7** Fig. (**4a**), with a piperidine linked to position 5 of the indole ring through etoxy chain. The compound presents *Ki* MT_1 = 11 nM and a *Ki* MT_2 = 19 nM [163]. Data about their *in vivo* effect are not reported in the patent.

Derivative **8** Fig. (**4a**) is a rigid melatonin analogue in which the β carbon of the 3-acylaminoethyl melatonin substitute is enclosed into a 6-membered ring, forming a 1,3,4,5-tetrahydrobenzeno[*cd*] indole-based compound. Its (S)-enantiomer is equipotent to melatonin (*Ki* MT_1 = 0.182 nM and a *Ki* MT_2 = 0.288 nM) and almost three orders of magnitude greater than (R)-enantiomer (*Ki* MT_1 = 106 nM and a *Ki* MT_2 = 126 nM) [164].

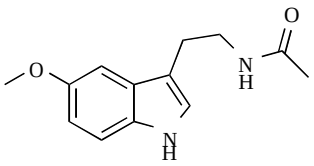
Recently, Alarma-Estrany and colleagues [154] have described new melatonin analogues able to activate MT_3 and MT_2 melatonin receptors which mediate reduction of IOP. The selective MT_3 receptor ligand 5-MCA-NAT Fig. (**4a. 2**) has been proved to be a potent ocular hypotensive agent [118, 125, 126] and the selective MT_2 receptor agonist N-butanoyl-2-(2-methoxy-6H-isoindolo[2,1-a]indol-11-yl) ethanamine (IIK7; Fig. **4a. 9**) also markedly decrease the IOP and was inhibited by selective MT_2 receptor antagonists [123](Table **2**). Furthermore, 5-MCA-NAT analogue exerts a long-term hypotensive effect by regulating the adrenoceptors and CA genes expression which are classical anti-glaucoma targets [150, 165]. Thus, both 5-MCA-NAT and IIK7 are presented as starting points for new classes of drugs to lower IOP, and for treating glaucoma. In this sense, INSPIRE Pharmaceuticals (recently acquired by Merck), synthesized compounds, starting from the indole core, that reduced IOP [154]. Among all the synthesized compounds the greatest reduction was achieved with the analogues termed INS48848 Fig. (**4a. 10**), INS48862 Fig. (**4a. 11**) and INS48852 Fig. (**4a. 12**). Common features are structural rigidity, frequent in the design of melatonin agonists and increased hydrophobicity on the eastern side of the molecule. These derivatives produce a dose-dependent decrease in IOP (pD2 INS48848 = 5.5 (8.19 ng), pD2 INS48862 = 5.7 (7.04 ng) and pD2 INS48852 = 5.5 (10.12 ng))(Table **2**). In addition and using melatonin receptor antagonists, it was demonstrated that INS48862 and INS48852 act preferentially via MT_2 melatonin receptors suggesting that INS48848 can activate mainly MT_3 melatonin receptors [154]. For these reasons, the compounds **10-12** have been claimed as potential compounds for glaucoma treatment. Having in mind that they activate different melatonin receptors they may be used in combination to render better hypotensive effects [154].

In other cases, changes in the indole moiety have been performed. Therefore, Elsner and coworkers [166] have synthesized a new class of melatonin receptor agonists with a pyrazolo[1,5-*a*]pyridine scaffold as indole bioisostere. These derivatives have the advantage of being more metabolically stable than melatonin. In these compounds, a 2-phenyl substitution produces higher binding affinity similarly occurring with the equal phenyl melatonin substitution i.e. Fig. (**4a. 13** *Ki* MT_1 = 0.58 nM and *Ki* MT_2 = 0.40 nM versus Fig. **4a. 14** *Ki* MT_1 = 12.3 nM and *Ki* MT_2 = 4.01 nM). In addition, the authors showed that shifting the 5-methoxy group into position 2 of the pyrazolo[1,5-*a*]pyridine and at the same time, keeping a N-butanoyl side chain, a MT_2 selective agonist (MT_1/MT_2 selectivity ratio of 76) with high MT_2 binding affinity (*Ki* MT_1 = 118 nM and a *Ki* MT_2 = 1.55 nM) was obtained see Fig. (**4a. 15**) [166]. Benzofuran and indane derivatives have been also proposed by Ferrer group [167, 168]. Binding affinity or functional data of these compounds is not reported. However, the molecules have better pharmacokinetic features than structurally similar ones. A benzofuran example is presented in Fig. (**4a. 16**). This compound has high metabolic stability (between 71 and 100%) tested on human microsomes and rat plasma levels of 98 ng/ml, whereas the reference analogue displays lower metabolic stability (<30%), plasma levels of 10.1 ng/ml and a brain/plasma ratio close to zero [167]. Compound **17** Fig. (**4a**), an indane derivative, shows a brain:plasma ratio of 1.5, nonetheless the ratio of reference analogue was close to zero [168]. Furthermore, indoline compounds have been also developed by Ferrer Company [169]. For example, compound **18** Fig. (**4a**) comprises the indoline nuclei with the acylaminoethyl chain on the nitrogen 1, instead of on carbon 3 as in melatonin. This derivative (at 100 nM) shows a greater MT_1 agonist activity (92%) than melatonin which was used as control (76.6%). It is also claimed as MT2 agonist but no data reported yet [169].

As we commented before, new naphthalene derivatives have been also synthesized. An example is the deuterated compound present in Fig. (**4b. 19**). Derivative **19** joins deuterium atoms in methoxy group and acetamide side-chain. Data about the binding affinity or function of these ligand classes have not been reported [170].

Recently, it has been tested the neuroprotective and hypotensive effect of the antidepressant agomelatine agent Fig. (**4a. 4**) see (Table **2**) [157-159]. Hence, and considering that many glaucoma patients also suffer from depression [148, 149] agomelatine-derivatives might be of interest for future treatment of glaucoma due to their possible triple ability: to help in depression, to reduce IOP and to neuroprotect.

Servier group has also developed agomelatine derivatives with a high affinity for MT1 and MT2 receptors and moderate 5-HT_{2C} affinity [156]. For example the compound **20** Fig. (**4b**) with a fluorine atom instead methoxy group of agomelatine position 7 (*Ki* MT_1 = 7 nM, *Ki* MT_2 = 0.6 nM and *Ki* 5-HT_{2C} = 6 μM) or the compound **21** Fig. (**4b**) with a fluorine atom connected to β-carbon though a methyl linker (*Ki* MT_1 = 0.1 nM, *Ki* MT_2 = 0.2 nM and



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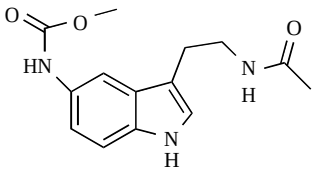

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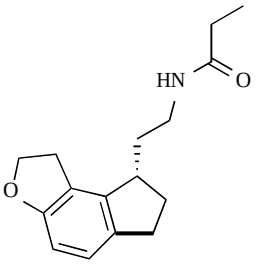

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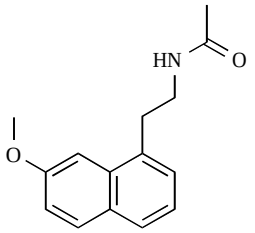

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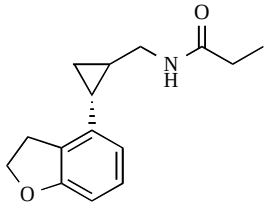

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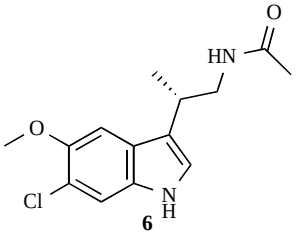

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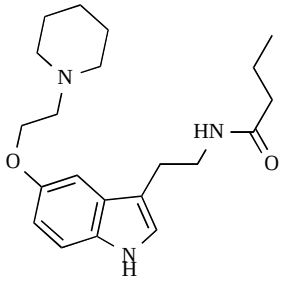

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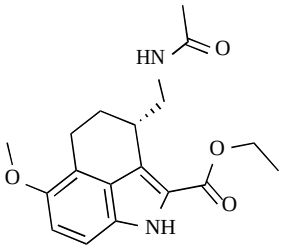

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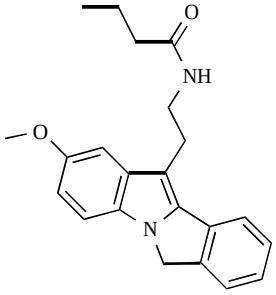

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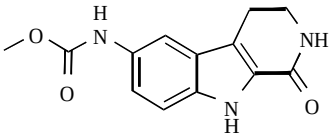

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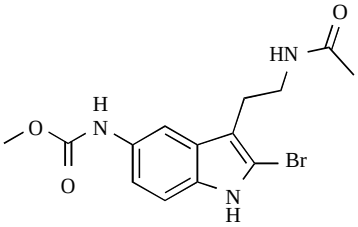

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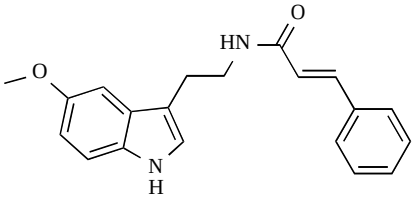

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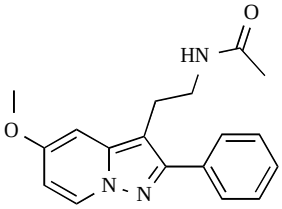

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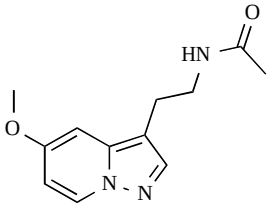

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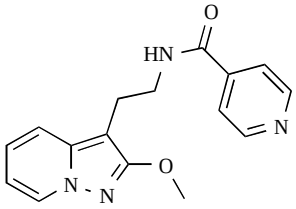

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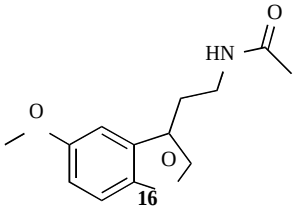

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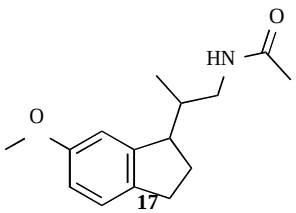

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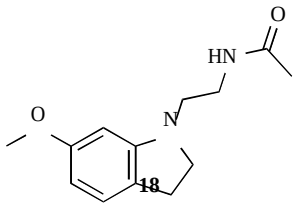

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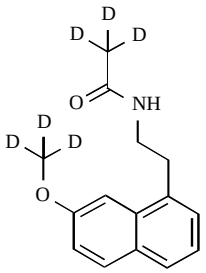

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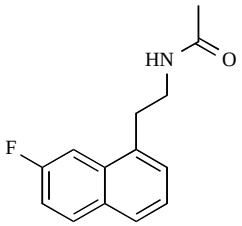

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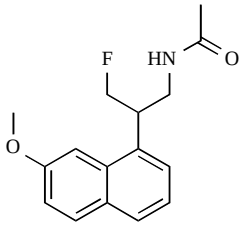

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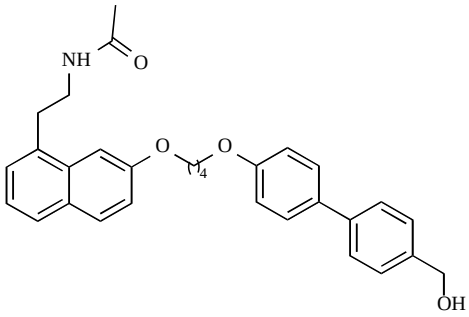

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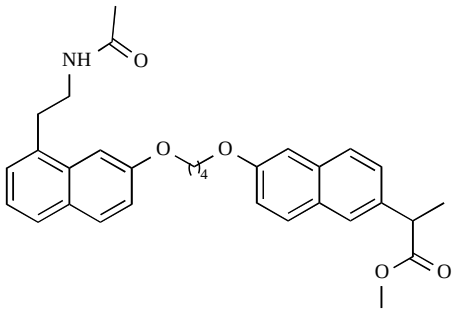

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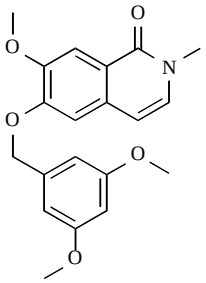

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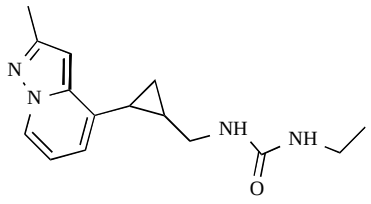

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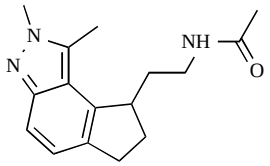

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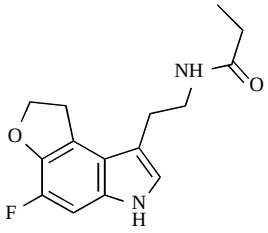

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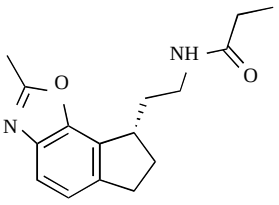

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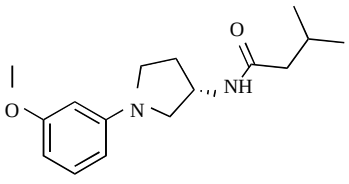

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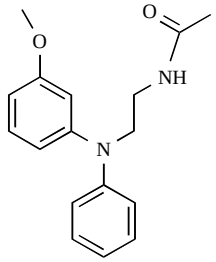

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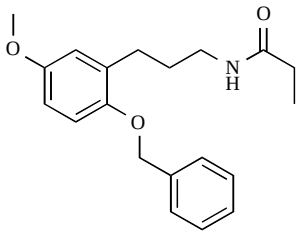

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Fig. (4). Melatonin, ligands approved or in clinical trials and new compounds.

Ki 5-HT_{2C} = 6 μM). Data about their effect *in vivo* are not included in the patents.

Méssangeau and colleagues [171], have recently developed a new class of melatonin receptor ligands formed by an agomelatine dimer or agomelatine-biphenyl dimer joined through an alkyl linker. These compounds present high MT_1-selectivity, with nanomolar or subnanomolar affinity. An example is the compound **22** Fig. (**4b**) with a *Ki* MT_1 = 0.09 nM and *Ki* MT_2 = 6.53 nM. In spite of its high affinity, compound **22** behave as partial agonist on MT_1 and MT_2 receptor subtype (EC_{50} MT_1= 2.37 nM and E_{max} MT_1 = 79%; EC_{50} MT_2 = 26 nM and E_{max} MT_2 = 22%; melatonin EC_{50} MT_1 = 2.2 nM and E_{max} MT_1 = 110 %; melatonin EC_{50} MT_2 = 0.49 nM and E_{max} MT_2 = 104 %). Another interesting compound of this new series is the **23** Fig. (**4b**), a full antagonist on both receptor subtypes with high MT_1 and MT_2 affinities (0.37 and 4.22 nM, respectively) but shows low selectivity (MT_2/MT_1 ratio 11).

Bicyclic Derivatives

In this class are grouped melatonin analogues with a bicyclic scaffold different to melatonin-like or agomelatine-like nucleus. Isoquinolone derivatives belong to this class of melatonin ligands. Wong and coworkers [172], have described the pharmacological profile of 2, 5, 6 and 2, 6, 7 substituted isoquinolones. In general, mono- and di-meta-methoxybenzyloxyl substituted isoquinolones resulted in very potent full agonists with high MT_2 selectivity. Because the activation of this melatonin subtype receptor mediate hypotensive actions [123], these derivatives seem to be interesting. An example is the compound **24** Fig. (**4b**) with a 3,5-dimethoxybenzyloxyl group at position 6 of isoquinolone ring. This derivative displays a potent melatonin agonist activity (pEC_{50} MT_1= 7.92 and pEC_{50} MT_2= 9.42) with high MT_2 selectivity (MT_2/MT_1 EC_{50} ratio 31.7) and similar MT2 response to that elicited by reference analogue.

Takeda Company has recently developed one or more types of bicyclic derivatives connected through cycloalkyl linker to an amide or urea group [156]. The bicyclic scaffold is formed by a nitrogen-containing 5-membered ring condensed to a 6-membered one. The cycloalkyl linker can be formed by 3, 4 or 5 carbon atoms and is joined to the 6-membered ring, in position ortho to the 5-membered one. Compound **25** Fig. (**4b**) is an example and it displays IC_{50} = 100 nM for MT_1. In this case, the amide side chain is connected to bicyclic nucleus by a trans-disubstituted cyclopropyl ring which is frequent in bicyclic melatonin analogues. Data about its agonist activity has not been reported [156].

Tricyclic Derivatives

These compounds are characterized by a central 6-membered ring fused with two additional rings formed by 5 atoms. One of the 5-membered rings mimicked the melatonin 5-methoxy group. For example, there have been claimed derivatives which tricyclic nucleus is joined to amide or urea group through an alkyl linker i.e. Fig. (**4b. 26**). This class of compounds present values of IC_{50} =100 nM for MT_1 and MT_2 receptors and no functional data are reported in the patents [156].

More recent are the 1,6-dihydro-2H-3-oxa-6-aza-as-indacene compounds described by Ferrer group [173]. These derivatives appear to be potent MT_1-agonists. Compound **27** Fig. (**4b**) is an example and shows an agonism percentage of 55.1% at 1 nM whereas melatonin and ramelteon show values of 48% and 47.4%, respectively. In addition, at 100 nM compound 14 presents an agonism percentage of 101.7% while melatonin and ramelteon display values of 102.6% and 117.5 %, respectively.

Another class of melatonin analogues with a tricyclic nucleus has been proposed by Takeda Company [156]. In these compounds, tricyclic scaffold is connected to an amide or urea group and present different substitutions at position 2. In general, all these derivatives appear to be agonists for MT_1/MT_2 melatonin receptors although functional data are not provided. An example is present in Fig. (**4b. 28**). Compound **28** shows IC_{50} MT_1 = 0.03 nM and IC_{50} MT_2 = 0.049 nM and *in vitro* metabolic stability test shows an elimination rate of 2.6 %/min/mg.

Monocyclic Derivatives

This group presents structures having a monocyclic core, which replace the indole of melatonin, with other usual pharmacophore elements connected to this ring. Phenylpyrrolidine compounds described by Ferrer Company [156] belong to this class. A characteristic of these derivatives is their greater metabolic stability (comprised between 71-100%). Compound **29** present in Fig. (**4b**) is an example. Its MT_1 agonism at 1 nM is 50.1% versus melatonin MT_1 agonism of 51.8 %. In addition, its MT_1 agonism at 100 nM is 110.3 % compared to melatonin MT1 agonism of 100.5%. Regarding its metabolic stability, compound **29** shows plasma levels of 15.1 ng/ml 15 min after administration (versus 10.1 ng/ml of reference analogue), and a brain:plasma ratio of 1 (compared to ratio 0 showed by reference analogue).

Other types of monocyclic derivatives are the N-(substituted-anilinoethyl) amides [156, 174]. Data about binding affinity and intrinsic activity are provided. For example, compound **30** Fig. (**4b**) is a MT_1/MT_2-partial agonist (p*Ki* MT_1= 8.30, p*Ki*MT_2= 10.18, IA MT_1= 0.79 and IA MT_2= 0.61. In addition, its antidepressant and anxiolytic actions have been demonstrated.

Recently, Hu and colleagues [162], have described a novel class of melatonin ligands with high binding affinities and selectivity toward MT_2 receptors. These compounds are phenylpropyl amides with benzyloxyl substitutes on carbon 6 i.e. Fig. (**4b. 31**). The presence of benzyloxyl group at position 6 of 3-methoxyphenyl ring produces a considerable increase of MT_2-binding affinity. Compound **31** shows *Ki* MT_2= 0.00055 versus propionamide analogue *Ki* MT_2= 7.07. Furthermore, **31** present high selectivity for MT_2 (MT_2/MT_1 ratio 4.73x 10^5).

CONCLUDING REMARKS

As described above, agents commonly used to treat glaucoma may cause adverse side effects due to the high doses needed and their systemic absorption. Another existing disadvantage is the frequent dosage, because pharmacology treatment of glaucoma requires the application of drops at least once a day see (Table **1**). As a consequence, there is a continuous effort to improve the existing and to discover safer and more effective drugs.

In the last years, several potential new anti-glaucoma targets have been discovered even though for most of them there is lack of comprehensive physiological information and some drugs have not been clinically validated yet.

Topical application of melatonin and its analogues produce a potent reduction of IOP suggesting that they may be strong candidates for the development of a new family of drugs see (Table **2**). In this sense, the melatonin analogue 5-MCA-NAT is the most potent in reducing IOP when compared with other melatonin receptor agonists but also compared with other therapeutic drugs [126, 138]. This compound apart of reducing IOP provokes a long-term hypotensive effect through the regulation of the adrenoceptors [165] and CA genes [150] which codify for classic glaucoma-target proteins. This additional ability of 5-MCA-NAT, may indicate that its dosage and administration will follow a different pattern to the one indicated for the current glaucoma treatment eye drops. If the number of instillations is lesser or the dose is reduced, a reduction in side effects will be also possible.

Other melatonin ability straightly connected to glaucoma is its neuroprotective role [124, 136, 137]. This substance acts as direct and indirect free radical scavenger. Nevertheless, there is a lack of information in the literature concerning these benefits. From our point of view, this is a remarkable issue. Although the current management of glaucoma is mainly directed towards controlling the IOP [2, 5, 175, 176], a therapy preventing the death of retinal ganglion cells and optic nerve axon loss should be the main goal of the treatment. In fact, there are studies showing that in a considerable number of patients in which ocular hypertension is pharmacologically decreased, the glaucomatous damage was further progressing [176-178]. Therefore, if melatonin or its analogues can protect retina from glaucoma injury, the effect of this new pharmaceutical product could be doubled. On the one hand, it reduces IOP (both short and long term) with the corresponding benefits and on the other hand, it keeps the retina fully functioning.

Because there is a high prevalence of sleep disorders and depression between glaucomatous patients, melatonin and its analogues present an additional advantage in comparison to other anti-glaucoma drugs [144-149].

However, clinical use of melatonin is limited because of its short half-life (15-30 min) and lack of subtype receptor selectivity [153]. As a consequence, there is a considerable interest in the design and synthesis of novel melatonin analogues not only more metabolically stable, but also more subtype receptor selective.

A great number of new melatonin analogues structures have been recently patented [156]. Despite the emerging role of melatonin in glaucoma pathology, these patents are generally directed towards sleep disorders and/or circadian rhythm-related disorders but not to glaucoma treatment [156]. In this sense only 5-MCA-NAT, IIK7, agomelatine, INS48848, INS48862 and INS48852 derivatives have been tested for their *in vivo* hypotensive effect see (Table **2**) [123, 126, 154, 159]. In the case of agomelatine a neuroprotective action has also been demonstrated [157, 158]. Furthermore, this compound has been recently proved to treat depression as previously indicated.

In addition and from a clinical point of view, some advances have been carried out to achieve efficient ocular formulations of 5-MCA-NAT analogue. Glaucoma medicines must be well tolerated by the ocular surface and reduce eye drop-induced toxicity. Regarding to these facts, cellulose polymers are commonly used for artificial tear solutions because they reduce the drainage rate of the drug and improve its therapeutic efficacy [179]. In this sense, a combination of 5-MCA-NAT analogue with bioadhesive polymers has been performed to achieve potent hypotensive agents with ocular safety [179]. In addition, new and efficient formulations of 5-MCA-NAT analogue and polyethylene glycol (accepted for ocular topical treatments) have been performed [180].

In conclusion, a remarkable progress in the development of new melatonin analogues has been achieved with a potential use for the treatment of ocular hypertension and glaucoma. A number of issues need to be solved before we see a melatoninergic compound joining the currently available group of commercial compounds. In this sense, a lot of effort is needed to develop compounds with improved solubility, oral bioavailability and ligand protection against metabolic degradation.

Finally, to achieve the most effective glaucoma treatment, is necessary to perform not only intraocular pressure studies but also neuroprotective assays with existing and novel melatonin analogues.

CONFLICT OF INTEREST

It is certified that there is no actual or potential conflict of interest in relation to this article.

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Artículo sobre Rho Kinasas

Potential Role of Rho-Associated Protein Kinase Inhibitors for Glaucoma Treatment

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Abstract: Rho kinase inhibitors are widely considered as a new treatment for glaucoma. Rho kinase inhibition has been shown *in vitro* and *in vivo* to lower intraocular pressure. Furthermore in the first clinical reports involving healthy human subjects, the results were quite promising. The potential of this new class of medicines is enormous in a field where there were not many developments lately. The inhibition of Rho kinase lowers the intraocular pressure by increasing the outflow through the trabecular meshwork. Increased blood flow to the optic nerve and a possible delay of optic nerve cell death has also been reported. As a consequence, the exploration of pharmacological inhibitors of Rho kinase signaling is actively being pursued by a number of pharmaceutical companies such as Senju Pharmaceuticals, Novartis, Kowa, Santen, Aerie, Inspire and others. In this article, we review the latest patents in this field, with their corresponding literature, regarding Rho kinase inhibitors for the treatment of intraocular pressure and summarize the many roles of Rho kinase signaling in the eye.

Keywords: Glaucoma, heteroaromatic compounds, inhibitors, intraocular pressure, Rho-kinase, trabecular meshwork.

INTRODUCTION

Glaucoma is a group of diseases characterized by retinal and optic neuropathy, and progressive visual field loss associated with chronic elevation of intraocular pressure (IOP). Sufficiently high and persistent IOP is believed to result in damage to the optic disc at the juncture of the optic nerve and retina, resulting in degeneration of retinal ganglion cells and blindness. According to estimations, there are about 61 million people with glaucoma, which is expected to rise to around 80 million by 2020. Glaucoma is the second leading cause of blindness in the world. There are many risk factors for but the most important factor is the high IOP (> 21 mmHg). The disease classification is based on the mechanism leading to the increase in IOP. In the normal eye, there is a balance between aqueous humor formation and its drainage through the major outflow pathway (trabecular meshwork and Schlemm´s canal) [1].

There are several types of glaucoma. The two main types are open-angle and angle-closure. In angle-closure glaucoma, the peripheral iris blocks the anterior chamber angle by apposition or synechiae, preventing the drainage of the aqueous humor. In the case of open-angle glaucoma (POAG), the anterior chamber angle is open but the trabecular meshwork is almost shut (especially in the juxtacanalicular tissue regions). Finally primary glaucoma is not associated with pre-existing disease whereas secondary glaucoma is a consequence of another ocular or systemic disease, trauma, or from drug effects [2,3].

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The efforts towards preparing a drug to stop glaucoma progression are focused in lowering IOP. In such a case, prostaglandin analogues and adrenergic α1 receptor agonists are lowering IOP by increasing the outflow of aqueous humor. In the other hand, the β receptor blockers and carbonic anhydrase inhibitors are inhibiting aqueous humor production. The last group of existing drugs, pilocarpine and other miotic agents are increasing the aqueous outflow, contracting the ciliary muscle (CM). Ultimately, a new group of compounds is proved to lower IOP, the Rho kinase inhibitors. Rho-associated kinase (ROCK) belongs to the AGC family of serine / threonine kinases and is the first downstream effector of small GTPase.

Up to now, two isoforms have been identified: ROCK-I (also called ROKβ and p160ROCK) and ROCK-II (also known ROKα). Both ROCK-I and ROCK-II present a high sequence homology including ATP binding site [4]. Rho A and its downstream kinase (ROCK) have been implicated in a number of important roles such as regulation of smooth muscle contraction, cytoskeleton rearrangement, cell migration and proliferation [5].

Rho kinases have a part in the signaling pathways leading to formation of actin stress fibers and focal adhesions and it has been shown to be expressed in ocular tissues, including the trabecular meshwork (TM) and CM. Inhibition of ROCK activity has been shown to induce alterations in TM cellular responses such as migration, adhesion, and changes in cell shape [6]. A new class of drugs that enhance aqueous drainage is emerging exploiting the characteristics of the specific ROCK inhibitors. The new class of inhibitors could modulate changes in the actin cytoskeleton and cellular motility of the trabecular meshwork, Schlemm's canal, and ciliary muscle. Probably, these inhibitors they lower the aqueous out-

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flow resistance by decreasing myosin light-chain phosphorylation, leading to cellular relaxation in human trabecular meshwork and Schlemm's canal cells [7,8].

Several target molecules have been identified as Rho kinase inhibitors. Most of them are heteroaromatic compounds mimicking guanine itself [9].

Below, we present the most recent developments of the main players in the pharmacological industry.

AERIE 6-AND 7-AMINO ISOQUINOLINE (AR-12286) INHIBITORS

An interesting Rho-kinase inhibitor is the AR-12286 of Aerie Pharmatheuticals Inc. Even though is not the most potent one, the company believes that can offer a completely different mechanism to be combined with the others already available, to increase the opportunities of lowering the IOP in patients, avoiding the severe side effects resulting by using the current drugs.

Initially the company chose as first candidate, from the amides group, the AR-11236 (100 nM in the PTM assay) but it was found it was not soluble in PBS and it was not functioning *in vivo*. SAR (Structure-Activity Relationship) analysis indicated 4 molecular regions that could be optimized. Moving or removing the adjacent aromatic ring from the amine improved water solubility, but decreased potency. Molecules in the AR-11771 series were soluble but not potent (1.7 mmHg decrease at 1%). Finally, moving the aliphatic or aromatic ring of AR-12080 to the alpha position resulted in molecules being soluble and potent. Hydrolytic stability of the initial leads was also poor. The stability of these compounds was enhanced by adding alkyl groups to the amine, as exemplified by AR-12162. Finally, the clinical candidate, AR-12286 a 6-aminoisoquinoline compound Fig. (**1-1**), was selected possessing optimized elements and a superior *in vivo* profile [10]. To protect its invention the company filled a series of patents concerning the compounds and pharmaceutically acceptable carriers. The series of consecutive patents US20070173530, US20107671205, US201001-37364, US20110319390, US20118034943, US20110183965 [11] with title "6-aminoisoquinoline compounds" and the US20100144713 [12] with title "6-and 7-amino isoquinoline Fig. (**1-2**) compounds and methods for making and using the same" where filled in that sense. AR-12286 is a heteroaromatic compound mimicking guanine itself incorporating hetero-cyclic nitrogen-containing rings that probably mimic the guanine from GTP.

According to National Institutes of Health, AR-12286 completed phase II trials in August 2011 (Trial ID: NCT01060579). It was conducted a double-masked, randomized, multi-center, active-controlled, crossover comparison of the addition of AR-12286 or timolol to latanoprost in the treatment of elevated IOP. In the US double-blind study in 88 patients, patients were given AR-12286 or eye drop once in the morning, once at night, or twice a day. Each dosing regime was administered for one week for a total of three weeks. IOP was measured for each dosing program. The maximum change in mean IOP from baseline was 28% for the 0.25% solution of AR-12286 dosed twice daily. The company has not disclosed the *p*-value, but said the mean change from baseline was statistically significant for all dose arms. During these tests AR-12286 showed a favorable effect on IOP and no serious side effects were reported. (Searle J. IOVS 2011; 52: ARVO E-Abstract 217) [13]. Currently, the company is recruiting patients for a Phase IIb trial testing AR-12286 in combination with Latanoprost. The study will include once-daily dosing of AR-12286 as a monotherapy. Aerie also is testing AR-13165 which is dual-acting compound, inhibits Rho kinase and a second undisclosed target. For this compound the company did not fill a patent yet.

INSPIRE/MERCK (INS-117548) INHIBITORS

Inspire Pharmatheuticals was asking for a Rho-kinase inhibitor in order to increase the trabecular outflow.The Rho family of GTPases is a fundamental component for the actin cytoskeletal regulation. They participate in the signal downstream pathways coordinating and directing the cellular movements. This cellular ability is regulated mainly by the actin cytoskeleton. Rho regulates the actin-myosin filament bundles to form stress fibers which provoke the cell movement. Rho kinases are the mediators of the actin rearrangements after the polymerization of the actin cytoskeleton. The actin is polymerized with the addition of actin monomers to the free barbed ends of an actin filament. In the other hand the depolymerization is possible using actin-depolymerizing agents, such as latrunculin [14]. Latrunculins are macrolides from marine sponges that inhibit actin polymerization by sequestering monomeric actin in a highly specific manner [15]. Consequently, latrunculin is provoking a loss of mechanical integrity of the trabecular meshwork and is increasing the trabecular outflow[16].

Inspire Pharmaceuticals filled a series of patents US20118071779 [17] for cytoskeletal active Rho kinase inhibitor compounds, the US20087320974, US20107666861, US20118039465 [18] for cytoskeletal active compounds and the US20107785624 [19] for pharmaceutical latrunculin formulations. The pharmaceutical compound patented is named INS-117548. The invention is directed to synthetic cytoskeletal active compounds related to natural latrunculin A Fig. (**1-3**) and B Fig. (**1-4**) that are inhibitors of Rho-associated protein kinases linked to the cytoskeletal reorganization. The cytoskeletal active compound is effective to influence the actomyosin interactions, by leading to cellular relaxation and alterations in cell-substratum adhesions. Additionally the company filled the patents US20087414137 and US20117947850 [20] concerning the high-yielding synthetic processes for preparation of 3,4-disubstituted-thiazolidin. The compounds prepared are useful in the synthesis and manufacture of latrunculins and their analogs. Furthermore, they filled the patent US20110144150 [21] for bridged bicyclic Rho kinase inhibitor compounds Fig (**1-5**). A bridge is an unbranched chain of atoms or an atom or a covalent bond connecting two bridgeheads in a polycyclic compound. A bicyclic molecule is a molecule that features two fused rings. Bicyclic molecules occur widely in organic and inorganic compounds. These compounds can inhibit the actomyosin interactions. The company doesn't provide further details on the efficacy of the compound.

Inspire Pharmaceuticals which has been acquired by Merck, put INS-117548 in clinical trials Phase I, in 2008

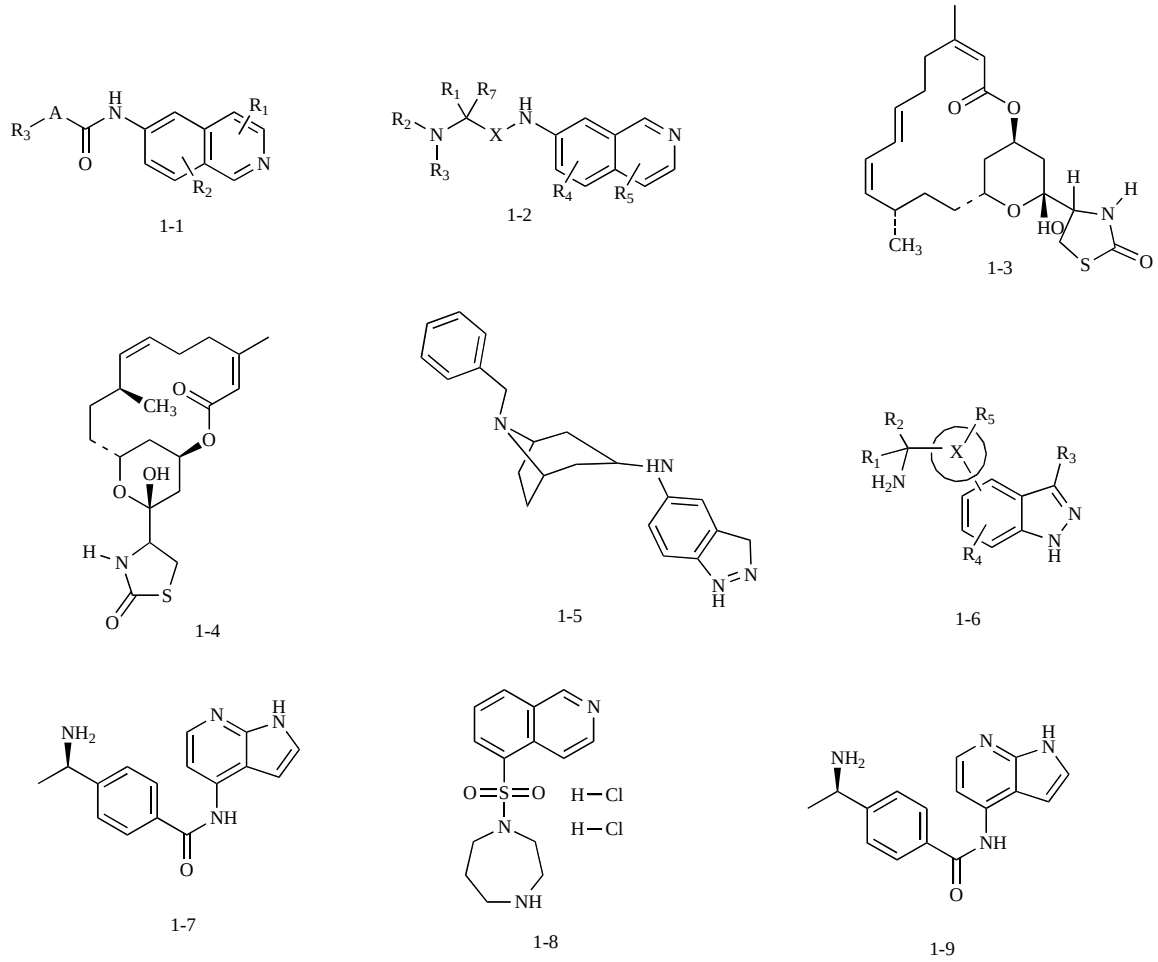

Fig. (1). Compounds used from Aerie, Inspire and Ube/Santen companies.

(Trial ID: NCT00767793). The company reported top-line results in September from the Phase I trials showing the compound demonstrated "mild" IOP-lowering effects in 84 randomized patients. Additionally were reported some dose-related adverse effects, specifically ocular burning and stinging and some safety problems concerning tolerability. After these results, the company is evaluating future directions in their glaucoma program.

The same company has another similar compound named INS-115644, a latrunculin B compound, which evolution started in 2004 and reached Phase I in clinical trials, but its results were not sufficient, reduced IOP only at the 0.02% and 0.05% doses, producing a maximal 12 hour decrease of 4 mm Hg on day 3 in patients treated with the 0.02% dose [22].

UBE INDUSTRIES AND SANTEN PHARMACEUTICAL INHIBITORS

In the year 2006, Ube Industries and Santen Pharmaceutical agreed to jointly develop DE-104, a compound that inhibits the activity of Rho-kinase. The same year started Phase I/II testing of its DE-104 safety and efficacy study (Trial ID: NCT00650338 and NCT00657579) but the results were far from promising and the companies had to abandon further tests of the compound last year.

In 2009 with the patents US20097563906 [23], US20107855222 and the US20110039891 [24] the companies presented a Rho kinase inhibitor which is an indazole derivative, an heterocyclic aromatic organic compound Fig. (**1-6**). The characteristics of the compound are the following: it has an indazole ring as a main skeleton, a ring X (benzene or pyridine ring) directly bonded to an indazole ring and an amino group located at 1-position of the alkyl group or the cycloalkyl group. The ring X has an amino-substituted alkyl group or cycloalkyl group. The carbon atom where is positioned the amino group, is not an asymmetric carbon atom. This detail is exhibiting a particularly good Rho kinase inhibiting action. According to the companies the compound demonstrated an excellent IOP-reducing action but they not give further details.

With the consequent patents US20100063060 [25], US20100041671 [26] and the US20120040994 [25], the two companies presented an agent consisting of a combination of a Rho kinase inhibitor and a prostaglandin analog. The inhibitors used were from the group consisting of (R)-(+)-N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4-(1-aminoethyl)-benzamide

dihydrochloride Fig. (**1-7**) (Compound A) and l-(5-isoquinolinesulfonyl) homopiperazine dihydrochloride Fig. (**1-8**) (Compound B). The doses of Rho kinase inhibitor were administered generally within 0.025 to 10,000 μg daily from once to several times. As prostaglandin was used isopropyl unoprostone or latanoprost. The doses of prostaglandin varied depending on the type of prostaglandin. The typical daily dose was within a range of 0.1 to 1.000 μg, which was administered from once to several times a day. The compounds were administered to Japanese white rabbits and cynomolgus monkeys. According to data provided from the company, the combination of the compounds provoke a maximum change in mean IOP from baseline far better than of prostaglandin or the inhibitor alone. There are no details for possible side-effects. In a parallel development in the patent US20110263638 [27] is described a similar combination comprising of Rho kinase inhibitor and a β-blocker. The Rho kinase inhibitor used is (R)-(+)-N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4-(1-aminoethyl)benzamide Fig. (**1-9**) and the β-blocker is befunolol, carteolol, nipradilol, betaxolol, levobunolol or metipranolol. Like in the previous combination, the company reported that the compounds provoke a maximum change in mean IOP from baseline significantly better than of timolol or the inhibitor alone. Best results are obtained 1 hour to 2 hours after administration of the drug. The IOP returns to its normal status after the 4th hour. The company mentions an improved persistence, but according to the data provided this is not obvious. Again they provide no details about possible side-effects.

RHO-ASSOCIATED PROTEIN KINASE INHIBITORS FROM KOWA PHARMACEUTICALS

The pharmaceutical company Kowa prepared a Rho kinase inhibitor called K-115 and put it in study. According to three posters presented in ARVO 2011 [28-30], the compound is now in Phase II clinical development in patients with POAG or ocular hypertension (Trial ID: JPRN-JapicCTI-090708 and JPRN-JapicCTI-101015). The purpose of the test was to investigate the efficacy and safety of the compound, over 24 hours in POAG patients and ocular hypertension patients.

The patients received each of K-115 0.2%, 0.4% or placebo twice a day at 9:00 and 21:00 in each dosing period separated by a sufficient washout period. The baseline IOP of each dosing period ranged from 20.3 $\pm$ 3.7 (mean $\pm$ SD) to 20.9 $\pm$ 3.9 mmHg. The largest mean IOP reduction from baseline was observed 2 hours after the instillation at each dose level. IOP of patients on K-115 0.4% treatment decreased by -6.4 $\pm$ 2.0 mmHg at 11:00 and -4.3 $\pm$ 2.2 mmHg at 21:00 over 12 hours in the first administration, and the analogue fashion of IOP reduction was seen in the second administration at night. Similarly, K-115 0.2% developed the IOP reduction of -5.3 $\pm$ 2.3 mmHg at 11:00 and -4.2 $\pm$ 2.4 mmHg at 21:00. The only adverse event of note was conjunctiva hyperemia seen in all patients but the degree of hyperemia was mild and transient. The reported adverse events in this study were mild to moderate, and the most frequently observed adverse event was a minor transient conjunctival hyperemia (65.3% of patients of K-115 0.4%). Only one case of mild conjunctival hemorrhage was reported at the 0.1% dose and considered drug-related.

The results suggest that the effect of K-115 on IOP is due to the increment of aqueous humor outflow via trabecular meshwork route by inhibition of ROCKs.

The company in 2009 filled two patents using their Rho kinase inhibitor in combination with carbonic anhydrase inhibitors or α1-blockers. In the patent US20090118299 [31] according to the company is provided an agent with a potent ocular hypotensive effect and prolonged duration. The agent is comprising of the Rho kinase inhibitor (S)-(-)-1-(4-fluoro-5-isoquinolinesulfonyl)-2-methyl-1,4-homopiperazine Fig. (**2-1**) and a carbonic anhydrase inhibitor. According to the data provided, the lowering of IOP is significant in comparison to the individual carbonic anhydrase inhibitor administration. The persistence of the action after the administration of the combination is considerably higher than the persistence obtained by the carbonic anhydrase inhibitor.

In the similar patent US20090082338 [32], the company is providing two different Rho kinase inhibitors. These two inhibitors were tested in combination with bunazosin. When it was used the(S)-(-)-1-(4-fluoro-5-isoquinolinesulfonyl)-2-methyl-1,4-homopiperazine inhibitor Fig. (**2-1**), the hypotensive action remained even after 3 or 4 hours of the administration. Similarly when it was tested hexahydro-1-(5-isoquinolinesulfonyl)-1H-1,4-diazepine inhibitor Fig. (**2-2**) a superior ocular hypotensive effect was observed and the action remained even after 5 hours. When the inhibitors were tested alone and not in combination with bunazosin the ocular hypotensive effect disappeared earlier.

Usually, such combinations are far from satisfactory in view of the potency of the ocular hypotensive effect and the duration of action. In particular, it is more difficult to lower the IOP in patients with normal tension glaucoma rather than lower elevated IOP. These combinations have limitations in the treatment of normal tension glaucoma, and enhancement of the ocular hypotensive action is needed in the clinical setting. In the case of the above mentioned patents, the company interesting results that are contradicting the common problems for combination drugs.

Finally, the same company filled the patent US20100-233287 [33] about a compound with a strong action of reducing IOP concerning eye drops containing their successful Rho kinase inhibitor (S)-(−)-1-(4-fluoro-5-isoquinolinesulfonyl)-2-methyl homopiperazine Fig. (**2-3**) combined with phosphoric acid. According to data provided by the company, the mixture exhibits superior action of reducing the IOP. The combination is effective even reducing normal IOP. The inhibitor alone or one preparation containing alginic acid instead of the phosphoric acid is not having the same level of results. The combination was tested in animals in comparison with the widely used commercially available timolol eye drops and it showed better ability of lowering the IOP and for longer period.

ALCON/NOVARTIS INHIBITORS

Last year, Alcon Laboratories was absorbed by Novartis Pharmatheuticals. Alcon is already producing travoprost since 2001, which is a prostaglandin analog acting as agonist to prostanoid FP receptors. In collaboration with Senju Pharmaceutical Co. Ltd., they produced a ROCK inhibitor

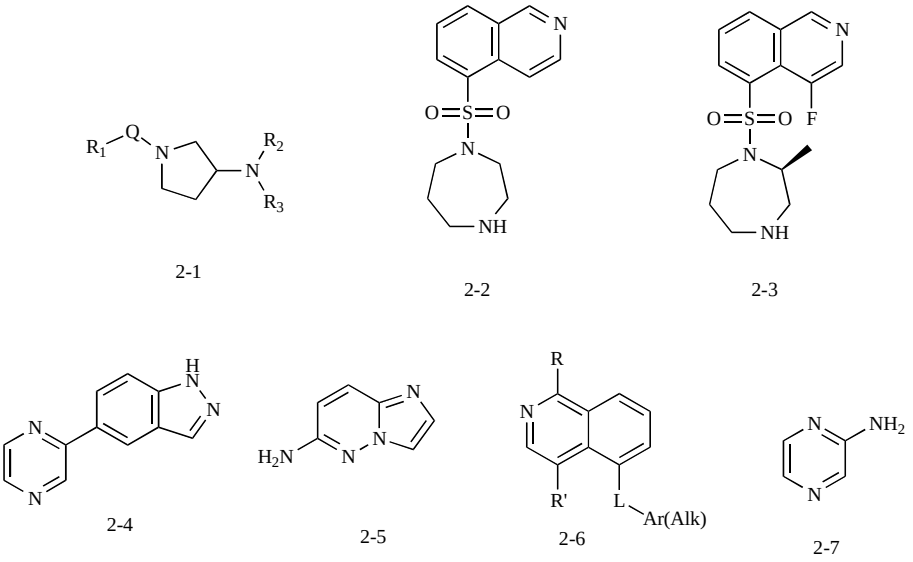

Fig. (2). Compounds used from Kowa and Alcon/Novartis companies.

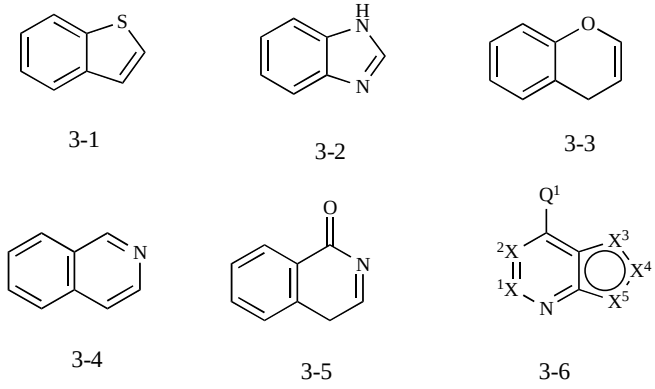

Fig. (3). Compounds used from other companies.

called Y-39983 with code name SNJ-1656/RKI-983 which is currently in Phase II of clinical trials (Trial ID: NCT00846989 and Trial ID: NCT00515424, Novartis Pharmaceuticals). The companies still have it under development, but looks like it has encountered efficacy and tolerability problems [30]. The inhibitor Y-39983 has a mechanism of action by increasing outflow of aqueous humor through the trabecular meshwork. Another solution under development from Novartis is US20100022517 [34] an ophthalmic formulation of Rho kinase inhibitor compound. The aqueous pharmaceutical formulation comprises 0.01-0.4% w/v of ROCK inhibitor (s), a non-ionic surfactant in an amount of 0.01-2% w/v, and a tonicity agent to maintain a tonicity between 220-360 mOsm/Kg, at a pH between 6.3 to 7.8, wherein the ROCK inhibitor, the surfactant, and the tonicity agent are compatible in the formulation. The aqueous ophthalmic formulations, prepared in this way, have an increased ocular bioavailability and/or aqueous humor concentrations without a concomitant increase in systemic concentrations.

Alcon presented the Rho kinase inhibitor US2007-0149548 [35], US20107655662 [36] using (indazol-5-yl)-pyrazines Fig. (**2-4**) and (1,3-dihydro-indol-2-one)-pyrazine-sand another one US2008079880 [37], US20107820670 [38] using 6-aminoimidazo[1,2-B]pyridazine and its analogs Fig (**2-5**). The IOP reduction obtained is no less than -21.6% in the 3rd hour of application and -22% in the 6th hour.

Another similar Rho kinase inhibitor of the company uses US20117867999 [39] hydroxyamino- and amino-substituted pyridine analogs Fig. (**2-6**). The method of administering includes applying 1 to 2 drops of a the composition comprising from about 0.01 percent weight/volume to about 5 percent weight/volume of the compound 1 to 4 times daily. There are no details about the efficacy of the compound.

The company has presented two patents US20080269249 [40] US20100216777 [40] concerning the use of aminopyrazine analogs as Rho kinase inhibitors. The analogs could be 2-aminopyrazine or 5-substituted 2,3 diaminopyrazines and derivatives Fig. (**2-7**). The compound will normally be contained in an amount 0.01 to 5 percent by weight/volume, but preferably in an amount of 0.25 to 2% weight/volume %. Thus, for topical presentation 1 to 2 drops of the formulation would be delivered to the surface of the eye 1 to 4 times per day.

Table 1. Patents Reviewed in This Review Article.

Patent App. Number	Title	Company
US20110319390	6-Aminoisoquinoline compounds	Aerie Pharmaceuticals, Inc.
US20110183965	6-Aminoisoquinoline compounds	Aerie Pharmaceuticals, Inc.
US20118034943	6-Aminoisoquinoline compounds	Aerie Pharmaceuticals, Inc.
US20100144713	6-And 7-amino isoquinoline compounds and methods for making and using the same	Aerie Pharmaceuticals, Inc.
US20100137364	6-Aminoisoquinoline compounds	Aerie Pharmaceuticals, Inc.
US20100233287	Preventing or treating agent for glaucoma	Kowa Co., Ltd.
US20090118299	Agent for prevention or treatment of glaucoma	Kowa Co., Ltd.
US20090082338	Preventive or remedy for glaucoma	Kowa Co., Ltd.
US20120040994	Therapeutic agent for glaucoma comprising Rho kinase inhibitor and prostaglandin	Santen Pharmaceutical Co., Ltd.
US20110263638	Therapeutic agent for glaucoma comprising Rho kinase inhibitor and beta blocker	Santen Pharmaceutical Co., Ltd.
US20110077244	Novel thiophenediamine derivative having urea structure	Santen Pharmaceutical Co., Ltd.
US20110039891	Methods for treating a disease in which Rho kinase is involved	Santen Pharmaceutical Co., Ltd.
US20100063060	Therapeutic agent for glaucoma comprising Rho kinase inhibitor and prostaglandin	Santen Pharmaceutical Co., Ltd.
US20100063045	Novel pyridinecarboxylic acid (2-aminophenyl) amide derivative having urea structure	Santen Pharmaceutical Co., Ltd.
US20100056522	Intraocular pressure-lowering agent comprising compound having histone deacetylase inhibitor effect as active ingredient	Santen Pharmaceutical Co., Ltd.
US20100041671	Methods for treating glaucoma	Santen Pharmaceutical Co., Ltd.
US20097563906	Indazole derivatives	UBE Industries, Ltd. Santen Pharmaceutical Co., Ltd.
US20107855222	Methods for treating a disease in which Rho kinase is involved	UBE Industries, Ltd. Santen Pharmaceutical Co., Ltd.
US20100209402	Agent for promoting corneal endothelial cell adhesion	Senju Pharmaceutical Co., Ltd.
US20087414137	Process for the preparation of 3,4-disubstituted-thiazolidin-2-ones	Inspire Pharmaceuticals, Inc.
US20107785624	Pharmaceutical latrunculin formulations	Inspire Pharmaceuticals, Inc.
US20117947850	Process for the preparation of 3,4-disubstituted-thiazolidin-2-ones	Inspire Pharmaceuticals, Inc.
US20118039465	Cytoskeletal active compounds, composition and use	Inspire Pharmaceuticals, Inc.
US20118071779	Cytoskeletal active Rho kinase inhibitor compounds, composition and use	Inspire Pharmaceuticals

(Table 1) Contd….

Patent App. Number	Title	Company
US20107666861	Cytoskeletal active compounds, composition and use	Inspire Pharmaceuticals, Inc.
US20087320974	Cytoskeletal active compounds, compositions and use	Inspire Pharmaceuticals, Inc.
US20110144150	Bridged bicyclic Rho kinase inhibitor compounds, composition and use	Inspire Pharmaceuticals
US20107807355	Diagnostics and therapeutics for glaucoma	Alcon, Inc. University of Iowa Research Foundation
US20107655662	(Indazol-5-yl)-pyrazines and (1,3-dihydro-indol-2-one)-pyrazines for treating glaucoma and controlling intraocular pressure	Alcon Research, Ltd.
US20107820670	6-Aminoimidazo[1,2-b]pyridazine analogs as Rho kinase inhibitors for the treatment of Rho kinase-mediated diseases and conditions	Alcon Research, Ltd.
US20117867999	Hydroxyamino- and amino-substituted pyridine analogs for treating Rho kinase-mediated diseases and conditions	Alcon Research, Ltd.
US20070149473	RNAi-mediated inhibition of Rho kinase for treatment of ocular disorders	Alcon Research, Ltd.
US20070149548	(Indazol-5-yl)pyrazines and (1,3-dihydro- indol-2-one)-pyrazines for treating Rho kinase-mediated diseases and conditions.	Alcon Research, Ltd.
US20080153903	Inhibitors of protein kinase c-delta for the treatment of glaucoma	Alcon Research, Ltd.
US20080269249	Aminopyrazine analogs for treating glaucoma and other Rho kinase-mediated diseases and conditions	Alcon Research, Ltd.
US2008079880	6-Aminoimidazo[1,2-B]pyridazine analogs as Rho kinase inhibitors for the treatment of glaucoma and ocular hypertension	Alcon Research, Ltd.
US20100120851	Prenyltransferase inhibitors for ocular hypertension control and the treatment of glaucoma	Alcon Research, Ltd.
US20100216777	Aminoipyrazine analogs for treating glaucoma and other Rho kinase-mediated diseases and conditions.	Alcon Research, Ltd.
US20100022517	Ophthalmic formulation of Rho kinase inhibitor compound	Novartis Ltd.
US20090318485	Novel inhibitors of Rho kinase	Kalypsys Inc.
US20110038835	Anilides and analogs as Rho kinase inhibitors	The Scripps Research Institute
US20110052562	Benzimidazoles and analogs as Rho kinase inhibitors	The Scripps Research Institute
US20110150833	Benzopyrans and analogs as Rho kinase inhibitors	The Scripps Research Institute
US20110190341	Substituted isoquinolines and isoquinolinones as Rho kinase inhibitors	Sanofi-AventisInc
US20117964613	Sulfonamide compound	Asahi Kasei Pharma Corporation
US20128093266	Rho kinase inhibitors	BoehringerIngelheim International GmbH
WO2007001439	Fused heterobicyclic kinase inhibitors	Osi Pharmaceutical, Inc.

Again there are no data concerning the efficacy of the inhibitor.

Finally, Alcon has filled the patent US20070149473 [41] based on the use of a RNAi-mediated inhibitor of Rho

kinase. The interfering RNA has a length of 19 to 49 nucleotides and is prepared in a pharmaceutically acceptable carrier. The interfering RNA presents a sense nucleotide strand plus an antisense nucleotide strand, with a region of a near-perfect contiguous complementarity with a minimum of 19 nucleotides. There are no details about the efficacy of the method.

OTHER INHIBITORS

The company Altheos recently presented its Rho inhibitor named ATS907 that is now in phase IIa clinical trials for glaucoma/OHT. In ARVO 2011 presented the results [42] of the *in vitro* and *in vivo* preclinical studies. These studies were conducted to evaluate pharmaceutical profile (i.e., potency and selectivity, cell permeability, absorption and metabolism) and safety and efficacy. Standard protocols were used to assess of ATS907 (parent) and its primary metabolite, ATS907M1, against 18 kinases in isolated enzymes *in vitro*. To evaluate the cell permeability in caco-2 cells *in vitro* and the generation of ATS907M1 in S9 fraction of liver homogenates from human, cynomolgus monkeys, dogs, and Japanese white rabbit using LC-MS and pharmacokinetic profile of ATS907 and ATS907M1 in aqueous humor and in plasma following topical administration to rabbits. The first in human clinical study, a Phase I/IIa was recently listed on www.clinicaltrials.gov. It is employing a two-stage adaptive design, intended to evaluate a number of different doses, and dosing regimens of ATS907 ophthalmic formulation versus placebo (vehicle) and to provide preliminary information on safety, tolerability and efficacy (reduction in IOP) after 28 days of administration. Altheos is preparing for the second stage of this trial (N =135), a comparison of 1-2 doses of ATS907, selected from stage 1 and latanoprost (a prostaglandin analogue), closely following completion of the first stage. According to the company, the drug shows excellent ocular surface penetration, and is rapidly converted into a more active form in the anterior chamber after topical dosing.

The company Kalypsys Inc. has filed a patent US20090318485 [43] for its novel Rho kinase inhibitor which is using pyridine and benzothiophene Fig. (**3-1**). The active ingredient for topical administration comprises from 0.001% to 10% w/w (by weight) of the formulation. The dose range for adult humans is generally from 5 mg to 2 g/day. The compounds can be administered in various modes, e.g., orally, topically, or by injection.

The Scripps Research Institute has several patents on ROCK kinase inhibitors. Among them are the US2011-0038835[44] using anilides and analogs the US20110052562 [45] using benzimidazoles and analogs Fig. (**3-2**) and the US20110150833[46] benzopyrans and analogs Fig. (**3-3**) as Rho kinase inhibitors.

Sanofi-Aventis Inc. has the patent US20110190341 [47] of substituted isoquinolines Fig. (**3-4**) and isoquinolinones Fig. (**3-5**) as Rho kinase inhibitors.

Asahi Kasei Pharma Corporation has filled the patent US20117964613 [48] about aforementioned sulfonamide compounds which according to the company, are strong Rho kinase inhibitors. The compound gave an IOP reducing degree not lower than 20% when it was administered on Japanese white rabbits. The results on cynomolgus monkeys were promising as well.

Finally, the company Osi Pharmaceutical, Inc. presented a fused heterobicyclic Fig. (**3-6**) kinase inhibitor WO2007-001439 [49] but they didn't provide any details about its efficacy.

CURRENT & FUTURE DEVELOPMENTS

The existing anti-glaucoma drugs may cause adverse side effects due to the high doses needed for drug-washed out or tear dilution and their systemic absorption. In addition, its poor compliance due to the requirement of at least once per day drop application is also a problem in glaucoma management. As consequence, there is a great interest not only in improving the current anti-glaucoma medicines but also in discover safer and more effective drugs. In the last years, several potential new anti-glaucoma targets have been discovered although for most of them there is limited physiological information or the drugs have not yet been clinically validated. Recently, an emerging role of Rho protein kinase in glaucoma pathology has been described by various authors and even, its inhibitors are being evaluated in clinical trials. ROCK inhibitors facilitate aqueous drainage, decreasing myosin light-chain phosphorylation and subsequently evoke cellular relaxation in trabecular meshwork and Schlemm´s canal. However, the high structural homology existing between different kinases limits its specificity.

During the clinical trials conducted with Rho kinase inhibitors some failed so far because of several side effects found. It was discovered that Rho kinase inhibitors are suffering from tolerability issues, some of them related with vasodilation. In this sense, the principal side effect is transient hyperemia (redness) noticed to some patients. Another significant drawback is the requirement of at least twice-daily dosing, which is exacerbating the side effects.

The solution of these difficulties of selectivity and the limited tolerability is the combination of existing IOP lowering drugs with the mentioned above ROCK inhibitors. Combined use of drugs having actions of reducing IOP to treat glaucoma has already been extensively studied and there are some plenty of reports on the studies. The action of reducing intraocular pressure is increased and/or the persistence of the action is improved by combining these drugs compared with a case where each drug is used alone. The mentioned drug mixtures did not reach for the moment the clinical trial level; hence we are not aware of the side effects they provoke. According to the pharmatheutical companies involved, the effects should be mild and there is a lot of expectation for this proposed solution.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

It is certified that there is no actual or potential conflict of interest in relation to this article.

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Artículo 1 sobre el tratamiento de Ojo Seco

EXPERT OPINION

An update on dry eye disease molecular treatment: advances in drug pipelines

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Introduction: Dry eye disease is a common disorder provoking changes in tear film and ocular surface. Untreated dry eye could cause ocular infections, corneal ulcer and blindness. Only a few drugs are authorized so far for the treatment of dry eye disease and the possibilities of evolution in this sector are immense. Consequently, a significant number of new potential solutions are under development or placed in the pharmaceutical pipeline, promising better results and lesser side effects.
Areas covered: In this article, the corresponding literature and recent Phase III clinical trial data and the corresponding literature, for dry eye disease treatment are reviewed, revealing the new strategic movements in drug pipelines.
Expert opinion: From the clinical trial results, the advancement in tear substitutes and secretagogues in addressing specific deficiencies of tear components even though not resolving the underlying conditions of the disease is evident. The vast majority of new compounds under development are anti-inflammatories, steroids, non-steroids and antibiotics; however, there are also some novel lubricating drops and mucin-tear secretagogues. A future aggressive therapy for dry eye, depending on the severity of the symptoms, would include combinations of soft steroids, anti-inflammatories, such as cyclosporine A, with the addition of the new polyvalent mucin and tear secretagogues.

Keywords: anti-inflammatory, corticosteroids, dry eye, keratitis sicca, keratoconjunctivitis sicca, mucin secretion, NSAID, tear secretion, xerophthalmia

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1. Introduction

Dry eye disease is a chronic ocular disorder affecting approximately 10 – 20% of the population worldwide, being relatively frequent among women especially after the menopause [1,2]. This pathology is closely related to the aging and it is progressively affecting larger parts of the population. Autoimmune disease patients are prone to suffer from dry eye inflammation, which is a key factor in the onset of severe dry eye disease [3]. Tear film forms a moist natural barrier separating eye from the external media. This consistent film is formed mainly from a triplet of aqueous, mucous and lipid layers, providing the necessary equilibrium for maintaining healthy the ocular surface. The pathology occurs when the ocular surface tear protective layer is weakened which could be the result of insufficient or atypical production of one or more tear components [4]. The main functions of this film are to lubricate the ocular surface, transfer nutritional elements to the cornea, eliminate foreign matter and cellular debris generated on the ocular surface by tear flow and blink process and act as the first line of defense against ocular surface infections [5]. In some cases, the imbalance of the tear film composite layers leads to reduced tear secretion, exposure

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Article highlights.

- Most of the drugs presented have already passed safety tests and have been approved as treatment for other diseases.
- Development and inclusion of improved objective methods in dry eye disease clinical trials can simplify the analysis of results obtained from diverse treatments.
- Recent developments in the research of tear film physiology and pathology made the advancement possible in a variety of tear substitutes capable of addressing specific deficiencies of tear components.
- NSAIDs and antibiotics are improving dry eye signs and symptoms, and they are safer than corticosteroids for long-term use, even though they do not demonstrate the rapid anti-inflammatory effect of steroids.
- The novel multipurpose eye lubricants are a safe and effective solution for moderate dry eye cases.
- In severe dry eye cases, the combined use of soft steroids with cyclosporine or other types of anti-inflammatories seems to be a worthy solution to achieve faster, prolonged and effective results with a better safety profile.

This box summarizes key points contained in the article.

of the eye surface, dryness and damage of the surface cells [3]. The imbalance of the composite layers could also be the result of Sjögren's syndrome tear secretion deficiency and/or tear film instability due to the use of contact lenses [3,6,7]. Dry eye disease types are classified according to etiological perspectives and environmental influences such as aqueous-deficient dry eye (ADDE) and evaporative-tear dry eye (EDE) [3,8]. The two forms of dry eye are not mutually exclusive and often coexist [9]. The proportion of subjects exhibiting signs of EDE resulting from meibomian gland dysfunction (MGD) far outweighs that of subjects with pure ADDE. In a general clinic-based patient cohort, asymptomatic is more common than symptomatic MGD [10,11].

1.1 Methodologies to diagnose dry eye disease

The clinical diagnosis is challenging since dry eye is a pathology characterized by an extensive variety of signs and symptoms and because of the ambiguity in the etiology and pathophysiology of the disease [12]. Other factors are the invasive nature of some of the diagnostic tests, which can make the interpretation of the results challenging or the use of individual tests in very dissimilar scenarios, suggesting the application of different protocols. Further, tear film is a dynamic, open system subject to numerous internal and environmental variations, leading frequently to misinterpretations of the obtained results [13]. In general, the variety of causative agents and the high number of ocular conditions with similar signs and symptoms are challenging the accurate and differential diagnosis, especially due to lack of association between signs and symptoms. Signs and symptoms can vary from patient to patient, sometimes with little or no correlation between them and a large part of dry eye population does not even notice symptoms. The asymptomatic patients who exhibit some of the objective features of dry eye may be entitled to diagnosis, suggesting that in the absence of symptoms, some objective criteria of dry eye may still be satisfied, such as tear hyperosmolarity, the presence of interpalpebral ocular surface staining, reduced tear production or tear instability [14,15]. The discrepancy between signs and symptoms represents a dilemma in dry eye clinical research and practice. Various researchers conducted clinical studies to verify the level of this unreliability. During these studies it was found that existing clinical tests could not predict the frequently reported symptoms, and there were not observed significant correlations between signs and symptoms after adjustment for age and artificial tear use, demonstrating an absence of direct association between the number or severity of symptoms and the degree of ocular surface damage or tear deficiency [6,16]. These results strongly support the evidence that diagnosis and treatment of moderate dry eye require a detailed assessment of self-perceived symptoms and that objective clinical testing alone may be insufficient. The optimal diagnosis of dry eye disease, therefore, depends on the results of several tests leading to a reliance on symptom-based diagnosis for dry eye [6,9,17,18]. Conventional examinations include Schirmer's test, tear break-up time (TBUT) and ocular surface staining, which are considered as having a low degree of standardization. The use of combinations of tests is advisable, even though for the moment a definitive combination does not exist among them and the creation of better objective tests with precise diagnostic value is essential.

There are several proposals for new tests such as molecular markers [19,20]. It is reported that objective tests of tear osmolality, ocular surface integrity and lid-parallel conjunctival folds sum severity scores, are usually more efficient than other clinical measures at predicting the symptoms [16,21]. Other reliable novel markers having the potential to be practical and which are useful additions to clinical practice are the MMP-9 and the lipid layer interferometry. With MMP-9, the inflammation associated with dry eye is assessed through the detection of elevated levels of MMP-9 in the tears [22]. Lipid layer interferometer measures the thickness of the lipid layer, since in dry eye the likelihood of a relatively thin lipid layer is significantly increased [23]. For the moment, tear film osmolarity test is considered as one of the best objective biomarkers for a correct diagnosis [24,25]. With tear film osmolarity, adequate objective results of the disease severity across normal, mild/moderate and severe categories are obtained [26].

During clinical trials, difficulties on selecting optimal drug doses or suitable outcome measures is common, and the new drugs are accounting for significant delays or denial in their approval process, not because they are unsafe or ineffective but because the information supplied is insufficient for the decision making. The reasons for this frequent failure could be justified by the complicated nature of the disease, the use of unsatisfactory end points or sometimes because of the excessive mandated requirements of the regulatory agencies. Often responsible for this failure is the lack of repeatability

of many clinical tests in common use, as recurring measures of the same test on the same patients at different times are not repeated [14]. Another reason is the inconsistency of the outcomes, having to do with the difficulty of the diagnostic tests to interpret the results after the application of the drug, as symptoms evaluation is insufficient as a measurement factor [27].

1.2 Current dry eye treatment

As already mentioned, there are only few pharmaceutical treatments for dry eye. In some countries such as Japan, there are commercialized more dry eye disease treatment drugs than in the USA as the approval rules are not so rigorous. Another important factor concerning dry eye disease treatment is that, in most of the cases, the biotechnology companies are trying to sort out the problem based on 'over the counter' strategies, trying to relieve symptoms rather than getting to the cause of the disease. Consequently, for the symptomatic relief of dry eye, the following treatments are currently used: supplements called 'artificial tears' which are synthetic lubricants, characterized by hypotonic- or isotonic-buffered solutions containing electrolytes, surfactants and several types of viscosity agents [28]. The 'artificial tears' permit not only the increase in tear quantity but also to keep the ocular surface moistened and to relieve discomfort, even though their use has disadvantages such as the high frequency in drop dosage and the loss of efficiency after long-term treatment [29]. The current treatment for severe cases of dry eye is mainly focused on addressing eye inflammation and tear restoration [30]. Dry eye disease is the outcome of many factors resulting in inflammation of cornea and conjunctiva. The dysfunction of the tear secretory glands leads to changes in tear composition such as hyperosmolarity which stimulates the production of inflammatory mediators on the ocular surface. This inflammation can be initiated either by chronic irritative stress, such as contact lens wearing, or from a systemic inflammatory autoimmune disease, such as rheumatoid arthritis [31,32]. Anti-inflammatory drugs are widely used for the treatment of eye inflammation with topical corticosteroid drops being considered as the most common therapy. Corticosteroids can relieve moderate or severe dry eye symptoms and signs rapidly and effectively [33]. Steroids, on the other hand, may cause severe side effects, especially after prolonged use. The effects vary from bacterial or fungal infection, intraocular pressure elevation and cataract formation. Additionally, steroids locally suppress the immune response of the patients with an already compromised ocular surface. Therefore, steroids are typically used for a limited period of time [30]. Due to the above-mentioned disadvantages, NSAIDs, credited as causing less severe side effects, are considered as a potential dry eye treatment. The NSAIDs could decrease inflammation and eye discomfort due to their analgesic effect, but on the other hand might induce dry eye disease, decreasing sensitivity. In 2002, the US FDA approved topical cyclosporine 0.05% (Allergan, Irvine, CA, USA) as the first prescription medicine specifically indicated to increase tear production [34]. Other types of drugs used are the antibiotics such as azithromycin and tetracycline. Further, some research groups are studying the use of serum tears and intense pulse light as potential treatments [30]. In the following sections, the latest trends on the future treatments from Phase II up to their commercialization stage are presented.

2. Compounds reaching the end of the pipeline – Phase III

2.1 Anti-inflammatory drugs

2.1.1 *Corticosteroids and NSAIDs*

2.1.1.1 *Dexamethasone phosphate (EGP-437)*

Eyegate Pharmaceuticals, Inc., presented EGP-437 (dexamethasone phosphate 40 mg/ml, DP formulated for iontophoresis) (**Table 1** Figure 1 and **Table 2**) as a possible treatment for dry eye disease [35]. Dexamethasone is a glucocorticoid showing anti-inflammatory and immunosuppressive properties. The company is using an ocular iontophoresis device for drug delivery in which the drug is ionized by an electrical field, modifying the permeability of the cells, enabling an easier delivery to the targeted areas. With this delivery system, dexamethasone levels distributed into the eye are significantly higher than using the glucocorticoid alone. Phase III (ClinicalTrials.gov Identifier: NCT01129856) randomized safety and efficacy studies were conducted from in May 2010, providing 40 mg/ml of EGP-437 in 103 dry eye patients [36]. The study employed a prospective, single-center, double-masked design using a controlled adverse environment. Compared to placebo, ocular iontophoresis treatments with EGP-437 had both a rapid onset of action and long-term effectiveness. The patients showed statistically significant improvements in dry eye signs and symptoms at various time points, but the primary end points were not achieved. The adverse events were mild and no severe effects were observed [36]. Despite the benefits and lack of side effects, there is a difficulty on the application of corticoid medications and iontophoresis is a complicated technique to be 'self-administered'. It seems that the company needs a better delivery vehicle for the compound.

2.1.1.2 *Rimexolone (AL-2178)*

Rimexolone 1% (**Table 1** Figure 2 and **Table 2**) is a glucocorticoid steroid developed by Alcon (AL-2178) as eye anti-inflammatory. Rimexolone inhibits T-cell proliferation as well as cytokine production of activated $CD4^+$ T-cells producing anti-inflammatory and immunomodulatory effects. In 2008, Alcon successfully completed the Phase III trial (ClinicalTrials.gov Identifier: NCT00471419) and now rimexolone is marketed as 1% eye drop suspension. Additionally several authors have reported the anti-inflammatory efficacy and safety of rimexolone after cataract surgery [37-40]. More recently, Amon and Busin concluded that rimexolone, loteprednol etabonate and difluprednate demonstrate a similar

Table 1. Chemical formulas of drugs in Phase III clinical trials for dry eye syndrome.

Figure 1 Dexamethasone phosphate (EGP-437)

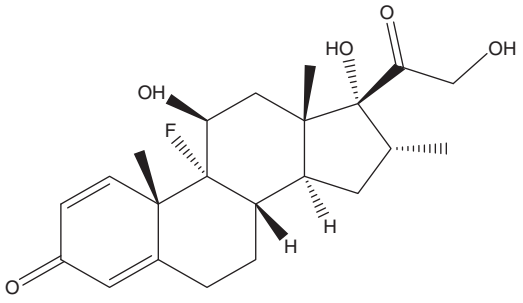


Figure 2 Rimexolone

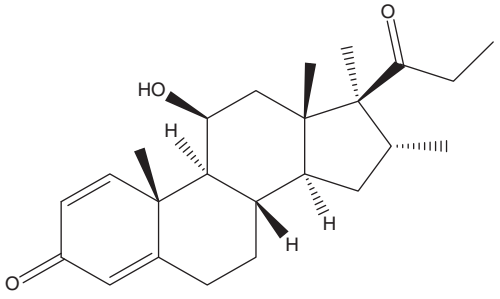


Figure 3 Bromfenac

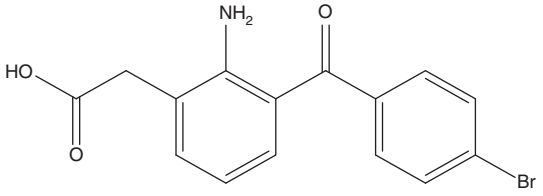


Figure 4 IB-MECA (CF101)

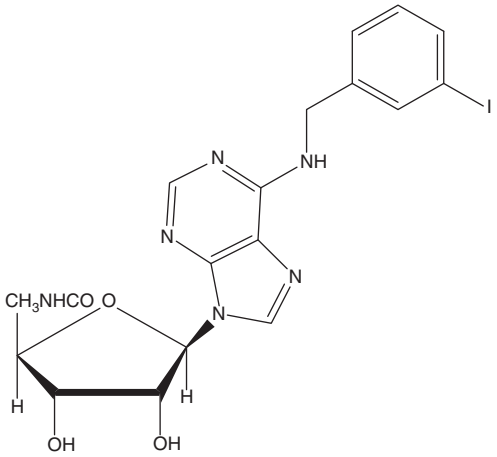


Table 1. Chemical formulas of drugs in Phase III clinical trials for dry eye syndrome (continued).

Figure 5 Cyclosporine A, NOVA22007

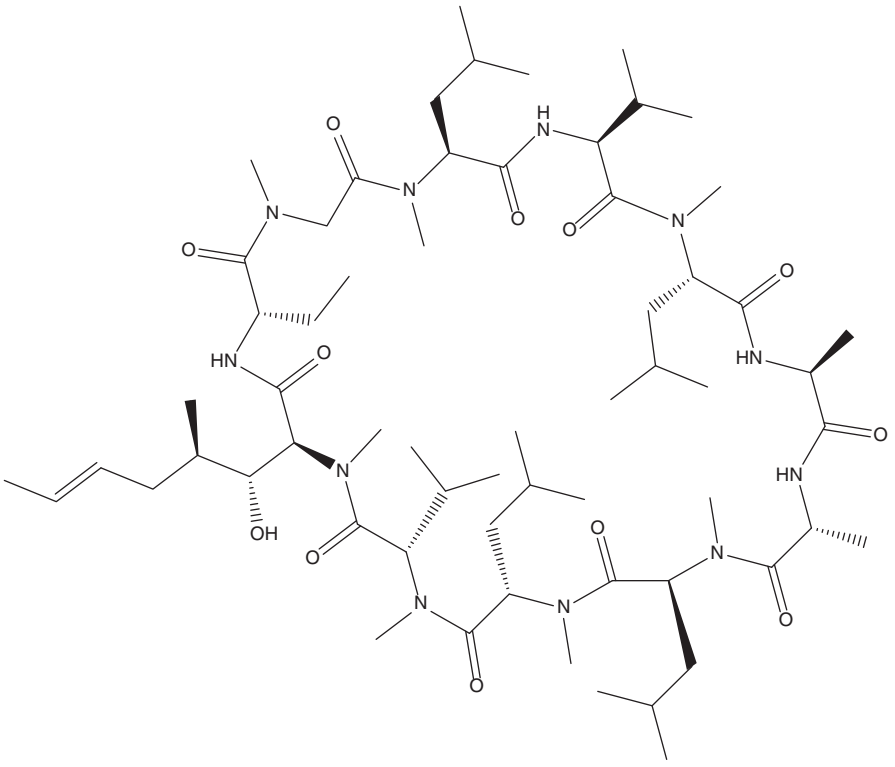


Figure 6 Lifitegrast (SAR1118)

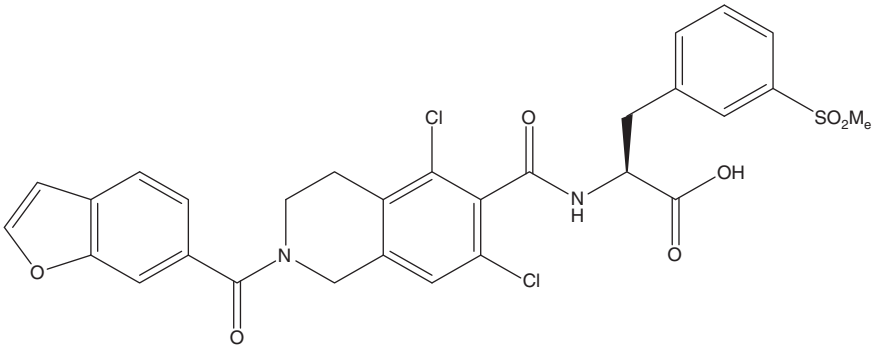


Figure 7 Azithromycin

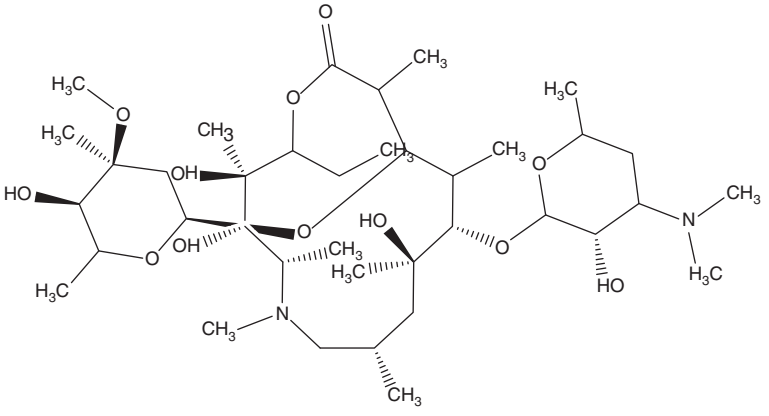


Table 1. Chemical formulas of drugs in Phase III clinical trials for dry eye syndrome (continued).

Figure 8 Hydroxychloroquine

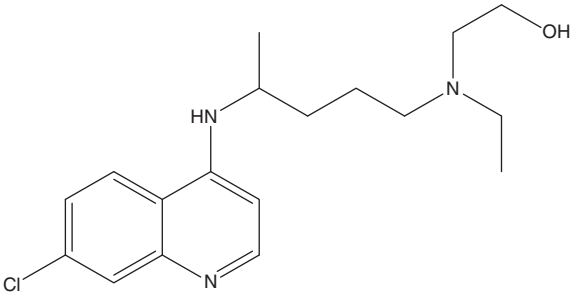


Figure 9 MIM-D3

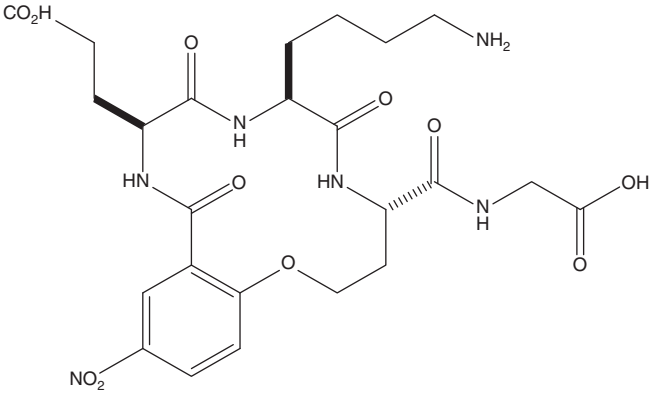


Figure 10 Rebamipide (OPC-12759)

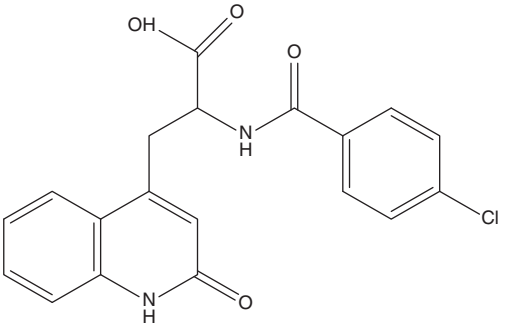


Figure 11 Ecabet sodium

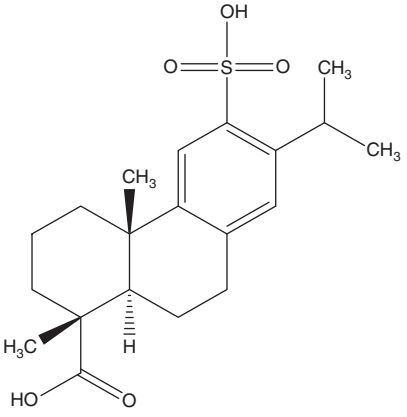


Table 1. Chemical formulas of drugs in Phase III clinical trials for dry eye syndrome (continued).

Figure 12 Diquafosol tetrasodium (INS365, DE-089) or UppppU

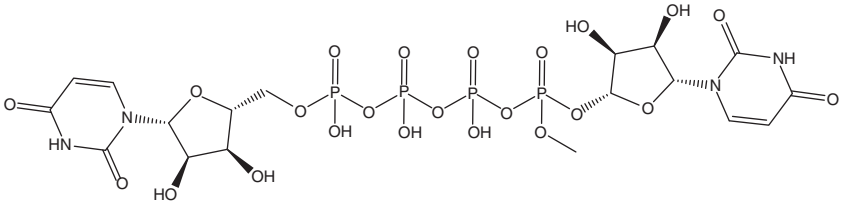
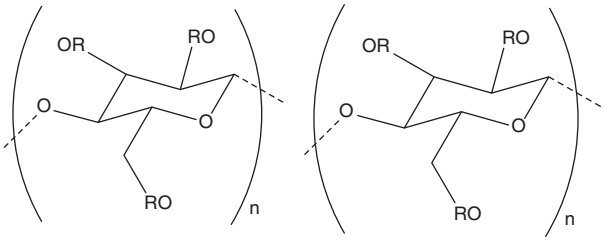


Figure 13 Carboxymethyl cellulose and hydroxypropyl methylcellulose



R = H or CH_2CO_2H R = H or CH_3 or $CH_2CH(OH)CH_3$

Figure 14 Sodium hyaluronate

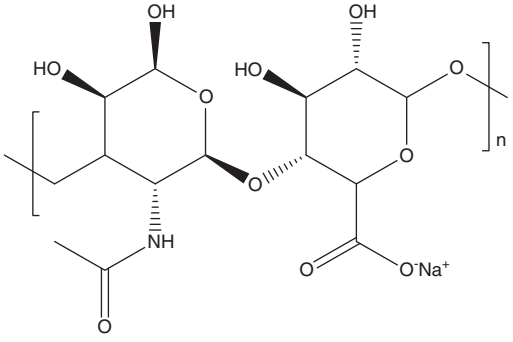
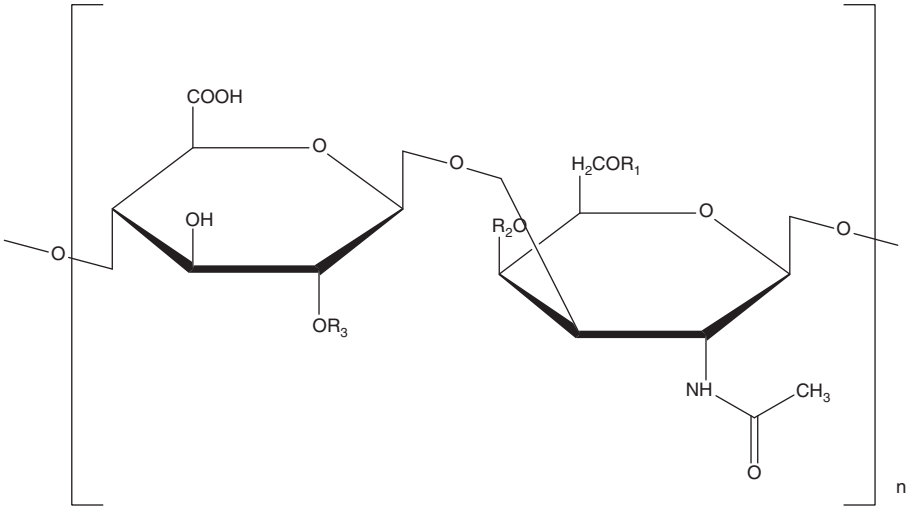


Figure 15 PRO-148



R_1 = H; R_2 = SO_3H; R_3 = H. Chondroitin-6-sulfate: R_1 = SO_3H; R_2, R_3 = H.

Table 2. Drugs in Phase III clinical trials for dry eye syndrome and their properties.

Drug name and Company	Phase	Properties	Clinical trial identifier – primary and secondary efficacy parameters	Results
Dexamethasone phosphate (EGP-437) of Eyegate Pharmaceuticals, Inc.	Phase III	Anti-inflammatory	NCT01129856 Primary: change in CFS Secondary: symptom, ocular discomfort pre- and post-CAE (Ora Scale)	Statistically significant improvements in dry eye signs and symptoms relative to the placebo group in CAE challenge, CAE recovery and environmental conditions were observed
Rimexolone (AL-2178) of Alcon, Inc.	Phase III	Anti-inflammatory	NCT00471419 Primary and secondary: CFS	NA
Bromfenac of ISTA Pharmaceuticals	Phase III	Anti-inflammatory	NCT01212471 Primary: sign and symptom measures of dry eye disease	Reported severe side effects
EBI-005 of Eleven Biotherapeutics	Phase III	Anti-inflammatory	NCT01998802 Primary: NEI score for total CFS Secondary: Total OSDI score	NA
Tocilizumab of F. Hoffmann-La Roche Ltd	Phase III	Anti-inflammatory	NCT01782235 Primary: enhancement superior or equal to 30% of the ESSPRI score	NA
IB-MECA (CF101) of Can-Fite BioPharma	Phase III	Anti-inflammatory	NCT01235234 Primary: complete clearing of corneal staining by fluorescein staining Secondary: complete central corneal clearing by fluorescein staining	NA
Cyclosporine A- NOVA22007 of Novagali Pharma SAS	Phase III	Anti-inflammatory	NCT00814515 Primary: dry eye signs, change in CFS Secondary: dry eye symptoms, mean change in global score of ocular discomfort using a VAS	Statistically significant improvements in CFS were observed at 1, 3 and 6 months. No statistically significant difference was observed compared to the vehicle at 6 months
Cyclosporine (Haporine-S) of DH Bio Co. Ltd and BTO Pharm Co. Ltd	Phase III	Anti-inflammatory and tear secretagogue	NCT01804361 Primary: corneal staining and CFS assessed at first day and at 4 and 12 weeks Secondary: OSDI, TBUT, Schirmer's score, drug compliance and DEWS level	NA
Lifitegrast (SAR1118) of SARcode Bioscience	Phase III	Anti-inflammatory	NCT01636206 NA	Superiority over placebo in the improvement of inferior and total corneal staining scores from baseline to week 12 ($p = 0.0007$ and $p = 0.0148$) was observed. The mean ocular discomfort score and mean eye dryness score were lower than in the placebo group at week 12 ($p = 0.0273$ and $p = 0.0291$)

CAE: Controlled adverse environment; CFS: Corneal fluorescein staining; CMC: Carboxymethyl cellulose; DEWS: Dry Eye Workshop; ESSPRI EULAR: Sjögren's syndrome patient reported index; LGCS: Lissamine green conjunctival staining; LGS: Lissamine green staining; NA: Not available; Na-HY: Sodium hyaluronate; NCT: National clinical trial; NEI-CSS: National Eye Institute Corneal Staining Scale; OCI: Ocular comfort index; OSDI: Ocular surface disease index; OsPr: Osmoprotective; TBUT: Tear break-up time; VAS: Visual analog scale.

Table 2. Drugs in Phase III clinical trials for dry eye syndrome and their properties (continued).

Drug name and Company	Phase	Properties	Clinical trial identifier – primary and secondary efficacy parameters	Results
Azithromycin of Merck & Co., Inc./Inspire Pharmaceuticals, Inc.	Phase III and Phase IV	Anti-inflammatory	NCT01014078 and NCT01105624 Primary: subject-reported comfortable contact lens daily wear time Secondary: subject-reported comfortable contact lens daily wear time	Statistically significant increase in mean comfortable contact lens wear time from baseline was observed. No significant differences were observed between the groups for total wear time, low contrast visual acuity or tear osmolarity
Hydroxychloroquine of Assistance Publique and Hôpitaux de Paris, Sanofi-Aventis	Phase III	Anti-inflammatory	NCT00632866 NA	The results demonstrated that the patients with dry eye symptoms and α-fodrin antibodies had a significant increase in tear production ($p = 0.001$)
Rituximab of IDEC Pharmaceuticals	Phase III	Anti-inflammatory	NCT00740948 30% improvement between the values on 2 of the 4 VAS measuring global scores of the disease	Confirmed efficacy and tolerance for primary Sjögren's disease
MIM-D3 of Mimetogen Pharmaceuticals, Inc.	Phase III	Stimulates mucin secretion	NCT01670357 Primary: change from baseline of CFS score Secondary: change from baseline of TBUT	NA
Rebamipide (OPC-12759) of Acucela, Inc. and Otsuka Pharmaceutical, Inc.	Phase III	Stimulates mucin secretion	NCT00885079 Primary: Change in CFS Secondary: Change in LGCS	CFS score mean ± standard deviation -3.5 ± 2.2 LGCS score mean ± standard deviation -4.0 ± 2.8
Ecabet sodium of Bausch & Lomb, Inc. and ISTA Pharmaceuticals	Phase III	Stimulates mucin secretion	NCT00198536 Lacrimation assessed by Schirmer's test in a time frame of 6 weeks	Results approached statistical significance versus placebo. No serious adverse events and no serious ocular or systemic adverse events were observed
Diquafosol tetrasodium or Up_4U (INS365, DE-089) of Merck & Co., Inc.	Phase III	Stimulates tear-mucin secretion	NCT01101984 Primary: dry eye signs, change in fluorescein and in rose bengal CFS Secondary: dry eye symptoms, changes in TBUT	Not approved by FDA for patient use, as having failed to demonstrate statistically significant improvement as compared to placebo in its primary end point
CMC (Optive) and hydroxypropyl methylcellulose of Allergan, Inc.	Phase III	Eye lubricant	NCT01335126 and NCT01711424 Primary: change from baseline in global ocular staining score at day 35 Secondary: change from global ocular staining at day 35	The primary efficacy analysis was per protocol (OsPr-CMC, n = 37; Na-HY, n = 29). Ocular staining score at day 35: -2.0 (0.33) with OsPr-CMC vs -1.7 (0.37) with Na-HY. Overall improvement in comfort and an improvement in tear stability was reported but a statistically significant increase in tear turnover rate was not shown

CAE: Controlled adverse environment; CFS: Corneal fluorescein staining; CMC: Carboxymethyl cellulose; DEWS: Dry Eye Workshop; ESSPRI EULAR: Sjögren's syndrome patient reported index; LGCS: Lissamine green conjunctival staining; LGS: Lissamine green staining; NA: Not available; Na-HY: Sodium hyaluronate; NCT: National clinical trial; NEI-CSS: National Eye Institute Corneal Staining Scale; OCI: Ocular comfort index; OSDI: Ocular surface disease index; OsPr: Osmoprotective; TBUT: Tear break-up time; VAS: Visual analog scale.

Table 2. Drugs in Phase III clinical trials for dry eye syndrome and their properties (continued).

Drug name and Company	Phase	Properties	Clinical trial identifier – primary and secondary efficacy parameters	Results
Na-HY of River Plate Biotechnology	Phase III	Eye lubricant, anti-inflammatory, wound healing	NCT01382225 Primary: change from baseline in LGS total score at day 7 Secondary: change from baseline in LGS total score at day 14	Baseline 4.9 ± 2.2, change from baseline at day 7 -0.8 ± 1.8 Baseline 4.9 ± 2.2, change from baseline at day 14 -1.2 ± 2.0
PRO-148 of Laboratorios Sophia	Phase III	Eye lubricant, wound healing	NCT01541891 Primary: evaluation of ocular signs, corneal and interpalpebral conjunctival staining Secondary: evaluation of ocular symptoms and signs	Significant reduction in the mean scores for the ocular symptoms of dryness, gritty/sandy feeling and burning
Linseed extract and polyvinylpyrrolidone (T-Clair SPHP700-3) of Sinclair Pharmaceuticals, Inc.	Phase III	Eye lubricant, tear secretagogue	Clinical trial in UK	Safe and effective in mild–to-moderate dry eye, improving tear film stability, ocular surface lubrication and ameliorating overall dry eye symptoms

CAE: Controlled adverse environment; CFS: Corneal fluorescein staining; CMC: Carboxymethyl cellulose; DEWS: Dry Eye Workshop; ESSPRI EULAR: Sjögren's syndrome patient reported index; LGCS: Lissamine green conjunctival staining; LGS: Lissamine green staining; NA: Not available; Na-HY: Sodium hyaluronate; NCT: National clinical trial; NEI-CSS: National Eye Institute Corneal Staining Scale; OCI: Ocular comfort index; OSDI: Ocular surface disease index; OsPr: Osmoprotective; TBUT: Tear break-up time; VAS: Visual analog scale.

anti-inflammatory effect, after reviewing the published clinical data of these drugs [41].

2.1.1.3 *Bromfenac*

ISTA Pharmaceuticals is developing bromfenac a NSAID for topical short-term use in dry eye patients (**Table 1** Figure 3 and **Table 2**) [42]. As possible mechanism of action is mentioned its ability to block prostaglandin synthesis, inhibiting COX-1 and -2. It is already approved as a medicine (ophthalmic solution bromfenac 0.07 – 0.09%) for the treatment of postoperative inflammation and reduction of ocular pain in patients who have undergone cataract surgery. ISTA Pharmaceuticals and Bausch & Lomb, Inc. conducted a Phase III, dose-ranging study to evaluate the safety and efficacy of bromfenac ophthalmic solution for dry eye disease (ClinicalTrials.gov Identifier: NCT012 12471). A total of 990 patients were enrolled, and the study was completed in August 2012. Various side effects were noticed, a known disadvantage of this type of anti-inflammatory drugs, such as temporary irritation, burning, stinging of the eye, temporary blurred vision, watery eyes and headache. These results are surprising since the anti-inflammatory efficacy and safety of bromfenac have been demonstrated, as we have commented above, for use after cataract surgery [43,44]. Additionally, Prasher reported a case of acute corneal melt after cataract surgery in a patient treated with bromfenac eye drops [45].

2.1.2 *Other anti-inflammatory drugs*

2.1.2.1 *Direct inhibitors of cytokines or cytokines receptors*

2.1.2.1.1 *EBI-005*

Eleven Biotherapeutics is developing EBI-005, a chimeric IL-1 receptor type I (IL-1 RI) antagonist, as an anti-inflammatory for dry eye treatment [46]. EBI-005 is an IL-1 R blocker, single-domain protein, optimized for topical ocular delivery. It is modulating cellular signaling response to IL-1 RI and is binding to its receptors (**Table 2**) [47]. According to data presented in association for research in vision and ophthalmology 2012, it was created from the combination of IL-1 R binding the subdomains IL-1β and IL-1Ra [48]. EBI-005 has been validated in clinical proof-of-concept studies in which IL-1 blockage was shown to be safe and well tolerated without significant adverse side effects. During preclinical trials in mouse models, EBI-005 was shown to be more active than topical cyclosporine [49]. Topical delivery resulted in distribution to multiple eye compartments but very low systemic exposure in rabbits [48]. EBI-005 was 9°C more thermally stable than anakinra [50]. Eleven Biotherapeutics presented promising preliminary clinical data from initial clinical trials. In November 2013, Phase III trials (ClinicalTrials.gov Identifier: NCT01998802) have started to evaluate EBI-005 as a topical ophthalmic solution for subjects with moderate-to-severe dry eye disease, applied three times daily for 12 weeks.

2.1.2.1.2 Tocilizumab

F Hoffmann-La Roche Ltd and the French University Hospital of Strasbourg are evaluating tocilizumab a recombinant humanized monoclonal anti-IL-6 R antibody as possible treatment of primary Sjögren's syndrome (**Table 2**). The hospital started randomized, double-blind, placebo clinical Phase III trials in January 2013 with 80 patients, for evaluating the efficacy of tocilizumab in primary Sjögren's syndrome (ClinicalTrials.gov Identifier: NCT01782235).

Tocilizumab is already approved in Japan as treatment for Castleman's disease and several types of arthritis and in EU for the treatment of moderate-to-severe rheumatoid arthritis [51]. Recently, Sato *et al*. have reported a case of acute anterior uveitis after discontinuation of tocilizumab treatment in a patient with rheumatoid arthritis [52]. Moreover, and unexpectedly, the efficacy of tocilizumab to treat refractory uveitis has been shown [53,54].

2.1.2.1.3 IB-MECA (CF101)

The Israeli company Can-Fite BioPharma presented the anti-inflammatory CF101 (**Table 1** Figure 4 and **Table 2**) for treatment of dry eye disease. CF101 is a specific agonist of the A3 adenosine receptor, inducing an anti-inflammatory effect in experimental animal models [55]. CF101 downregulates the NF-κB–TNF-α signaling pathway, inhibiting cytokine production and apoptosis of inflammatory cells. In November 2010, Can-Fite BioPharma started Phase III safety and efficacy studies (ClinicalTrials.gov Identifier: NCT01235234) on 240 dry eye patients, with oral doses of 0.1 or 1 mg, twice daily for 24 weeks. There were a statistically significant proportion of patients who achieved more than a 25% improvement in corneal staining at week 12 and in the clearance of corneal staining among the CF101-treated group and the placebo group. CF101 was well tolerated with no serious adverse events reported [56,57].

2.1.2.2 Inhibitors of T-cell activation

2.1.2.2.1 Cyclosporine A (NOVA22007)

The French company Novagali Pharma SAS, which was acquired by Santen SAS, presented an anti-inflammatory compound named NOVA22007 [58]. NOVA22007 is a cationic emulsion of cyclosporine A (CsA), (**Table 1** Figure 5 and **Table 2**) [59]. Preclinical studies demonstrated that NOVA2 2007 could improve CsA absorption in cornea and conjunctiva [59,60]. This is an interesting point concerning cyclosporine solubility and absorption problems in other ocular and anti-inflammatory medicines. Daull *et al*. compared the ocular and systemic distribution of CsA in rabbits after the instillation of preservative-free CsA cationic and anionic emulsions [60]. Single-dose pharmacokinetic data demonstrated that NOVA2 2007 0.1 or 0.05% delivered higher CsA concentrations to the cornea compared to cyclosporine with a C_{max} of 2692, 1372 and 748 ng/g, respectively, having a better exposition (AUC). In general, CsA cationic emulsions were more effective than cyclosporine in delivering to target tissues [60]. Initial trials demonstrated acceptable safety and tolerance profiles of the formulation and promising preliminary trends toward efficacy. The company started Phase III, safe and efficacy studies (ClinicalTrials.gov Identifier: NCT00814515) of NOVA22007 (cyclosporine 0.1%) in December 2008 on 482 patients and safe and efficacy studies in pediatric patients with active severe vernal keratoconjunctivitis (ClinicalTrials.gov Identifier: NCT01751126) since December 2012. During the NCT008 14515 trials, the compound demonstrated statistically significant improvements in corneal fluorescein staining (CFS). Even though the results in the mean visual analog scale score from baseline were encouraging, statistically significant difference at month 6 was not observed, compared to the emulsion vehicle [61]. Recently, new methods for the subconjunctival administration of CsA were developed, such as microspheres, implants and liposomes, for enhancing its efficiency [62]. NOVA22007 is a clear example of a drug with interesting properties already in use for other diseases, which is redirected to treat dry eye. New compositions have been found to be more effective when changing delivery vehicle and making emulsions, enhancing the absorption and the anti-inflammatory effect.

2.1.2.2.2 Cyclosporine (Haporine-S)

The Korean University Hanyang, in cooperation with the companies DH Bio and BTO Pharm Co. Ltd, is developing Haporine-S, a new anti-inflammatory for dry eye disease (**Table 2**). Haporine-S eye drops contain cyclosporine 0.05% as immunosuppressive ocular inflammatory regulator of immune cell activation and generation, suppressing lacrimal gland inflammation. The university is applying nanotechnology in order to improve solubility and absorption rate. Conventional cyclosporine is normally formulated as an opaque non-uniformed particle size compound. Haporine-S eye drops are divided to a great number of clear colorless nano-sized particles which are evenly distributed, improving cyclosporine usage and reducing the side effects. This compound represents a new tendency of the pharmaceutical industry. Nanoparticle medicine has experienced substantial growth in the past half decade. The key benefits of the technology include, among others, improved biocompatibility, increased absorption rate, dose reduction, faster compound formulation and increased performance through variable administration routes. According to Hanyang University, Haporine-S normalizes the lacrimal gland, increasing tear production. The compound entered Phase III trials in 2013 (ClinicalTrials.gov Identifier: NCT01804361) to determine efficacy and safety of topical Haporine-S versus Restasis® eye drops, on 90 patients with moderate-to-severe dry eye disease applying 1 or 2 drops twice a day at 12-h interval for 12 weeks.

2.1.2.2.3 Lifitegrast (SAR 1118)

SARcode Bioscience is developing a synthetic lymphocyte functional antigen-1 (LFA-1) antagonist as eye anti-inflammatory (**Table 1** Figure 6 and **Table 2**) [63]. Lifitegrast (SAR1118) is a small-molecule integrin (ICAM-1) antagonist

mediating the chronic inflammatory cascade associated with dry eye disease. It inhibits T-cell receptor inflammation while blocking the binding of two key surface proteins LFA-1 and ICAM-1 [64]. Phase II results of SAR1118 (0.1%, 1.0% 5.0%) eye drops indicate significant improvements ($p < 0.05$) in corneal staining score, total ocular surface disease index (OSDI) and visual-related function OSDI scores. Adverse events were mild and transient in nature [65]. A Phase III randomized, double-blind, safety study (ClinicalTrials.gov Identifier: NCT01636206) on 300 patients was started in February 2013. On the other hand, the University of Colorado developed a type of sustained-release microspheres as a vehicle to deliver SAR 1118, intended for the treatment of eye vascular complications. According to the university, the microspheres showed a high loading efficiency, low burst release and a sustained release duration of up to 6 months [66].

2.1.2.3 *Inhibitors of mRNA translation*

2.1.2.3.1 *Azithromycin and polycarbophil*

Azithromycin (**Table 1** Figure 7 and **Table 2**) is an azalide that binds to the 50S ribosomal subunit of susceptible microorganisms and interferes with microbial protein synthesis, and apart from antibiotic properties, it also demonstrates anti-inflammatory and immunomodulatory capability [67]. Further, azithromycin is considered as an emerging treatment option for MGD [68,69]. Merck/Inspire Pharmaceuticals, Inc. is evaluating a combination of azithromycin ophthalmic solution and 1% polycarbophil. Polycarbophil is an aqueous mucoadhesive polymer contained in DuraSite® that permits azithromycin to deliver high and prolonged concentrations in a variety of ocular tissues, including conjunctiva, cornea and particularly the eyelid. FDA approved the azithromycin–polycarbophil compound, in 2007, for the treatment of bacterial conjunctivitis [70,71]. This is another example of a second use of an existing drug, demonstrating interesting results especially on contact lens wearers. It is important to notice that the polycarbophil vehicle is very viscous and, when applied on the ocular surface, produces a foreign object sensation lasting for some minutes, thus complicating the treatment. Merck conducted a Phase IV study in October 2009 (ClinicalTrials.gov Identifier: NCT01014078) for azithromycin ophthalmic solution 1% on 112 dry eye patients and in April 2010 another Phase IV study (ClinicalTrials.gov Identifier: NCT01105624) of 1% azithromycin ophthalmic solution versus rewetting drops on 50 patients suffering from contact lens-related dry eye was conducted. The drug was well tolerated and resulted in a significant improvement in comfortable contact lens wear time in the patients [71,72].

2.1.2.4 *Inhibitors of hemozoin biocrystallization*

2.1.2.4.1 *Hydroxychloroquine*

The Hospital Publique-Hôpitaux de Paris in collaboration with Sanofi-Aventis is developing a compound containing hydroxychloroquine (HCQ) (**Table 1** Figure 8 and **Table 2**) for the treatment of dry eye disease. HCQ has been already used for many years to treat malaria and various other diseases. It probably works by blocking the Toll-like receptors on plasmacytoid dendritic cells, decreasing their signaling and the inflammatory process [73]. Sanofi-Aventis conducted a Phase III (ClinicalTrials.gov Identifier: NCT00632866) study from February 2008 to July 2012 to evaluate the use of HCQ for the treatment of dry eye associated with Sjögren's syndrome. The results demonstrated that the patients with dry eye symptoms and α-fodrin antibodies had a significant increase in tear production ($p = 0.001$) [74]. The estimated enrollment for the study was 120 patients who received 200 mg/day of HCQ for 24 weeks. Response is defined by the improvement of ≥ 30%on at least two out of three of the following visual analog scales: most disabling dryness, fatigue and pain. Additionally, Yavuz *et al*. suggested that HCQ may alleviate symptoms and signs of dry eye in patients with primary Sjögren's syndrome by decreasing the tear fluid B-cell activating factor levels [75]. In contrast with the positive results, it is reported that HCQ causes severe side effects, such as toxic retinopathy and toxic maculopathy. It is also necessary to bear in mind that it may produce other side effects such as nausea, stomach cramps, loss of appetite, diarrhea, dizziness or headache [76,77].

2.1.2.5 *Immune system B cells antibodies*

2.1.2.5.1 *Rituximab*

Rituximab, developed by IDEC Pharmaceuticals, is a chimeric mAb against B-lymphocyte antigen (CD20) that is found on immune system B cells surface. It was initially approved for treatment of patients with $CD20^+$ B-cell non-Hodgkin's lymphoma and later as drug for various autoimmune diseases, including Sjögren's syndrome (**Table 2**) [78]. Even though their role it is not fully understood, there is evidence that B cells contribute to the pathogenesis of Sjögren's syndrome [79]. The company in collaboration with University of Brest started Phase III clinical trials (ClinicalTrials.gov Identifier: NCT00740948), in 2009, to study the tolerance and efficacy of rituximab injection on Sjögren's disease patients. The results were not published, but in 2013, Gottenberg *et al*. in a similar clinical study confirmed the efficacy and tolerance of rituximab as a potential drug for the syndrome [80].

2.2 Mucin secretagogues

2.2.1 *MIM-D3*

Mimetogen Pharmaceuticals, Inc. is developing a mucin agonist called MIM-D3, which is a small molecule mimicking the nerve growth factor (NGF) activity (**Table 1** Figure 9 and **Table 2**). NGF stimulates the conjunctival cell glycoconjugate secretion, and during *in vivo* trials it demonstrated therapeutic efficacy on dry eye disease [81]. NGF plays an important role in ocular surface maintenance, corneal wound healing, displaying a mucin secretagogue activity in conjunctival cells [82]. The use of compounds promoting mucus production and release is important, in particular when there is

a lack of mucin production because these compounds have a tendency to revert dryness condition. Jain *et al*. during evaluation studies demonstrated that MIM-D3 1% increased the glycoconjugate concentration, improving the quality and stability of the tear film and the corneal healing [83]. Mimetogen conducted Phase II safety and efficacy studies (ClinicalTrials.gov Identifier: NCT01257607) from December 2010 to October 2012 and the results demonstrated that treatment with topical ophthalmic 1% and 5% MIM-D3 reduced patient-reported diary symptoms, with a favorable safety profile. Especially the 1% MIM-D3 application resulted in significant improvements versus placebo ($p < 0.05$) in inferior fluorescein and lissamine green staining after 14 and 28 days. The adverse events were assessed as mild [84]. In October 2013, the company started Phase III clinical trials (ClinicalTrials.gov Identifier: NCT01960010) of 1% MIM-D3 ophthalmic solution.

2.2.2 *Rebamipide (OPC-12759)*

Acucela, Inc. and Otsuka Pharmaceutical, Inc. are evaluating a rebamipide-based solution for treatment of patients with dry eye. Rebamipide is an amino acid derivative of 2-(1H)-quinolinone (**Table 1** Figure 10 and **Table 2**) known for its ability to protect mucosa [85,86]. Urashima *et al.* reported that the compound increases mucin levels in the tear film covering the conjunctiva and cornea [87]. It acts by improving the mucosal defenses, scavenging free radicals and temporarily activating genes encoding COX-2. In 2012, OPC-12759 was commercialized in Japan for the treatment of dry eye disease [88]. Phase II clinical trials revealed that OPC-12759 was effective in terms of change from baseline to last observation carried forward (LOCF) for fluorescein corneal staining (FCS), lissamine green conjunctival staining (LGCS) and TBUT scores, with no significant response in baseline to LOCF in Schirmer's test values. No serious adverse events were reported except for an observed photophobia. According to the results, OPC-12759 was effective and well tolerated in this 4-week study, with 2% rebamipide doses proving more effective than 1% doses [89]. In a 2009 Phase III confirmatory trial (ClinicalTrials.gov Identifier: NCT00885079) to compare OPC-12759 ophthalmic suspension 2% with hyaluronate ophthalmic solution 0.1%, a change in LGCS score from baseline -4.5 ± 3.2 for OPC-12759 against -2.4 ± 2.5 for hyaluronate was obtained with no serious adverse events detected. In 2012, the two companies announced a Phase III (ClinicalTrials.gov Identifier: NCT01632137) clinical study to determine the efficacy and safety of 2% rebamipide ophthalmic suspension in subjects with dry eye disease with the instillation of one drop into each eye four times a day for 4 weeks. The study has been completed but the results have not been published.

2.2.3 *Ecabet sodium*

ISTA Pharmaceuticals is developing a mucous secretion stimulant of goblet and epithelial cells, called ecabet (**Table 1** Figure 11 and **Table 2**). Sulfodehydroabietic acid monosodium salt pentahydrate or ecabet sodium represents a new class of small diffusible molecules capable of increasing the quantity and quality of mucin produced by conjunctival goblet cells and corneal epithelia [90]. This ability makes the compound quite interesting as treatment for mucodeficient dry eye because it effects on basal tear secretion, which is one of the main factors in the etiology of the disease. Ecabet probably targets the prostaglandin E2 pathway, inhibiting the pepsin formation, increasing blood flow and downregulating the reactive oxygen species on the ocular surface [91]. In Japan, it is marketed by Senju Pharmaceutical as an oral agent for gastric ulcers and gastritis treatment [92]. Bausch & Lomb organized, from April 2005 to January 2013, Phase III efficacy and safety studies of ecabet ophthalmic solutions 2.83% and 3.70% (ClinicalTrials.gov Identifier: NCT00198536) in 159 patients. According to ISTA, the analysis of responders from anesthetized Schirmer's test revealed a strong trend for ecabet sodium, which approached statistical significance versus placebo. There were not reported serious ocular or systemic adverse events. The compound is still pending FDA approval for its use in the USA market.

2.2.4 *Diquafosol tetrasodium*

Merck developed a formulation of diquafosol tetrasodium, a second-generation P2Y receptor agonist, for the treatment of dry eye disease. Diquafosol tetrasodium (Up_4U, INS365, DE-089) (**Table 1** Figure 12 and **Table 2**) is a uridine nucleotide analog of $P2Y_2$ purinergic receptor agonist, working through the rehydration, stimulating the fluid and mucin secretion of the ocular surface [93-95]. The drug was designed by Inspire, Inc., in collaboration with Allergan and Santen, before its merging with Merck. It is validated through a series of clinical trials, the most recent being performed by Santen (ClinicalTrials.gov Identifier: NCT01101984) on 400 patients and completed in November 2012. The studies demonstrated that topical diquafosol 0.1% was well tolerated and was superior to placebo, reducing corneal staining and relieving certain patient symptoms. Takamura *et al.* confirmed that diquafosol (3%) and sodium hyaluronate (SH) (0.1%) exhibit similar efficacy in improving FCS, whereas, diquafosol is superior in improving rose bengal staining scores [96]. Additionally, Kamiya *et al*. and Koh *et al*. suggested that when SH monotherapy was insufficient, diquafosol tetrasodium was effective in improving objective and subjective symptoms [97,98]. For the moment, diquafosol is not approved by FDA for patient use, as having failed to demonstrate statistically significant improvement as compared to placebo in its primary end point, during the trials. On the contrary, in April 2010, the Japanese Ministry of Health, Labor and Welfare granted approval for diquafosol tetrasodium 3% as a dry eye ophthalmic solution [99]. Diquafosol tetrasodium is a remarkable compound. In clear difference with other drops quoted in this article, apart from being tear secretagogue, it stimulates mucin secretion. Compounds like this should be a reference for dry eye treatment considering that in many cases dry eye

presents not only a decrease in tear volume but also deficiencies in mucin content.

2.3 Artificial tears and reepithelialization agents

2.3.1 *Carboxymethyl cellulose and hydroxypropyl methylcellulose*

Allergan developed ophthalmic drops, containing carboxymethyl cellulose (CMC) 0.5% and glycerin (0.9%) as active ingredients (**Table 1** Figure 13 and **Table 2**). The drops contain as well interesting inactive ingredients such as levocarnitine (L-carnitine) and erythritol. The combination of glycerol, erythritol and L-carnitine claim an osmoprotective role as hyperosmolarity causes cell damage and nerve stimulation, triggering inflammatory cascades [100]. CMC or carmellose, a high molecular weight polysaccharide is one of the most common viscous polymers used in artificial tears to allow them to achieve prolonged residence time on the ocular surface [101]. CMC is well known for its mucoadhesive and viscous properties having the ability to bind on corneal epithelial cells and stimulate epithelial cell migration through its binding to matrix proteins. The enhancement of cell attachment to the matrix suggests CMC as a potential reepithelialization agent for clinical use [102]. Between 2009 and 2012, Optometric Technology Group Ltd (London), in collaboration with Allergan conducted a safety and efficacy, Phase III, randomized study, comparing osmoprotective CMC (OsPr-CMC) with SH in dry eye patients (ClinicalTrials.gov Identifier: NCT00987727). A total of 82 patients received OsPr-CMC or SH artificial tears. From the results, the efficacy of OsPr-CMC and the viability of osmoprotection were verified. The primary efficacy analysis was per protocol and there were noticed improvements in tear osmolarity, Schirmer's-I test score, OSDI and ocular staining. The majority of the patients preferred OsPr-CMC compared to SH because their eyes felt comfortable, considering the treatment as easy to use. Both treatments were well tolerated, with no serious treatment-related adverse events [103]. In October 2012, OsPr-CMC was released by adding castor oil to carmellose and glycerol, mimicking the trilaminar nature of the natural lipid layer, offering OsPr-CMC the advantage of lipid enhancement. Allergan started an observational study of the new drug for the treatment of dry eye disease (ClinicalTrials.gov Identifier: NCT01711424) with the recruitment of 2000 patients and was completed in February 2013. Additionally, Allergan is developing another eye lubricant containing hydroxypropyl methylcellulose (2-hydroxypropyl cellulose ether and cellulose methyl ether) which is used as a component in contact lens wetting solutions. In April 2011, the company started Phase III trials (ClinicalTrials.gov Identifier: NCT013 35126) to evaluate the tolerability and clinical performance of emulsion-type artificial tears containing hydroxypropyl methylcellulose. In both eye solutions mentioned above, an improvement in comfort and a growth in tear stability was reported but a statistically significant increase in tear turnover rate was not demonstrated [104,105].

2.3.2 *Sodium hyaluronate*

River Plate Biotechnology is evaluating SH 0.18% in an ophthalmic solution for the treatment of moderate dry eye disease (**Table 1** Figure 14 and **Table 2**). The compound has been marketed in Europe as a viscoelastic lubricant eye drop. This is an example of a compound which was originally designed as a lubricant but which showed interesting anti-dry eye properties including anti-inflammatory, wound healing and water-retention actions [106]. The point now is to discover which mechanism is responsible for these positive properties. There are various reports confirming the efficacy of SH to manage dry eye and supporting its well-known safety profile [107,108]. Additionally, Aragona *et al*. reported that SH may improve ocular surface damage caused by dry eye [109]. Alcon Laboratories realized a Phase III trial to evaluate its efficacy and safety from June 2011 to June 2012 (ClinicalTrials.gov Identifier: NCT01382225). A total of 304 patients were instilled one or two drops of SH 0.18% in each eye, two to four times per day for over 84 days. The compound caused a statistically significant improvement in a subjective end point (symptom frequency score) and an objective end point (FCS) and it was well tolerated resulting in low incidence of adverse events [110]. In addition, Dumbleton *et al*. have demonstrated a greater efficacy of SH in the improvement of dry eye disease symptoms than CMC [111].

2.3.3 *PRO-148*

The Mexican company Laboratorios Sophia is developing the sterile artificial tear solution PRO-148 as an ophthalmic combination diminishing the loss of topically administered drug on the ocular surface. PRO-148 contains a polyanion (chondroitin sulfate) (**Table 1** Figure 15) and xanthan gum (**Table 2**). Chondroitin sulfate is a major component of articular cartilage, affecting the mobility and flexibility of the joints in a decisive way [112]. Zorzi *et al*. restored mucin concentration on the ocular surface, using hybrid cationized gelatin nanoparticles containing chondroitin sulfate to transfect ocular epithelial cells [113]. After Phase II clinical trials (ClinicalTrials.gov Identifier: NCT01541891), the company announced that PRO-148 can improve dry eye disease clinical signs and symptoms and promote epithelial repair. The results demonstrated a significant reduction in the mean scores for the ocular symptoms of dryness, gritty/sandy feeling and burning [114]. In 2013, the company started Phase III clinical trials (ClinicalTrials.gov Identifier: NCT01657253) to evaluate the efficacy and safety of PRO-148 versus polyethylene glycol 400 0.4% and propylene glycol 0.3%.

2.3.4 *Linseed extract and polyvinylpyrrolidone (T-Clair SPHP700-3)*

SPHP700-3 is a long-chain polysaccharide combined with a polymer, designed by Sinclair Pharmaceuticals, Inc., for dry

eye treatment (**Table 2**). It is already approved as medical device class I (sterile) in EU and its status is under review in the USA by FDA. SPHP700-3 is a preservative-free formulation of linseed extract and polyvinylpyrrolidone. Soluble polymers are used to increase viscosity, binding large quantities of water and delivering moisture to the eye surface, since the regulation of their concentrations is increasing the duration of the effect [115]. Published Phase III results demonstrated that the compound is safe and effective in mild-to-moderate dry eye. Additionally the compound recovers tear film stability, ocular surface lubrication, improves overall dry eye symptoms and is compatible with contact lens use. No severe adverse events were reported [116].

3. Conclusion

Developing a new drug for the treatment of dry eye has become a key target for the pharmaceutical industry. Even though dry eye is the most widespread eye disorder, is not life-threatening and its symptoms are not severe, there is limited offer of effective therapeutic agents. Dozens of companies have placed under development a vast variety of new drug candidates in their pharmaceutical pipelines, but the overarching complexity of the disease makes it challenging to manage.

Most of these drugs have already passed safety tests and have been approved as treatment for other diseases. It is well known that identical biological pathways can be active in different ways in various diseases. Discovering new uses for old drugs can ensure important financial benefits, for both patients and biotechnology companies, by lowering costs and shortening approval timelines. Earlier treatments were usually proved ineffective as it intended to address the symptoms rather than the cause of dry eye, and the majority of the compounds were mere tear substitutes. The compounds analyzed in the article center their action mainly toward controlling inflammation or restoring the normal amount of tears and mucins but, again, are vaguely addressing the causative mechanisms of dry eye. This is due to the fact that the etiology of the disease is very complex, and almost all eye components such as cornea, conjunctiva, meibomian glands, goblet cells and lacrimal glands are linked to tear secretion and any malfunction of the components affects the remaining. The inflammation of these components leads to apoptosis of the tear-producing epithelial cells in the lacrimal glands, diminishing tear production.

4. Expert opinion

As it was mentioned above, dry eye disease presents a great variability of clinical signs and symptoms, frequently not correlated. End points used in dry eye disease clinical trials are also variable, hampering the comparison between different treatments. It is believed that the development and inclusion of improved objective methods in dry eye disease clinical trials can simplify the analysis of results obtained from diverse treatments.

In the article, some compounds stimulating mucin secretion, most of which have derived from older drugs for other diseases, were presented. There is one drug that promotes mucin and water production and a variety of new eye polyvalent lubricants. From the lubricants presented, it is concluded that recent developments in the research of tear film physiology and pathology made possible the advancement in a variety of tear substitutes capable of addressing specific deficiencies of tear components. Although the use of artificial tears can improve dry eye symptoms, there is no evidence that they can resolve the underlying conditions producing the corneal and conjunctival inflammation. In most patients, the application of artificial tears alone is not sufficient and an anti-inflammatory therapy is required to target the inflammatory component of the pathology. There are many opinions about the eye inflammation and especially if the inflammation is occurring in the beginning, initiating the disease or in the middle as a consequence of the evolution of the events. In any case, inflammation damages ocular structures intensifying the signs and symptoms; therefore, the current tendency is toward addressing the underlying immune-based inflammation-restoring neuronal control. The anti-inflammatory compounds mentioned above include steroids, non-steroids immunomodulatory and antibiotic agents which have also shown promising results in dry eye therapy. Most of the anti-inflammatories under development are corticosteroids because of their noteworthy anti-inflammatory properties and rapid action. The steroids mentioned are working through the increase of goblet cell density, reducing the inflammatory cells on ocular surface, manipulating the ocular surface NGF, blocking prostaglandin synthesis or inhibiting T-cell receptors. Normally they are used for a limited period of time generally limited to 2 – 4 weeks, depending on the severity of the case, but when the treatment pauses the patient frequently returns to the initial inflammatory condition, even though in some patients an increase in the density of conjunctiva goblet cell is noticed. Also, it is necessary to bear in mind that steroidal compounds present important side effects, such as increase in intraocular pressure and posterior subcapsular cataract [117] making them unsuitable for chronic dry eye therapy. Nevertheless, there are steroids with an acceptable safety profile as it is quickly metabolized to inactive metabolites [118]. Another important factor is the penetration in the eye tissues. For example, dexamethasone is very potent but has low penetration ability even though presenting a strong side-effect profile. In such a case, the iontophoresis method proposed (EGP-437) looks like a promising solution.

On the other hand, immunosuppressant compounds, NSAIDs and antibiotics are improving dry eye signs and symptoms, and they are safer than corticosteroids for long-term use, even though they cannot demonstrate the rapid anti-inflammatory effect of steroids. CsA is the first FDA-approved

drug for the treatment of dry eye disease and at least two NSAIDs are under development in ISTA Pharmaceuticals and InSite Vision, combining high-level penetration and potency with lower dosage. They are effective and comparatively well-tolerated in the treatment of dry eye disease but the uncertainty of their long-term results and their high therapy cost could be included in their limitations. Consequently, the use of softer steroids is suggested to avoid side effects such as elevation of the intraocular pressure. Depending on the severity of the disease, for mild-to-moderate symptoms, future treatment could include eye lubricants, CsA, mucin secretagogues or soft topical steroids. For severe cases, an aggressive therapy would be needed, combining topical steroids with oral cyclosporine or other types of anti-inflammatories. The addition of novel multipurpose eye lubricants seems to be a worthy solution to achieve faster, prolonged and effective results.

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Declaration of interest

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Artículo 2 sobre el tratamiento de Ojo Seco

REVISIÓN

Dry eye disease compounds currently under evaluation in clinical trials

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ABSTRACT

Dry eye syndrome is a common disorder provoking changes in tear quantity and composition being the most common ophthalmic manifestation of systemic inflammatory diseases. Untreated dry eye could cause increased risk of ocular infection, corneal ulcer and blindness. Only a few drugs are authorized so far for the treatment of dry eye disease and the possibilities of evolution in this sector are immense. Accordingly, the future tendency is towards the development of drugs to control the inflammation or stimulate the mucin and tear secretion. A significant number of new potential solutions are under development or placed in the pharmaceutical pipeline, promising better results and lesser side effects, but still leaving unattended the main causes of the disease. In this article, we review the corresponding literature, recent clinical trials data and patents concerning future pharmaceutical compounds for dry eye disease treatment, presenting the new strategic movements of the pharmaceutical industry.

Keywords: Dry eye; anti-inflammatory; keratoconjunctivitis sicca; keratitis sicca; xerophthalmia; mucin secretion; tear secretion; NSAID.

RESUMEN

Fármacos de la enfermedad de ojo seco actualmente en evaluación de ensayos clínicos

El síndrome de ojo seco es un trastorno bastante común, provocando cambios en la cantidad y la composición de la lágrima, siendo la manifestación oftálmica más común de enfermedades inflamatorias sistémicas. El ojo seco no tratado puede provocar un aumento del riesgo de infección ocular, úlceras corneales y en casos extremos ceguera. Actualmente sólo unos pocos fármacos están autorizados para el tratamiento de la enfermedad y las posibilidades de evolución en este sector son inmensas. La tendencia del futuro está dirigida hacia el desarrollo de fármacos que controlan la inflamación o estimulan la secreción de las mucinas y de las lágrimas. Un número significativo de nuevos compuestos están en desarrollo o ya están en ensayos clínicos, con aparentes mejores resultados y efectos secundarios menos adversos, pero una vez más dejando desatendidas las principales causas de la enfermedad. En este artículo revisamos la literatura correspondiente, los últimos datos de los ensayos clínicos y las patentes relativas a futuros compuestos farmacéuticos para el tratamiento de la enfermedad del ojo seco, desvelando los nuevos movimientos estratégicos de la industria farmacéutica.

Palabras clave: Ojo seco, anti-inflamatorios, queratoconjuntivitis sicca, queratitis seca, xeroftalmia, secreción de mucinas; secreción de lágrima; antiinflamatorios no esteroideos.

INTRODUCTION

Dry eye disease is a chronic ocular disorder affecting approximately 10-20% of the population worldwide, being relatively frequent among women especially after the menopause [1, 2]. This pathology is closely related to the aging and it is progressively affecting larger parts of the population. Almost everyone experiences ocular irritation or the symptoms of dry eye under certain conditions, such as prolonged working on a computer, being in a dry environment or after using certain drugs [3-5]. Patients with autoimmune diseases are prone to suffer from dry eye inflammation, which is a key factor in the onset of severe dry eye disease [6].

The pathology occurs when the ocular surface tear protective layer is weakened which could be the result of insufficient or atypical production of one or more tear components [7]. Tear film forms a moist natural barrier separating eye from the external media. This consistent film is formed mainly from a triplet of

aqueous, mucous, and lipid layers providing the necessary equilibrium for the healthy maintenance of the ocular surface (Figure 1). The main functions of this film are: lubrication of the ocular surface, transfer of nutritional elements to the cornea, elimination of foreign matter and cellular debris generated on the ocular surface by the tear flow and the blink process, and acting as the first line of defense against ocular surface infections [8]. In some cases, the imbalance of the tear film composite layers leads to reduced tear secretion, exposure of the eye surface, dryness and damage of the surface cells [6]. The imbalance of the composite layers could be caused by Sjögren's syndrome tear secretion deficiency and/or tear film instability due to the use of contact lenses [6, 9, 10]. Dry eye syndrome types are classified according to etiologic perspectives and environmental influences as: aqueous deficient dry eye (ADDE) and evaporative-tear dry eye (EDE) [6, 11]. ADDE is divided to: related to Sjögren's syndrome (primary and secondary) and non-related to Sjögren's syndrome conditions (lacrimal disease, lacrimal obstruction and malfunctioning blinking reflex) [6]. EDE conditions are: oil deficient, lid related and caused by an ocular surface alteration [6]. Signs and symptoms of these complications can vary from patient to patient, sometimes with little or no correlation between them, even though they are generally related to the tear film composition. The signs and symptoms could include keratitis, conjunctival and corneal staining, redness, blurry vision, decreased tear film break-up time, decreased tear production, volume, and flow, increased conjunctival redness, excess debris in the tear film, ocular dryness, ocular grittiness, ocular burning, foreign body sensation in the eye, excess tearing, photophobia, ocular stinging, refractive impairment, ocular sensitivity, and ocular irritation [12, 13].

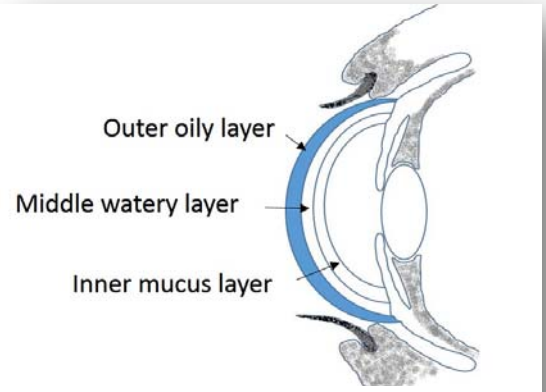

Figure 1.- Tear film layers

The clinical diagnosis of dry eye is challenging since dry eye is a pathology characterized by an extensive variety of signs and symptoms, mentioned above, related to ocular dryness and the ambiguity in the etiology and pathophysiology of the disease is contributing to the difficulty of a precise diagnosis [14]. Conventional tests include Schirmer test, tear break-up time (TBUT) and ocular surface staining,

some of them considered as invasive and having a low degree of standardization. Another negative factor is the lack of knowledge on the pathophysiology of the disease and the unclear symptoms which could be confused with the symptoms of other conditions , such as conjunctivochalasis (which can easily induce an unstable tear film) or delayed tear clearance (which is a frequent cause of ocular irritation) [15]. Other negative factors are the invasive nature of some of the diagnostic tests, which can make the interpretation of the results challenging or the use of the individual tests in very dissimilar scenarios, suggesting the application of different protocols. Nevertheless, tear film is a dynamic, open system subject to numerous internal and environmental variations, leading frequently to misinterpretations of the obtained results [16]. In conclusion the variety of causative agents and the high number of ocular conditions with similar signs and symptoms make an accurate, differential diagnosis difficult, especially due to lack of correlation between signs and symptoms. Accurate testing and diagnosis of dry eye is crucial to the correct management of the condition and it is advisable the use of combinations of tests and sequences. Consequently, the creation of objective tests with precise diagnostic value is essential. The International Dry Eye Workshop (DEWS) diagnosis subcommittee classifies tests into five fundamental functional groups: Questionnaires, ocular surface staining tests, tear film stability tests, tear volume tests and tests to measure biological components [2].

Currently there are only a few pharmaceutical treatments for dry eye. In some countries like Japan, are commercialized more DED treatment drugs than in Europe and the USA as the approval rules are not so rigorous. Another important factor concerning DED treatment is that in most of the cases, the biotechnology companies are trying to sort out the problem based on “over the counter” strategies, trying to relieve symptoms rather than getting to the cause of the disease. Consequently, for the symptomatic relief of dry eye we could mention the following treatments currently used: Supplements called “artificial tears” which are synthetic lubricants, characterized by hypotonic or isotonic buffered solutions containing electrolytes, surfactants and several types of viscosity agents [17]. The “artificial tears” permit not only the increase of tear quantity, but also to keep the ocular surface moistened and relieve discomfort. Another type of treatment is the tear retention devices/implants also known as punctal plugs. They have been developed to occlude the lacrimal puncta and they can be absorbable and non-absorbable [18]. These devices keep tears longer on the ocular surface relieving the patient’s from the undesirable symptoms. Other type of treatment is the moisture chamber

spectacles. It has been reported that increased periocular humidity can cause the tear film lipid layer to thicken and that spectacle wearers with dry eye have a longer inter-blink interval than the non-spectacle wearers [19]. Current treatments are mainly focused on addressing inflammation and tear restoration [20]. Dry eye disease is the outcome of many factors resulting in inflammation of cornea and conjunctiva. The dysfunction of the tear secretory glands leads to changes in tear composition such as hyper-osmolarity which stimulates the production of inflammatory mediators on the ocular surface. This inflammation can be initiated either by chronic irritative stress like wearing contact lens wearing or from a systemic inflammatory autoimmune disease like rheumatoid arthritis [21, 22]. Anti-inflammatory drugs are widely used for the treatment of the inflammation produced by diseases. Topical corticosteroids can relieve moderate or severe dry eye symptoms and signs rapidly and effectively [23]. Steroids on the other hand may cause severe side effects after prolonged use. The undesired effects vary from bacterial or fungal infection, elevated intraocular pressure and cataract formation. Additionally, steroids suppress locally the immune response in patients with already compromised ocular surface. Therefore, steroids are typically used only for a limited period of time in dry eye patients [20]. Due to the above-mentioned reasons, non-steroidal anti-inflammatory drugs (NSAID) credited as causing less severe side effects are recently evaluated as a potential dry eye treatment. The NSAIDs could decrease inflammation and eye discomfort due to their analgesic effect, but they might induce DED decreasing sensitivity. In 2002 U.S. Food and Drug Administration (FDA) approved the drug *RESTASIS*® of the company Allergan as the first prescription medicine capable to increase tear production [24]. Topical *RESTASIS*® is an ophthalmic emulsion containing cyclosporine 0.05%. Other types of drug used are the antibiotics like azithromycin, and tetracycline. Furthermore, some research groups are studying the use of serum tears and the intense pulse light as potential treatments [20]. On the following pages, we present some future treatments currently passing clinical trials.

AL-2178/RIMEXOLONE

AL-2178/Rimexolone 1% (Figure 2) is a glucocorticoid steroid developed by Alcon, as a treatment for dry eye disease. Rimexolone inhibits T-cell proliferation as well as cytokine production of activated CD4+ T-cells in a similar manner to dexamethasone [25]. The compound passed successfully from Phase III clinical trials (ClinicalTrials.gov Identifier: NCT00471419) in May 2007 and now is

marketed as a 1% eye drop suspension under the trade name Vexol by Alcon Laboratories [26].

LX214/VOCLOSPORIN

LX214-voclosporin (Figure 3) is an immunosuppressant drug developed by Isotechnika and Lux Biosciences. Voclosporin is a calcineurin inhibitor, analog of cyclosporin with enhanced action against calcineurin and a greater metabolic stability. It is already used as a treatment of various forms of non-infectious uveitis [27]. The two companies are evaluating voclosporin ophthalmic solution as a treatment for dry eye disease in a Phase I clinical trial (ClinicalTrials.gov Identifier: NCT00851734), but did not publish results over its efficacy [28].

TOFACITINIB

Pfizer Inc. developed a JAK inhibitor called *Tofacitinib* (formerly CP-690550 - *Tasocitinib*) (Figure 4) capable to interfere the JAK-STAT signaling pathway, which transmits extracellular information into the cell nucleus, influencing DNA transcription [29]. The company concluded phase I/II trials (ClinicalTrials.gov Identifier: NCT00784719) in patients for various disorders including dry eye disease. The trials demonstrated improvement in the signs and symptoms on dry eye patients and the use of the drug was well tolerated and without severe side effects [30]. Huang and colleagues conducted a study to 82 patients with moderate to severe dry eye disease, treated for eight weeks with 0.0001% to 0.005% *tofacitinib* once or twice daily. The patients showed reduction in conjunctival cell surface HLA-DR expression, tear levels of pro-inflammatory cytokines and inflammation markers. The markers in tears included MMP-9, IL-15, IL-17A, and IL-12p70 [31].

NUTRILARM®-OMEGA-3 AND OMEGA 6 FATTY ACIDS-T1675

Omega-3 and omega-6 essential fatty acids (EFA) are the origin of a family of compounds called eicosanoids. Eicosanoids are signaling molecules produced by the oxidation of 20-carbon fatty acids and they are mediators of various inflammatory processes. In general, the omega-3 derived compounds, systemic linoleic (LA) and gamma-linolenic acid (GLA) are reducing eye inflammation, and n-6 pathway eicosanoids are considered as promoting inflammation [32, 33]. Omega-3 and omega-6 EFAs are used for dry eye treatment due to their anti-inflammatory effects

such as the drug *Nutrilarm*® of the French company Théa Laboratoires, which is already commercialized in Europe. This medicine uses a supplementation of omega-3 and omega-6 fatty acids, which according to the company reduce expression of the conjunctival inflammatory marker human leucocyte antigen-DR (HLA-DR) and may help improving DED symptoms. The compound past in 2006 from Phase II clinical trials in US (ClinicalTrials.gov Identifier: NCT00357201) but the company decided to continue the pre-clinical development to resolve efficacy and doses issues [34-36].

ORAL OMEGA-3-ACID ETHYLESTERS

Pennsylvania State University and the American Society of Cataract and Refractive Surgery Foundation (ClinicalTrials.gov Identifier: NCT01107964) started in April 2010 a clinical study, based on the hypothesis that oral omega-3-acid ethyl esters (Lovaza, GlaxoSmithKline) will decrease the dry-eye related symptoms as well as clinical markers associated to dry eye disease. The preliminary results of the study are positive but more research is needed to define the composition and dosing of the treatment. From the results it was confirmed the relationship between EFA supplementation and improvement in dry eye symptoms in patients [37]. Nevertheless before any anti-inflammatory therapy is necessary a detailed examination of a patient due to the complexity of the disease and to provide individual and successful treatment [38].

SECUKINUMAB AND CANAKINUMAB

Novartis Pharmaceuticals Inc. presented two novel antibodies, Secukinumab (AIN457) and Canakinumab (ACZ885). Secukinumab is a fully human monoclonal antibody designed to bind selectively, inhibiting the member A of interleukin 17cytokine family, a key driver of immune-mediated diseases. Canakinumab is a fully human monoclonal antibody, inhibiting solely interleukin 1β (IL-1β), the form of the interleukin-1 protein that is the main cause of various inflammatory diseases [39]. Currently both antibodies are under development as treatments of a wide range of auto inflammatory diseases. In November 2010 Novartis started a Phase II study on effects of a single intravenous administration of Secukinumab 10 mg/kg or Canakinumab 10 mg/kg on 72 dry eye patients (ClinicalTrials.gov Identifier: NCT01250171). The study reports suggest that both substances are well tolerated in most patients, and no serious adverse effects have been reported. The drugs

provide significant advantages over existing competitive therapies, including bimonthly administration and an approved use in children [40-42].

MIM-D3

Mimetogen Pharmaceuticals Inc. is developing MIM-D3, a small molecule mimicking the nerve growth factor (NGF) activity (Figure 5). NGF stimulates the conjunctival cell glycoconjugate secretion and during *in vivo* trials demonstrated therapeutic efficacy on dry eye disease [43]. NGF plays an important role in ocular surface maintenance, corneal wound healing, displaying a mucin secretagogue activity in conjunctival cells [44]. Jain and colleagues during evaluation studies demonstrated that a 1% MIM-D3 dose increased glycoconjugate concentration, improving the quality and stability of tear film and the healing on the ocular surface in dry eye [45]. Mimetogen conducted Phase II safety and efficacy studies (ClinicalTrials.gov Identifier: NCT01257607) from December 2010 to October 2012, on 150 patients with the application of 1% MIM-D3 and 5% MIM-D3 for 28 days. Unfortunately the target primary endpoints were not met completely. Despite this negative development the company continues the development considering MIM-D3 as a potent compound [46].

DIFLUPREDNATE

Alcon is evaluating the corticosteroid Difluprednate-Durezol, a butyrate ester of 6(alpha),9(alpha)-difluoro prednisolone acetate as a potential treatment for dry eye disease (Figure 6) [47]. This corticosteroid is preventing the phospholipid release and decreases the eosinophil action. *Durezol* received approval from the FDA in 2008 as the first ophthalmic steroid for the postoperative inflammation and pain. Alcon started clinical trials Phase II (ClinicalTrials.gov Identifier: NCT01276223) in January 2011 for the Difluprednate ophthalmic emulsion 0.05%, one drop in each eye twice per day, on 726 patients, as an anti-inflammatory treatment of dry eye disease. Mulki and colleagues are suggesting that difluprednate 0.05% ophthalmic emulsion exhibits enhanced penetration, decent bioavailability, rapid local metabolism and strong efficacy, with a low incidence of adverse effects and a comparable safety profile [48].

CIS-UROCANIC ACID

LaurantisPharma Ltd and Kuopio University Hospital are developing eye drops containing Cis-urocanic acid (cis-UCA) as treatment for ocular surface inflammation associated with moderate to severe dry eye syndrome. Cis-urocanic acid inhibits the SAPK/JNK signaling pathway in ultraviolet B (UV-B) exposed human corneal epithelial cells *in vitro,* having important implications in the management of inflammatory eye conditions since chronic stress is directly linked to cytotoxicity in epithelial cells. During tests it was demonstrated that is inhibiting the secretion of pro-inflammatory cytokines from human conjunctival and corneal epithelial cells after UV-B-induced stress reaction [49, 50]. Preclinical studies in relevant animal models of eye inflammation also demonstrated that cis-UCA may be a safer and more effective alternative to current drugs used for acute and long-term treatment of dry eye. From November 2011 to May 2012 Laurantis conducted Phase I (ClinicalTrials.gov Identifier: NCT01476332), safety, tolerability and pharmacokinetics studies of 0.5% and 2.5% Cis-UCA eye drops in adult healthy volunteers. The results are not published, but the company continues the development of the compound.

THYMOSIN BETA 4

RegeneRx Biopharmaceuticals is developing RGN-259 which is a 0.1% Thymosin Beta 4 (Tβ4) ophthalmic solution as a novel therapeutic treatment for dry eye [51, 52]. Thymosin Beta 4 is a synthetic version of a naturally occurring peptide, used clinically as a tissue repair and regeneration peptide [53]. In human body is a protein encoded by the TMSB4X gene (GenBank accession number: NC_000023.10). During preclinical evaluations it was demonstrated that Tβ4 is promoting corneal epithelial intercellular adhesions following injury in animal models of dry eye [54]. In June 2011 RegeneRx started Phase II safety and efficacy studies (ClinicalTrials.gov Identifier: NCT01387347) of the RGN-259 ophthalmic solution in patients with dry eye syndrome. The study has been completed in December 2012 and in ARVO 2012 the company presented the results, claiming the statistically significant reduction in central corneal fluorescein staining from baseline compared to placebo and also a greater reduction in exacerbation of ocular discomfort [55].

BELIMUMAB

Belimumab is a human monoclonal antibody inhibiting B-cell activating factor (BAFF) and is developed by the University of Udine as a treatment for Sjögren's syndrome. Sjögren's syndrome is a systemic autoimmune disease

characterized by an increase in BAFF (BLyS) levels resulting in B cell hyperactivity. B cells are involved in the pathogenesis of SS in both systemic and glandular features, and B cell down regulation may lead to a decrease of disease activity. Moreover, pathogenesis of SS is closed to that of systemic lupus erythematosus, where *belimumab* has been proven to be effective. Currently the compound is under Phase II clinical trials evaluation (ClinicalTrials.gov Identifier: NCT01160666) and the initial results are promising [56].

RITUXIMAB

Rituximab (CD20) is developed by IDEC Pharmaceuticals as a treatment for Sjögren's syndrome. CD20 antigen is targeting cells of the B-cell lineage, leading to transient blood B-cell depletion, leaving aside stem cells [57]. *Rituximab* is already approved by the U.S. Food and Drug Administration in 1997 as a treatment of B-cell non-Hodgkin lymphomas resistant to other chemotherapy regimens. The compound currently is tested in Phase III clinical trials (ClinicalTrials.gov Identifier: NCT00740948) to study the tolerance and efficacy of rituximab injection in Sjögren's disease [58, 59].

LOTEMAX

Bausch & Lomb, Inc. is re-purposing the already approved drug *Lotemax* with topical cyclosporine ophthalmic emulsion 0.05% (*Restasis*) as a possible treatment of dry eye. *Lotemax* (loteprednoletabonate-LE) (Figure 7) [60] is an ester corticosteroid with a high therapeutic index, containing an ester rather than a ketone, at carbon-20 of the prednisolone core structure. LE is indicated for the treatment of steroid responsive inflammatory conditions associated to the palpebral and bulbar conjunctiva, cornea, and anterior segment of the globe. Its mechanism of action is to block the inflammatory mediators treating the inflammation associated with the dry eye disease. Cyclosporine A as already mentioned above is a standard molecule against inflammation with efficacy in dry eye disease, enhancing or restoring the lachrymal gland secretion in patients suffering from this syndrome [61]. An advantage of topical loteprednol pretreatment is the reduction of Cyclosporine stinging in chronic dry eye disease [62]. *Lotemax* ophthalmic suspension, 0.5% completed Phase II studies (ClinicalTrials.gov Identifier: NCT00560638) in July 2011. From the results it was confirmed its ability to reduce the eye inflammation rapidly, causing limited side

effects. Additionally in 2012 Wan and colleagues performed a study on 34 patients, confirming the efficacy of topical 0.5% loteprednoletabonate ophthalmic suspension for the treatment of moderate dry eye [63].

ECABET-SODIUM

Bausch & Lomb Inc. is developing a prescription eye drop for the treatment of dry eye syndrome called *Ecabet* (Accession Number: DB05265). Sulfodehydroabietic acid monosodium salt pentahydrate or ecabet sodium, represents a new class of small diffusible molecules capable of increasing the quantity and quality of mucin produced by conjunctival goblet cells and corneal epithelia (Figure 8) [64]. This ability makes the compound quite interesting as a treatment for muco-deficient dry eye. *Ecabet* possible mechanism of action is through the targeting of the prostaglandin E2 pathway, inhibiting the pepsin formation, increasing blood flow and downregulating the reactive oxygen species on the ocular surface. [65]. It is marketed in Japan by Senju Pharmaceutical as an oral agent for gastric ulcers and gastritis treatment [66]. Bausch & Lomb organized from April 2008 to January 2013 Phase II efficacy and safety studies for *Ecabet* ophthalmic solution with the purpose of treating the dry eye syndrome (ClinicalTrials.gov Identifier: NCT00667004) in 183 patients. Bausch & Lomb Inc. did not yet distribute results information.

BOL-303242-X

Pharmaceutical R&D, Bausch & Lomb Inc., presented the drug BOL-303242-X (mapracorat) (Figure 9) a selective glucocorticoid receptor agonist, as a possible treatment for inflammatory skin and eye diseases. This agonist is binding to the glucocorticoid receptor with an affinity similar to dexamethasone. Zhang and colleagues demonstrated during *in vitro* and *in vivo* studies that BOL-303242-X inhibited interleukin-1β (IL-1β) and induced decreases of inflammation in human corneal epithelial cells. It is possible that BOL-303242-X is acting as an anti-inflammatory agent in various primary human ocular cells with similar activity to classical steroids. As mechanism of action it is suggested its interference in human ocular cells MAPK (p38 and JNK) and NFκB signaling pathways [67]. Vollmer T.R. and colleagues confirmed that mapracorat exerts its anti-inflammatory effects, at least in part, by augmenting MAPK phosphatase-1 (MKP-1) expression [68]. The compound has an improved side-effect profile, compared to classical glucocorticoids. On the other hand farther research is necessary due to reports of

skin atrophy produced in some patients [69]. Bausch & Lomb performed Phase II studies on 350 patients, to assess the safety and efficacy of 0.3%, 2% BOL-303242-X in dry eye syndrome, (ClinicalTrials.gov Identifier: NCT01163643) from July 2010 to November 2012 but did not publish results.

AL43546

AL43546 is an ophthalmic solution developed by Alcon. It is consisted of hydroxypropyl guar galactomannan 0.15% or 0.25% (Figure 10). Guar galactomannan is used to add viscosity to artificial tears. In September 2008 Alcon started a Phase II clinical pharmacological study of AL-43546 ophthalmic compound in subjects with shortened tear film break up time, to study the lacrimal fluid condition chronologically after a single dose of AL-43546 ophthalmic solution (ClinicalTrials.gov Identifier: NCT00760045) [70]. The results were not published.

SIROLIMUS-RAPAMYCIN

Sirolimus is an antibiotic demonstrating immunosuppressive and anti-inflammatory properties. Its mechanism of action is to block T-cell activation and proliferation, to activate p70 S6 kinase and to inhibit significantly the production of Th1 cytokines (IFN γ, IL-2 and TNF) [71]. Its name is derived from a native word from Easter Island, Rapi Nui (Figure 11). Santen Pharmaceutical is working with subconjunctival injections of sirolimus as a mTOR inhibitor to treat dry eye [72]. It is reported that the activation of rapamycinmTOR signaling mediates nerve growth factor (NGF) inducing cell migration [73]. From December 2008 to July 2010, Santen realized a Phase II dose-ranging clinical study (ClinicalTrials.gov Identifier: NCT00814944) to assess the safety and efficacy of subconjunctival injection of sirolimus on 143 patients with dry eye in a controlled adverse environmental (CAE) model. The doses applied were 220, 440 and 880 micrograms of rapamycin. The results indicated that sirolimus is safe and tolerable with no systemic adverse events noted, demonstrating bioactivity as immunomodulatory and corticosteroid-sparing agent. It is proved capable to reduce vitreous haze and cells, improving visual acuity [74].

ISV-101

ISV-101 is a new drug of the company InSite Vision incorporating a low dose of the non-steroidal anti-inflammatory (NSAID) bromfenac (Figure 12) and a flow-

able mucoadhesive polymer of *DuraSite* technology [75]. This combination enables a slow release of bromfenac over a longer period of time. Consequently the eye aqueous humor is absorbing and retains a higher dose of bromfenac. The extension of the duration of drug residence on the surface of the eye enables better penetration, improving efficacy, safety and dosing. Initial data from previous clinical studies evaluating this combination demonstrated a favorable safety profile. The company started Phase II clinical trials (ClinicalTrials.gov Identifier: NCT01478555) in July 2012 on 150 patients to evaluate the safety, tolerability, and efficacy in topical administration of different dose regimens of the ISV-101.

ESBA-105

ESBATechdeveloped ESBA-105 a single-chain antibody fragment that targets tumor necrosis factor alpha (TNF-α) [76]. Selective inhibition of TNF-α has the potential of modulating the inflammatory and immune response. Preclinical studies demonstrated that topically administered ESBA105 attains therapeutic levels in both the anterior and posterior segments of the eye without a need of a penetration enhancer. Consequently, its drug penetration and ocular bio-distribution appear highly attractive for clinical use to treat TNF-α connected eye diseases [77]. Alcon after the fusion with ESBATech conducted Phase II clinical trials (ClinicalTrials.gov Identifier: NCT01338610) in April 2011 up to February 2012 to evaluate the efficacy of ESBA-105 10 mg/mL in 90 patients with severe dry eye experiencing persistent ocular discomfort. The results demonstrated that the compound topically applied penetrated into the anterior chamber of the human eye at therapeutic levels [78].

DA-6034

Dong-A Pharmaceutical Co., Ltd is developing a MMP-9 inhibitor called DA-6034, consisting of 7-carboxymethyloxy-3',4',5-trimethoxy flavone monohydrate (Figure 13) as possible treatment for dry eye. DA-6034 is a synthetic derivative of eupatilin, a pharmacologically active flavone, capable to increase secretion of mucin-like glycoprotein. It may increase as well the secretion of some mucin species in conjunctiva and cornea. Mucin is a glycoprotein lubricating component of tear film able to weaken moisture loss from tear evaporation. Choi and colleagues verified that DA-6034 at concentrations above 100 microM increased mucin-like glycoprotein levels in animal models, in conjunctival and corneal epithelial cells [79]. Furthermore in human conjunctival epithelial cells, DA-6034 treatment in doses of 200 microM increased mucin secretion between the trans-membrane

mucins MUC1, MUC2, MUC4, MUC5AC, MUC5B, and MUC16 [79]. Seo and colleagues investigated the inhibiting effect of DA-6034 MMP-9 on inflammatory cytokines and the activation of the MAPK signaling pathway on rabbit inflammation models. It was demonstrated that DA-6034 could restore tear function and inhibit inflammatory responses reducing the phosphorylation of Jun N-terminal kinase (JNK) and p38 MAPK, inhibiting the nuclear factor kappa B cells (NF-kB) activation in corneal epithelial cells [80]. The NF-κB is a key transcription factor pathway, responsible for many key biological processes, such as inflammation, apoptosis, stress response, corneal wound healing, and angiogenesis [81]. The company Dong-A started a Phase II study of DA-6034 (ClinicalTrials.gov Identifier: NCT01670357) in January 2012 to determine the efficacy and safety of 3% or 5% DA-6034 eye drops on 150 patients with doses of one drop/each eye, four times/day, for four weeks.

RX-10045

Resolvyx Pharmaceuticals developed RX-10045 (Figure 14) a small lipid mediator as a product candidate for the treatment of ocular surface and anterior segment diseases [82]. In 2010 Celtic Therapeutics and Resolvyx Pharmaceuticals started to collaborate in the development of the compound. RX-10045 is a synthetic resolvin E1 (RvE1) analog formulated for topical application treatment of eye disease as a potent mediator of inflammatory resolution, endogenously produced by the human body from omega-3 fatty acid [83]. In pre-clinical dry eye models, RX-10045 has demonstrated potent efficacy. The compound successfully completed a Phase II study (ClinicalTrials.gov Identifier: NCT00799552) in dry eye patients and according to the companies it demonstrated significant symptom improvement from baseline (as assessed in subject diaries), and performed significantly better than placebo on the primary endpoint of the worst symptom score with a 75% reduction in controlled adverse environment-induced central corneal staining from baseline [84]. RX-10045 was evaluated in another Phase II study (ClinicalTrials.gov Identifier: NCT01675570) from August 2012 to February 2013 on 150 patients for the treatment of dry eye disease but the results are not yet published.

TOBRADEX

TobraDex was approved by FDA as dry eye treatment in 1988. *TobraDex* is a combination of tobramycin and dexamethasone (Figure 15). Tobramycin is an aminoglycoside antibiotic-antibacterial derived from streptomycestenebrarius and

is used to treat various types of eye infections. It is working through binding to a site on the bacterial 30S and 50S ribosome, preventing formation of the 70S complex, inhibiting mRNA to be translated into protein [85]. Dexamethasone is a potent synthetic member of the glucocorticoid class of steroid drugs used to reduce the inflammation and relieve the symptoms of the inflammatory eye [86]. The combination of tobramycin/dexamethasone might be an interesting solution for dry eye problems caused by inflammation and infection. Lately Alcon is evaluating a reformulation of *TobraDex* decreasing the amount of the steroid (from 0.1% to 0.05%) adding an inactive agent (xanthan gum), to stabilize the combination and to deliver more of each drug to the eye. The composition provides longer ocular retention for enhanced ocular bioavailability of tobramycin and dexamethasone and improved suspension of dexamethasone [87]. Alcon executed Phase III clinical trials (ClinicalTrials.gov Identifier: NCT00576251) in June 2007 for *TobraDex* ophthalmic suspension, containing 0.3% of tobramycin and 0.05% and now is marketing the product as an antibiotic to treat bacterial infections.

SYL1001

The Spanish biotechnology company Sylentis is developing the compound SYL1001, which is decreasing the pain related to dry eye disease, is acting by targeting the TRPV1gene expression on the ocular surface (interference RNA, RNAi) [84, 85]. This is an interesting approach as gene silencing is an attractive aspect avoiding the activity of some proteins by inhibiting its synthesis. In November 2010 Sylentis started a Phase II safety/efficacy study (ClinicalTrials.gov Identifier: NCT01776658) on patients with common mild to moderate dry eye symptoms and persistent daily symptoms for more than three months. The study is still ongoing but the initial results are very promising.

R932348

Rigel Pharmaceuticals is working on R932348 2,4-pyrimidinediamine, a small JAK3 molecule inhibitor, as a possible treatment for autoimmune diseases (Figure 16) [88]. During trials it was observed that systemic levels of IL-17, IL-22, IL-23, and TNF-α were significantly lower in mice receiving the compound, and T cells isolated from R932348-treated mice also showed reduced phosphorylation of Stat5 after stimulation with IL-2 [89]. The company in July 2013, started Phase II safety, tolerability and pharmacokinetics studies (ClinicalTrials.gov Identifier:

NCT01900249) for 0.2% and 0.5% R932348 ophthalmic solution in patients with dry eye disease.

EBI-005

Eleven Biotherapeutics is developing chimeric IL-1 receptor type I agonists and antagonists as dry eye treatment. These non-naturally occurring cytokine domains can modulate cellular signaling response to interleukin-1 receptor I (IL-1 RI), and to detect and or even bind on cellular receptors[90]. The drug started in December 2012 Phase I multi-center clinical trials (ClinicalTrials.gov Identifier: NCT01745887) with the name EBI-005-2, an IL-1 receptor blocker, single-domain protein, optimized for topical ocular delivery. The EBI-005 has been validated in clinical proof-of-concept studies in which IL-1 blockage was shown to be safe and well tolerated without adverse side effects. According to the results presented in ARVO 2012 [91] it was created by combining the IL-1 receptor binding the sub-domains IL-1β and IL-1Ra. During the pre-clinical trials EBI-005 was shown more active than topical Cyclosporine (the active ingredient of *Restasis*™ ophthalmic emulsion eye drops) in mouse models. Topical delivery resulted in distribution to multiple eye compartments, but very low systemic exposure in rabbits ($F < 0.2\%$). EBI-005 was 9°C more thermally stable than anakinra an interleukin-1 receptor antagonist [92]. Eleven Biotherapeutics presented promising preliminary clinical data from the trials.

CONCLUDING REMARKS

Many of the above mentioned potential drugs are anti-inflammatory compounds and some are mucin secretagogue, promoting mucin and water production, and also there are a few eye lubricants. Most of the anti-inflammatories are corticosteroids because of their noteworthy anti-inflammatory properties and rapid action. Normally they are used for a limited period of time and depending on the severity of the case, after treatment the patient returns to the initial inflammatory condition. This limitation makes them unsuitable for chronic dry eye therapy as steroidal compounds present important side effects, such as increase in intraocular pressure and posterior sub-capsular cataract [93]. A critical point to highlight is that all of the above-mentioned potential new drugs are centering their action mainly towards symptoms, controlling inflammation or restoring the normal amount of tears but none of them is addressing the causative mechanisms of the

disease leaving its cause unattended. Developing a new drug for the treatment of DED has become a key target for the pharmaceutical industry. Even though dry eye is the most widespread eye disorder, there is limited offer of effective therapeutic agents. Dry eye disease is not a life-threatening disorder and its symptoms are not severe, but the overarching complexity of the syndrome makes it challenging to manage. Many of the candidate dry eye agents are failing FDA testing, even though some of them are approved in Asia and Europe, mainly because of the tight clinical trials regulations in the USA. Other reasons for this frequent failure are the nature of the syndrome and the lack of an objective test for the diagnosis of the severity of the disease, because usually symptoms evaluation is insufficient as a measurement factor. There are several proposals for new, objective tests such as molecular markers [94, 95] and tear film osmolarity tests [96, 97] but at the moment none of them has advanced beyond the pre-clinical evaluation. Dozens of companies have placed under development a vast variety of new drug candidates in their pharmaceutical pipelines and most of these drugs have already approved as treatment for other diseases as identical biological pathways can be active in different ways in various diseases. Discovering new uses for old drugs can ensure important financial benefits, for patients as well as for biotechnology companies, lowering costs and shortening the approval timelines.

Conflict of Interest: It is certified that there is no actual or potential conflict of interest related to this article and the authors have no relationship with the companies described above.

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Table 1.- Drugs and properties

Drug	Company	Properties	Phase
AL-2178/Rimexolone	**Alcon/Novartis**	**Anti-inflammatory**	**Phase III**
LX214/Voclosporin	**Isotechnika and Lux Biosciences**	**Anti-inflammatory**	**Phase I**
Tofacitinib	**Pfizer Inc.**	**Anti-inflammatory**	**Phase II**
Nutrilarm®-Omega-3 and Omega 6 fatty acids	**ThéaLaboratoire**	**Anti-inflammatory**	**Phase II**
Omega-3-acid ethyl esters	**Lovaza, GlaxoSmithKline**	**Anti-inflammatory**	**Phase I**
Secukinumab and Canakinumab	**Novartis Pharmaceuticals**	**Anti-inflammatory**	**Phase II**
MIM-D3	**Mimetogen Pharmaceuticals**	**Mucin secretagogue**	**Phase II**
Difluprednate-Durezol	**Alcon/Novartis**	**Anti-inflammatory**	**Phase II**
Cis-urocanic acid	**LaurantisPharma**	**Anti-inflammatory**	**Phase I**
Thymosin Beta 4	**RegeneRx Biopharmaceuticals, Inc.**	**Anti-inflammatory**	**Phase II**
Belimumab	**University of Udine**	**Anti-inflammatory**	**Phase II**
Rituximab	**IDEC Pharmaceuticals**	**Anti-inflammatory**	**Phase III**
Lotemax	**Bausch & Lomb Inc.**	**Anti-inflammatory**	**Phase II**
Ecabet sodium	**Bausch & Lomb Inc.**	**Mucin secretagogue**	**Phase II**
BOL-303242-X (mapracorat)	**Bausch & Lomb Inc.**	**Anti-inflammatory**	**Phase II**
AL43546	**Alcon/Novartis**	**Artificial tears**	**Phase II**
Sirolimus-Rapamycin	**Santen Pharmaceutical**	**Anti-inflammatory and immunosuppressive**	**Phase II**
ISV-101	**InSite Vision**	**Anti-inflammatory**	**Phase II**
ESBA-105	**Alcon and ESBATech**	**Anti-inflammatory**	**Phase II**
DA-6034	**Dong-A Pharmaceutical Co.**	**Mucin secretagogue**	**Phase II**
RX-10045	**Resolvyx Pharmaceuticals**	**Anti-inflammatory**	**Phase II**
Tobradex (tobramycin/ dexamethasone)	**Alcon/Novartis**	**Anti-inflammatory**	**Phase I**
SYL1001	**Sylentis**	**Lowers dry-eye related pain**	**Phase II**
R932348	**Rigel Pharmaceuticals**	**Anti-inflammatory**	**Phase II**
EBI-005	**Eleven Biotherapeutics**	**Anti-inflammatory**	**Phase I**

Table 2.- Drug Chemical Structures

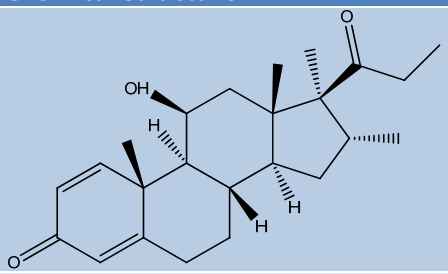
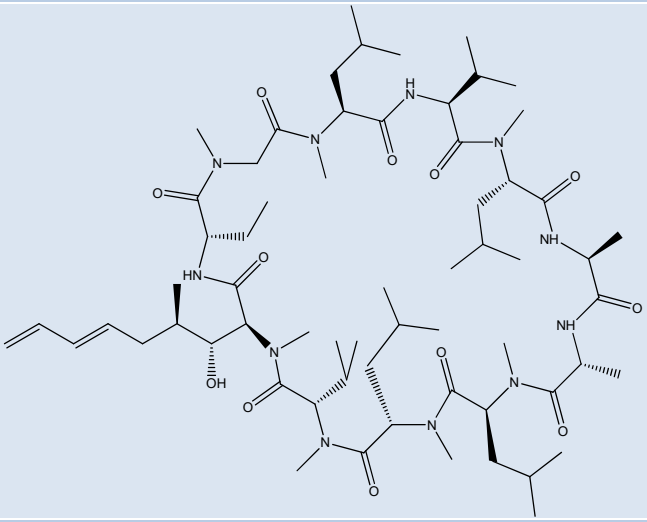
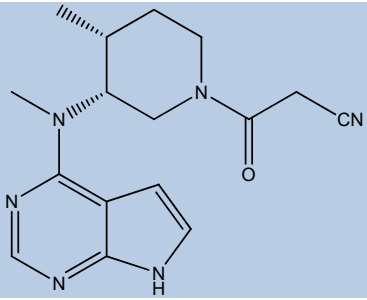
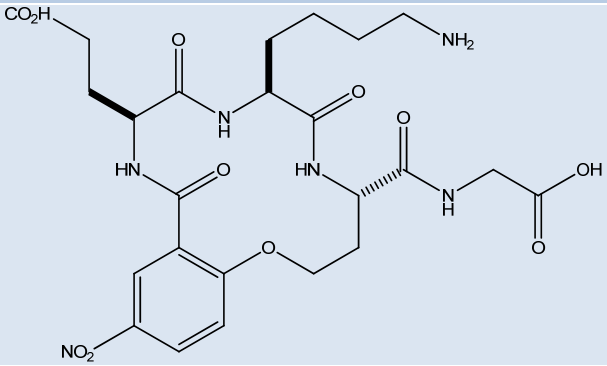
	Drug	Chemical structure
Fig. 2	**AL-2178/ Rimexolone**	
Fig. 3	**LX214/ Voclosporin**	
Fig. 4	**Tofacitinib**	
Fig. 5	**MIM-D3**	

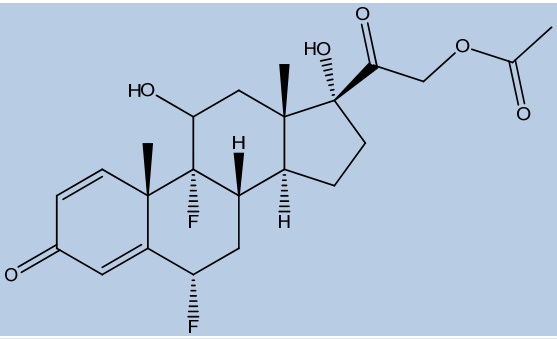
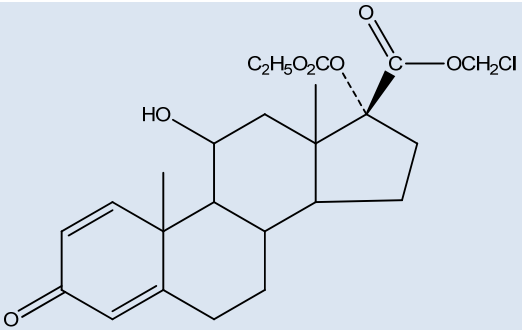
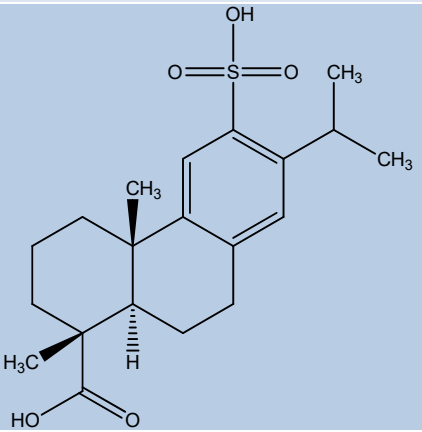
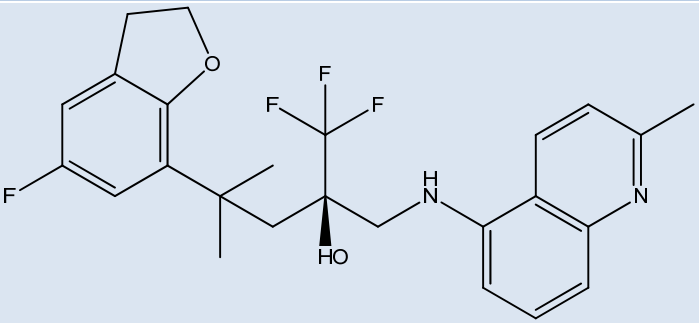
Fig. 6	**Difluprednate-Durezol**	
Fig. 7	**Lotemax**	
Fig. 8	**Ecabet sodium**	
Fig. 9	**BOL-303242-X (mapracorat)**	

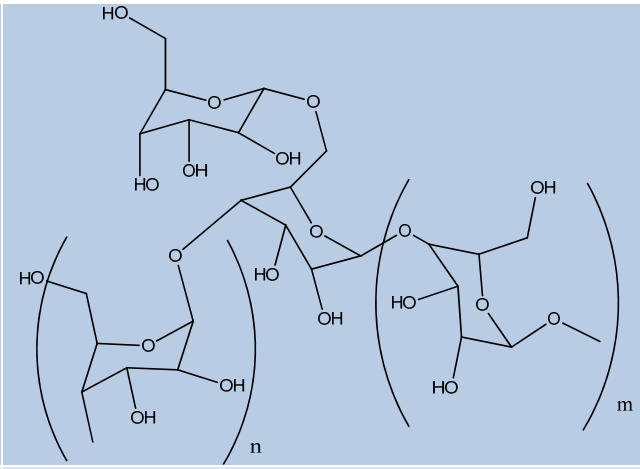
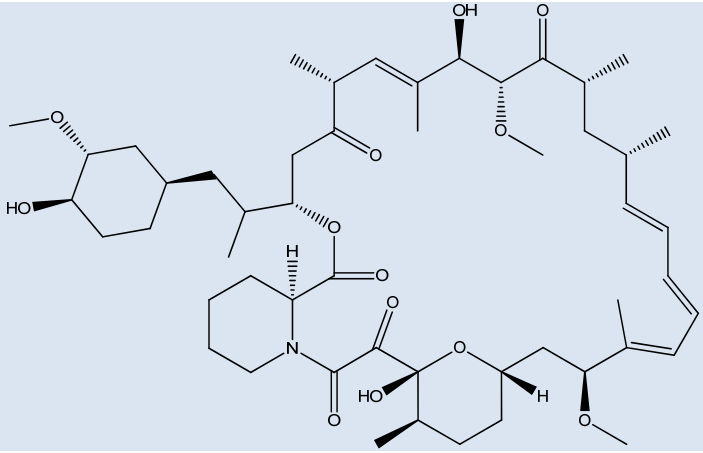
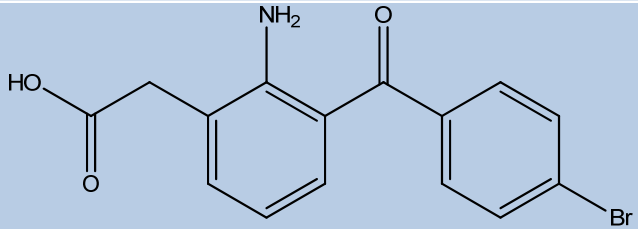
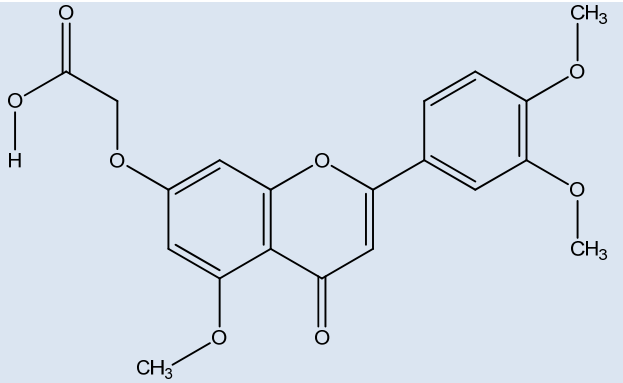
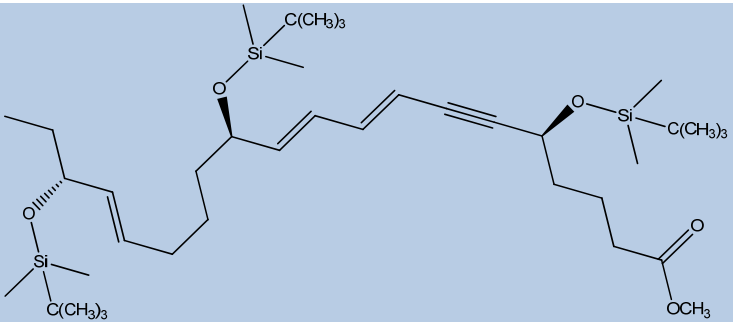
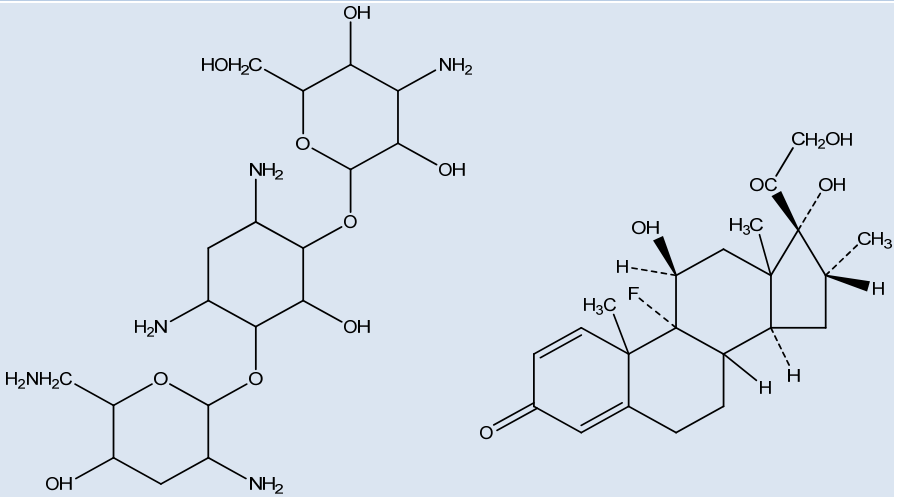
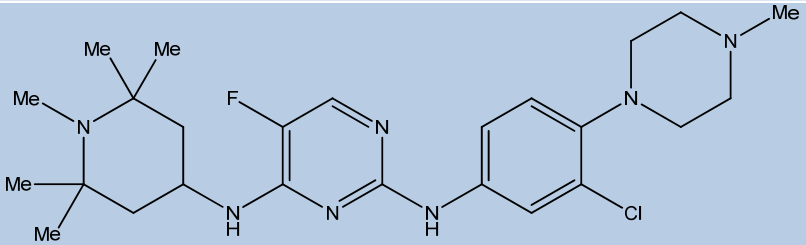
Fig. 10	**AL43546/ galactomannan**	
Fig. 11	**Sirolimus**	
Fig. 12	**ISV101/ bromfenac**	
Fig. 13	**DA-6034**	

Fig. 14	**RX-10045**	
Fig. 15	**Tobradex (tobramycin/ dexamethasone)**	
Fig. 16	**R932348**	

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5. DISCUSIÓN

Durante esta tesis doctoral, hemos buscado nuevas vías de investigación, que podrían ayudar a la industria farmacéutica-oftalmológica a organizar su estrategia a largo plazo, adaptándose a la evolución de las tendencias en I+D.

Consecuentemente, hemos investigado la dirección y el futuro del mercado de medicamentos y biomarcadores a nivel ocular, basándonos en el análisis de las patentes publicadas y de los ensayos clínicos en curso. El análisis de la información recabada revela la aparición de moléculas novedosas con las que actualmente se trabaja en los centros de investigación, las nuevas perspectivas del sector y los esfuerzos económicos y humanos de las investigaciones en curso. Los resultados muestran prometedoras estrategias a corto y largo plazo para fomentar la investigación y desarrollo de nuevos medicamentos oculares.

5.1 PATOLOGÍA GLAUCOMATOSA

5.1.1 DIAGNÓSTICO DE GLAUCOMA

El glaucoma, al igual que otras patologías asociadas a neurodegeneración, es difícil de diagnosticar antes de que la enfermedad se encuentre en un estado avanzado. Su diagnóstico, como fue mencionado en la introducción (apartado 2.5.1), se basa actualmente en la búsqueda de síntomas específicos asociados a esta enfermedad. La elevación anormal de la presión intraocular, la observación de los cambios característicos de la cabeza del nervio óptico y la pérdida del campo visual, son los principales indicadores de la aparición y evolución de esta patología [191] [192].

El principal inconveniente de la metodología actual reside en la necesidad de una colaboración absoluta por parte del paciente y en la dificultad de la evaluación del progreso de la enfermedad por parte del especialista. Esta situación hace que el diagnóstico y seguimiento de esta enfermedad se fundamente en criterios considerados como subjetivos [193]. Además, ningún de los métodos empleados es considerado como definitivo, haciendo necesarios múltiples exámenes.

Se hace patente, por lo tanto, la necesidad de nuevos métodos de diagnóstico, más precisos y objetivos. Adicionalmente, se pone de manifiesto la importancia de la obtención de resultados predictivos de la evolución de la enfermedad y la monitorización precisa de los nuevos fármacos frente al glaucoma y de su eficacia [194] [193].

La respuesta a estas cuestiones se centra fundamentalmente en la búsqueda de nuevos biomarcadores. Estas moléculas, señalizadoras de la patología, han ganado gran interés científico y clínico en todos los campos de la medicina, ya que son potencialmente útiles a lo largo de todo el curso normal de la enfermedad [195, 196]. Los biomarcadores podrían ser utilizados para evaluar el riesgo de contraer o desarrollar la patología para la que son específicos, y de esta forma poder prevenir o mejorar su evolución antes de la aparición de ésta (detección precoz). Durante la enfermedad, los biomarcadores pueden servir para determinar el estadio de la misma, para la selección de la una terapia inicial o adicional y para monitorizar la respuesta a la misma [20].

La gran capacidad de detección y discriminación específica de las técnicas en genética molecular, convierten a ésta en una valiosa herramienta en la comprensión de la fisiopatología y el tratamiento del glaucoma [197]. En este sentido, varios estudios genéticos en pacientes

afectados por esta dolencia han revelado la asociación de esta patología con un número importante de genes como *Myocilin*, *Optineurin*, *WD Repeat Domain 36* y locus cromosómicos [198].

Los estudios sobre las bases genéticas del POAG se han realizado mediante análisis de ligamiento, estudios de asociación y estudios de asociación del genoma completo, revelando que hasta el momento el glaucoma está relacionado con al menos 20 locus genéticos [199]. Pese al gran número de locus identificados, solamente un 5 % de los casos de POAG, que es el tipo más común de glaucoma, son debidos a mutaciones en un solo gen (herencia mendeliana). El 95% restante, se deben a una combinación de mutaciones genéticas y factores ambientales [200, 201]. Este hecho, dificulta enormemente el conocimiento de los mecanismos moleculares que conducen al glaucoma y por tanto el desarrollo de nuevos métodos de diagnóstico. Por otro lado, algunos estudios genéticos han dado resultados contradictorios, bien sea por diferencias en el diseño de los estudios o por la heterogeneidad genética de las poblaciones estudiadas[202]. Finalmente, los recientes resultados de Chen y colaboradores demuestran la existencia de individuos "resistentes" a enfermedades genéticas (individuos que presentaban mutaciones relacionadas con varias enfermedades mendelianas graves y que estaban sanos) lo cual cuestiona de nuevo la utilidad de los estudios genéticos como métodos diagnósticos y de los genes como biomarcadores [203].

Por todas estas razones, y para poder conocer todos los mecanismos moleculares que subyacen al glaucoma, es necesario complementar los estudios genéticos con otras aproximaciones tecnológicas. En este sentido, técnicas como la proteómica y metabolómica han permitido la identificación de nuevos biomarcadores de la susceptibilidad y progresión de la patología glaucomatosa, así como la respuesta a diferentes aproximaciones farmacológicas [204-206].

En muchos casos los biomarcadores identificados se encuentran relacionados con el estado de oxigenación, como en el caso de la hemoglobina, que regula la homeostasis del oxígeno a nivel de retina y su transporte a las células ganglionares o con el estrés oxidativo/inflamatorio como en el caso de algunos componentes del complemento o de la activación del factor potenciador de las cadenas ligeras kappa de las células B activadas (NF-κB) [207]. De hecho, el glaucoma se ha asociado frecuentemente con factores metabólicos como los marcadores plasmáticos de estrés oxidativo, tales como malondialdehído (MDA) y conjugado dieno (CD) [208] así como la reducción del estado antioxidante total (TAS) [209]. En conclusión, el estrés oxidativo es un factor

importante en la patogénesis de POAG, por lo tanto, puede ser un objetivo interesante para un diagnostico precoz de la enfermedad [205].

Ya que las proteínas son a la vez diana molecular de la enfermedad (debido a alteraciones funcionales en la interacción de proteínas) y pueden servir como biomarcadores para un diagnóstico precoz, son cada vez más frecuentes los estudios de proteómica en glaucoma [204] [210]. Sin embargo, la identificación de proteínas como biomarcadores tiene algunas limitaciones. Por ejemplo, las proteínas pueden sufrir modificaciones, lo que dificulta su identificación, y ser precisamente las proteínas modificadas las que tengan una mayor importancia a nivel de la patología. La literatura recoge varios ejemplos de esta problemática, como el caso de la hemoglobina glicosilada (HbA1c) que es un biomarcador de diabetes [210]. Otro inconveniente es la falta de métodos para amplificar proteínas de baja expresión aunque se han propuesto algunas estrategias para solventar dicho problema como el enriquecimiento de péptidos o proteínas mediante el uso de nanopartículas o los microarrays de proteínas de fase reversa [211] [212].

A pesar de la adquisición de las nuevas técnicas de proteómica y metabolómica, en la actualidad ninguna de las moléculas estudiadas puede considerarse como un biomarcador definitivo en la prevención y diagnóstico precoz de la ceguera glaucomatosa, ya que ninguna de éstas ha superado los ensayos clínicos [213]. A pesar de la gran cantidad de estudios básicos que hay sobre biomarcadores de glaucoma y por tanto de la gran expectativa que hay en este campo de investigación no hay una transferencia a la investigación clínica. En el glaucoma, como en otros campos de la oftalmología y la medicina, la transferencia de los biomarcadores del descubrimiento a la práctica clínica es un proceso con numerosas dificultades y limitaciones. Una posible razón del problema de la dificultad de trasladar estas técnicas al mercado del diagnóstico médico, es el coste de las técnicas que se emplean en ciencia básica y su poca aplicabilidad en clínica. Las dificultades más importantes consisten en la falta de una correcta caracterización y validación de biomarcadores, y en la elección correcta de técnicas de análisis utilizadas en ensayos clínicos [214].

Para la aprobación del uso clínico de un biomarcador, su uso ha de ser validado en un gran número de ensayos de forma altamente reproducible y demostrando una alta especificidad por la patología diana [215]. La muestra demográfica seleccionada, ha de ser representativa de los diferentes subtipos de la enfermedad y de su estadio; y englobar individuos con diferentes características demográficas, de tratamientos farmacológicos y ser estadísticamente comparable a individuos sanos tomados como población control [196].

De las pocas técnicas que han llegado hasta los ensayos clínicos es el DARC (Detection of Apoptosing Retinal Cells), del Western Eye Hospital of London RU, que ha sido evaluada en Fase I de ensayos clínicos en 2015, sin embargo, aún no han sido publicados los resultados. En este método se detecta la apoptosis de células ganglionares de la retina (CGR) *in vivo* en una etapa más temprana de la patología, para evitar la degeneración de las neuronas y la progresión de la enfermedad. Otro ensayo clínico de un posible biomarcador es de la Universidad Coreana de Chungnam, que en el año 2007-2009 ha realizado un estudio en 1224 pacientes de glaucoma. La hipótesis del estudio conectaba la elevación de la PIO con el polimorfismo rs7961953 del gen TMTC2 [196]. En el año 2014 los resultados del estudio fueron publicados, desacoplando dicho polimorfismo de la patología glaucomatosa, y proponiendo esta vez un nuevo polimorfismo del mismo gen (polimorfismo rs7098387) como causante de la enfermedad [216].

Debido a la complejidad etiológica de glaucoma, la existencia de diferentes subtipos de la enfermedad y la heterogeneidad entre pacientes, parece recomendable el uso de múltiples biomarcadores para mejorar la sensibilidad y la especificidad, en lugar de usar un solo biomarcador [196].

A la luz de la complejidad en el diagnostico debido a todos estos factores, creemos que son necesarias nuevas redes médicas capaces de compartir recursos, crear nuevas bases de datos y establecer directrices comunes para la investigación, con el fin de definir, con precisión, el perfil molecular del paciente con POAG. Todo esto puede conducir a una mejor evaluación de la validez de los biomarcadores actualmente propuestos como posibles criterios de la valoración indirecta del glaucoma.

Finalmente opinamos que los avances en la sensibilidad y la precisión de las técnicas de genómica, metabolómica y proteómica han generado varios candidatos biomarcadores moleculares con un potencial valor clínico. Por consiguiente, sería interesante, una plataforma que integra datos de la genómica, proteómica y metabolómica, para promover la integración y el descubrimiento de nuevos biomarcadores.

5.1.2 TRATAMIENTO DE GLAUCOMA

5.1.2.1 FARMACOLOGÍA CLÁSICA Y MEJORA DE LOS TRATAMIENTOS EXISTENTES

La introducción de nuevas clases de fármacos para reducir la PIO en la última década ha contribuido a un cambio en el patrón de prescripción de medicamentos. Los medicamentos actuales para el glaucoma son compuestos que reducen la PIO, tales como los agonistas α2-adrenérgicos, antagonistas β2-adrenérgicos, inhibidores de la anhidrasa carbónica y lípidos hipotensores, que se utilizan por separado o en combinación [86].

Los agonistas α2-adrenérgicos tales como la brimonidina y los antagonistas β2-adrenérgicos tales como el timolol, se usan comúnmente como medicamentos anti-glaucomatosos en tratamientos de larga duración. Las dianas de estos fármacos (los receptores α2- y β2-adrenérgicos) se encuentran localizadas en estructuras oculares implicadas directamente en la dinámica y regulación de la producción del HA (epitelio ciliar) razón por la cual actúan como hipotensores[217].

Las anhidrasas carbónicas (CA) son metaloenzimas que catalizan la conversión del CO_2 a HCO_3^- en presencia de H_2O. A nivel fisiológico estas enzimas intervienen en procesos tan variados como el mantenimiento del equilibrio ácido-base, el transporte y la secreción de fluidos. Dentro de las estructuras oculares las CAs han sido localizadas en los procesos ciliares, implicándolas directamente y de forma activa en los procesos responsables de la formación del humor acuoso en esta estructura [218]. Los inhibidores de CAs son un tratamiento ampliamente utilizado en el control del avance de las patologías glaucomatosas, ya que promueven una importante reducción de la PIO mediante la supresión de la secreción de humor acuoso [219, 220]. Los inhibidores sistémicos de CA han sido utilizados en la práctica clínica desde hace mucho tiempo, sin embargo, debido a los efectos secundarios asociados a su empleo, estas sustancias en la actualidad tienden al desuso. Entre los efectos secundarios notados se pueden destacar, malestar general, fatiga, depresión, parestesias, sabor metálico, náuseas, disminución de la libido, hipopotasemia, anemia aplásica, acidosis metabólica, y nefrolitiasis. El desarrollo de las CA tópicas reduce al mínimo estos efectos secundarios relativamente comunes, aunque se han informado pequeñas cantidades del medicamento para alcanzar la circulación sistémica [221].

Los lípidos hipotensores (análogos de prostaglandina y derivados de prostamidas) se encuentran entre los más potentes agentes hipotensores oculares tópicos disponibles en la actualidad. Los análogos de la prostaglandina $F_{2\alpha}$, como el latanoprost, se unen a diferentes receptores de prostaglandinas (PG) presentes en el músculo ciliar, produciendo un efecto

neuroprotector que es independiente de la estimulación del receptor PG [222, 223]. A diferencia de los análogos de postranglandinas, algunos derivados de la prostamida F2α como el bimatoprost, utilizan otros mecanismos de acción, uniéndose a un receptor específico de prostamida, localizado en las células de la malla trabecular [224]. Como consecuencia, el bimatoprost aumenta el flujo de drenaje convencional y no convencional del humor acuoso.

Los agonistas-antagonistas adrenérgicos, los inhibidores CAs y los derivados de prostaglandinas son en la actualidad las líneas farmacológicas de primera elección en el tratamiento de la patología glaucomatosa, proporcionando a los oftalmólogos amplias oportunidades para elegir dentro de una gran gama de fármacos hipotensores de la PIO.

Pese a ello, estos fármacos se asocian a una extensa variedad de efectos secundarios adversos, en parte, debido a las altas dosis necesarias para obtener una acción terapéutica, y a su absorción sistémica. Uno de los factores clave implicado en este fenómeno se haya estrechamente relacionado con la frecuencia de administración del principio activo, ya que el tratamiento farmacológico del glaucoma requiere la aplicación tópica en gotas, al menos, una vez al día [225]. Como consecuencia las empresas farmacéuticas realizan un esfuerzo continuo en la mejora de la medicación existente y en el desarrollo de fármacos más seguros y eficaces y que requieran una menor dosis diaria.

Actualmente, se puede constatar la existencia de tres líneas fundamentales o estrategias básicas encaminadas a reducir las desventajas asociadas a las terapias farmacológicas en el ámbito del glaucoma.

- La mejora de los medicamentos existentes.
- El desarrollo de nuevas combinaciones de fármacos.
- El diseño de nuevos fármacos con mecanismo de acción innovadores.

En este sentido, la industria farmacéutica continúa aumentando el arsenal farmacéutico disponible actualmente con el estudio y futura incorporación de nuevos fármacos. Adicionalmente, en los últimos años se nota un creciente interés de la industria farmacéutica en medicamentos inyectables. Varios ensayos clínicos están comenzando con productos intraoculares de liberación prolongada que eliminarían la necesidad de aplicación diaria de gotas oftálmicas [226].

Entre los fármacos que provienen de la mejora de medicamentos con dianas o vías de actuación clásica dentro de la farmacología anti-glaucomatosa, se encuentra la agmantina. Éste es uno de los pocos agonistas α2-adrenérgicos que están actualmente en desarrollo. Este

fármaco destaca por su importante acción hipotensora sobre la PIO, sin embargo, su más destacada cualidad es la de disminuir la pérdida de células ganglionares de la retina [227].

Siguiendo esta misma estrategia encontramos también ejemplos dentro de los antagonistas β-adrenérgicos; con el estudio y desarrollo de nuevos derivados del *timolol*© que incluyen en su formulación el óxido nítrico (NO). La adicción de los grupos NO, no sólo potencia los efectos hipotensores del compuesto original, sino que además puede posponer el daño del nervio óptico en los pacientes glaucomatosos. Otros estudios sin embargo contradicen esta visión. En 2009 un trabajo publicado por Chiroli et al, mostraba efectos contra-terapéuticos de esta substancia en la neurodegeneración glaucomatosa[228].

En este mismo grupo de β-bloqueantes se encuentra el *OT-730*, desarrollado por la empresa Othera, y entre cuyas interesantes ventajas destaca su capacidad de descomponerse a un producto inactivo al entrar en el torrente sanguíneo. Esta capacidad podría minimizar o erradicar los efectos secundarios cardiovasculares y respiratorios asociados a los fármacos β-bloqueantes, puesto que hasta ahora no era posible prescribir este tipo de compuestos a pacientes con problemas cardiovasculares [229] o respiratorios tan comunes como el asma.

En la actualidad, la empresa Sylentis está evaluando el compuesto SYL040012. Este fármaco mantiene como diana los receptores β-adrenérgicos, sin embargo el abordaje de esta diana clásica se realiza de una forma novedosa al valerse de pequeños fragmentos de ARN de doble cadena (siRNA), para el silenciamiento de los receptores β2-adrenérgicos, que permite la reducción a más largo plazo de la expresión de proteínas [230]. Este mismo abordaje novedoso ha sido seguido en nuestro laboratorio con la evolución de un inhibidor de los receptores $P2Y_2$ basado en la tecnología de los siRNAs, y que se patentó con el título “siRNA y shRNA para la elaboración de medicamentos frente a la presión intraocular elevada” (WO/2010/010208).

A los esfuerzos emprendidos por las empresas farmacéuticas en mejorar fármacos ya existentes basados en lípidos hipotensores y en adrenérgicos, hay que añadir los realizados en aquellos cuya diana son las anhidrasas carbónicas. Al igual que en los casos anteriores la industria farmacéutica busca valores añadidos como la capacidad neuroprotectora, además de centrar sus esfuerzos en la obtención de un inhibidor de anhidrasas carbónicas más potente y selectivo. En este sentido en la actualidad, se encuentra en fase de desarrollo un nuevo derivado de la dorzolamida y una nueva clase de sulfonamidas, con restos donantes de NO, que además de presentar un potente efecto hipotensor sobre la PIO, añade efectos anti-apoptóticos y anti-inflamatorios [231-233].

De los últimos análogos de PG reivindicados como agentes anti-glaucomatosos, la mayor parte se obtienen como derivados de la molécula PG $F_{2\alpha}$. Como ejemplo de estos nuevos análogos, el 15-ketolatanoprost ha sido probado en monos glaucomatosos, mostrando un efecto hipotensor equivalente, o incluso mayor, al obtenido con el latanoprost [234], ampliamente utilizado en la actualidad y perteneciente al mismo grupo de fármacos. Adicionalmente está en desarrollo un análogo de PG que libera NO, llamado BOL-303259-X, actuando como un potente agente hipotensor en los ensayos clínicos de Fase II [235]. En esta misma dirección la empresa Aerie Pharmaceuticals está actualmente desarrollando un nuevo agonista de PG $F_{2\alpha}$, denominado AR-102, que presenta 150 veces mayor selectividad y 30 veces mayor potencia que el latanoprost©. Existen además otras alternativas basadas en modificaciones no sustanciales de fármacos ya existentes, como el Travoprost APS© de Alcon, que es una solución de travoprost (análogo de PG $F_{2\alpha}$) que contiene un conservante alternativo o el Latanoprost sin conservante (cloruro de benzalconio) de Sun Pharma.

El PGE_2, puede inducir respuestas diversas de señalización celular mediante su unión a receptores $EP_{1\text{-}4}$ debidas a que el efecto de vaso-relajación está mediado por los receptores EP_2 y EP_4. Por lo tanto, la aplicación tópica de agonistas del EP_2 y EP_4 se puede utilizar como agente hipotensor. Este es el caso del CP-544326 de Pfizer que es un agonista potente y selectivo del EP_2 con efecto hipotensor comparable al latanoprost [236]. Igualmente, el CP-734432 de la misma empresa, que es un potente agonista del EP_4, produce una reducción de la PIO significativa en perros normotensos [237].

Otro enfoque para lograr la seguridad y la eficacia de estos medicamentos es mediante la mejora de los sistemas de liberación de fármacos. Para el tratamiento médico del glaucoma se utiliza la aplicación tópica de gotas para los ojos. La medicación tópica ocular tiene varias desventajas como la dilución del fármaco por la lágrima y el reflejo del parpadeo. Otro problema es la absorción sistémica de estos fármacos, debido a la baja permeabilidad del tejido corneal. Por eso se están desarrollando lentes de contacto de hidrogel cargados con brimonidina y timolol y geles cargados con nanopartículas y microesferas de timolol [238, 239]. Esto podría estar especialmente indicado en aquellos pacientes que usan lentes de contacto, con la limitación de que estas lentes deben ser reemplazadas con mayor frecuencia o los pacientes deben estar capacitados para volver a cargar las lentes con el compuesto hipotensor.

Esta misma línea de mejora se ha utilizado también con los inhibidores de CA, demostrando una mejora en la eficacia del tratamiento con estas substancias, de baja solubilidad en agua,

cuando forman complejos con ciclodextrinas [240]. Éste es un claro ejemplo de mejora en la eficacia hipotensora de un fármaco debido no sólo a cambios en el principio activo, sino también a la forma de vehiculización del mismo. De esta forma se abre la evidente posibilidad de mejora de muchos fármacos pobremente solubles mediante la utilización de otros mecanismos de trasporte que mejoren su vehiculización y entrega efectiva.

En este mismo sentido la empresa Vistakon ha introducido mejoras en los sistemas de liberación de fármacos derivados de agonistas del receptor PG, mediante la inclusión de un implante con *latanoprost* y *bimatoprost* [236].

Como ya hemos mencionado anteriormente, otra de las grandes estrategias seguida por las compañías farmacéuticas implica la introducción de mejoras en las terapias de combinación de fármacos ya existentes. En esta línea el número de agentes en desarrollo es actualmente muy alto. Se utilizan principalmente combinaciones de tratamientos de dos, tres o cuatro medicamentos de las cuatro clases principales de primera elección (Tabla 1). En la mayoría de los casos, la combinación es de un β-bloqueante con un inhibidor de anhidrasa carbónica o bien un lípido hipotensor. Por ejemplo, la combinación de brinzolamida con timolol ya está disponible bajo el nombre comercial de Azarga©. Una de las principales ventajas de la combinación de fármacos de diferentes clases de primera elección, es que se produce una disminución adicional de la PIO, que responde a una acción sinérgica de los fármacos empleados. Sin embargo los mecanismos y la magnitud de esta disminución adicional no ha sido aún bien estudiada [90]. Webers et al realizaron estudios en este sentido, aportando datos interesantes acerca del comportamiento farmacológico de algunas de estas combinaciones [90].

Webers y sus compañeros compararon combinaciones de inhibidores de la anhidrasa carbónica tópicos a un régimen de β-bloqueante, análogos de prostaglandinas a un tratamiento a base de betabloqueantes, agonistas de los receptores adrenérgicos α2, a un régimen de β-bloqueante y antagonistas de los receptores adrenérgicos β con análogos de la prostaglandina [90]. Los datos aportados por este estudio muestran claramente que la combinación de prostaglandinas y β-bloqueantes produce la disminución adicional más fuerte.

La combinación de fármacos puede ser una opción médica beneficiosa para pacientes en los que la terapia de primera línea es eficaz, pero es necesaria una reducción adicional de la PIO que no puede conseguirse mediante los fármacos de la terapia clásica. La combinación de estos fármacos tiene sin embargo inconvenientes, debido principalmente a que la mezcla de compuestos multiplica la probabilidad de la aparición de efectos secundarios [241].

5.1.2.2 NUEVOS TRATAMIENTOS

Los fármacos implantados en el mercado, están focalizados en su inmensa mayoría en promover una bajada de los valores anormalmente elevados de PIO, característicos de esta patología. Durante los últimos años se ha iniciado el desarrollo de los futuros medicamentos, utilizando aproximaciones innovadoras, con nuevas dianas y nuevos mecanismos de acción en el tratamiento del glaucoma. Estos agentes comparten con los fármacos clásicos su acción hipotensora, sin embargo actúan sobre dianas no utilizadas anteriormente; a través de la inducción de metaloproteinasas (MMPs), contracción de células de la MT, inhibición de la secreción de humor acuoso o activación del receptor CB1 (receptor de cannabinoides de tipo 1) [242, 243].

En esta misma dirección, una nueva clase de fármacos en fase de desarrollo pretende mejorar el flujo sanguíneo ocular, particularmente en la retina y la cabeza del nervio óptico [244, 245]. De igual forma, otra de las dianas novedosas surgida en los últimos años, y de gran relevancia en la detención del avance de la enfermedad, es la neuroprotección [246]. Aunque la idea de incluir la neuroprotección en el tratamiento es muy reciente, ya existe un número alto de agentes de esta clase en desarrollo, como por ejemplo los agentes melatoninérgicos que desarrolla nuestro laboratorio [247].

A pesar del descubrimiento de estas nuevas dianas farmacológicas en el tratamiento del glaucoma, la mayoría de éstas, adolecen de falta de caracterización de la misma o no han sido clínicamente validadas.

5.1.2.2.1 MELATONINA

La melatonina es una neurohormona secretada al torrente sanguíneo, principalmente por la glándula pineal o epífisis; presente en el en diencéfalo [248]. Los sistemas que regulan la producción de melatonina en la glándula pineal, están íntimamente relacionados con la función circadiana de esta neurohormona [249].

A pesar de que la glándula pineal es el principal órgano secretor de melatonina en los vertebrados, existen otras estructuras fisiológicas ligadas a su producción [250]. Un caso particular de producción extrapineal, lo encontramos en las estructuras anejas al ojo; como las glándulas de Harder [251] y estructuras oculares como el cristalino [252], la retina [253] el cuerpo ciliar [254] y la lágrima humana [255]. Al igual que en otros órganos, la producción de melatonina extrapineal en el ojo parece tener una marcada función local, entre las que destaca la protección frente al estrés oxidativo [256]; sin embargo, en el ojo, la síntesis de melatonina mantiene un ciclo circadiano.

Las acciones de la melatonina en los mecanismos reguladores y de señalización intracelular son muy diversas. Algunas de estas acciones se deben a una interacción directa de la melatonina con moduladores o intermediarios de señalización intracelular. La mayoría de las funciones de la melatonina están mediadas por receptores específicos de melatonina ya sean receptores nucleares o receptores de membrana.

De ellos los receptores de membrana son los mejor caracterizados. Estos receptores pertenecen a la familia de proteínas de siete dominios transmembrana acopladas a proteína G (GPCR) [257]. Actualmente han sido descritos tres tipos de receptores de melatonina en mamíferos, denominados, según nomenclatura adaptada a la International Union of Pharmacology (IUPHAR), como MT_1, MT_2 y MT_3 [258].

Tanto el receptor MT_1 [259] [260] como el MT_2 [261], han sido identificados y clonados. Un caso particular lo presenta el receptor MT_3, caracterizado farmacológicamente, pero cuya estructura molecular es desconocida [262].

La evidencia actual demuestra que la melatonina y sus análogos pueden reducir la presión intraocular tanto en animales de experimentación, como en seres humanos [263]. De igual forma hay indicios de que esta acción hipotensora se mantiene durante largos periodos de tiempo, y de la existencia de una acción neuroprotectora en estas sustancias [264] (Fig. 5). Asimismo, las acciones circadianas antidepresivas y la normalización del ritmo circadiano por los análogos de la melatonina pueden ser incluidas entre los efectos beneficiosos para los pacientes con glaucoma [265]. La aplicación tópica de melatonina y sus análogos producen una potente

reducción de la PIO, convirtiendo a estos compuestos en buenos candidatos para el desarrollo de una nueva familia de fármacos. En consecuencia, la investigación científica ha dado un nuevo progreso significativo en el desarrollo de nuevos ligandos de melatonina, más potentes y más selectivos [266].

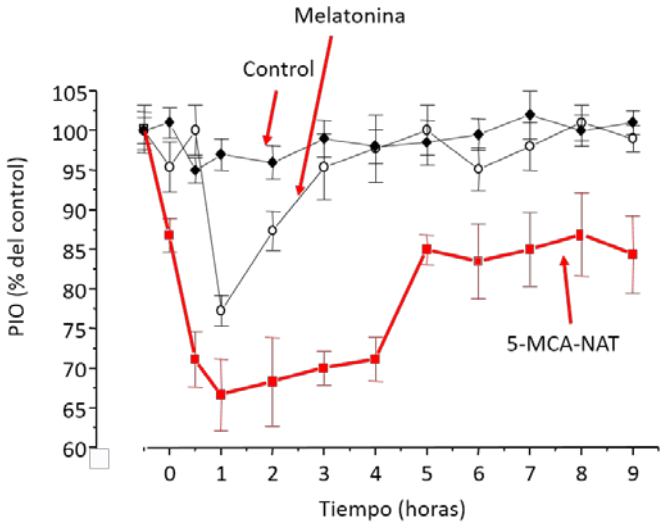

Fig. 10 La Melatonina modula la PIO

Sin embargo, el uso clínico de la melatonina está limitado debido a su corta vida media (15-30 min) y la falta de selectividad frente a los diferentes subtipos de receptor [267]. Como consecuencia, existe un interés considerable en el diseño y la síntesis de nuevos análogos de melatonina no sólo más estables metabólicamente, sino también más selectivos.

Un gran número de nuevos análogos de la melatonina han sido recientemente patentados [268]. Este hecho demuestra el interés económico que la melatonina ha despertado entre las empresas del sector farmacológico. A pesar del creciente papel de la melatonina en la patología glaucomatosa, estas patentes se dirigen generalmente a los trastornos del sueño y/o trastornos relacionados con el ritmo circadiano, pero no como tratamiento del glaucoma [267]. En este sentido, sólo el 5-MCA-N AT, IIK7, la agomelatina, el INS48848, el INS48862 y los derivados del INS48852 han sido probados por su efecto hipotensor *in vivo* [266, 269, 270]. En el caso de la agomelatina también se ha demostrado un efecto neuroprotector [271, 272]. Además, este compuesto ha demostrado recientemente su acción efectiva en el tratamiento de la depresión, como se ha indicado anteriormente.

De entre los diferentes análogos de la melatonina mencionados anteriormente, los resultados previamente publicados por nuestro grupo de investigación muestran claramente que el 5-MCA-N AT (Tabla 2) es el de mayor capacidad hipotensora, en comparación con otros agonistas de receptores de melatonina y en comparación con otros compuestos terapéuticos [273-275].

Asimismo, los trabajos realizados por nuestro grupo de investigación en la caracterización de estos análogos de melatonina [270, 276], han confirmado que la acción hipotensora del 5-MCA-NAT no sólo presenta una acción aguda (bajada rápida y muy significativa), sino que se mantiene durante días (efecto a largo plazo). Este efecto sostenido en las propiedades hipotensoras es debido a la modificación en la expresión de los receptores adrenérgicos [277] y de las anhidrasas carbónicas propiciadas por este compuesto [275] y que, en última instancia, regulan la formación del humor acuoso.

Como consecuencia de este mecanismo de acción hasta ahora desconocido para las drogas melatoninérgicas, una única dosis de 5-MCA-N AT, podría ser capaz de potenciar durante días, los efectos hipotensores del *Alphagan* (brimonidina-agonista adrenérgico α2-selectivo); utilizado comúnmente en el tratamiento del Glaucoma. Esta potenciación del efecto del *Alphagan* implicaría una dosificación menor en la posología de este fármaco, traduciéndose potencialmente en una disminución de los efectos no deseados asociados a la terapia antiglaucomatosa [274].

El resultado de los esfuerzos invertidos por nuestro grupo de trabajo sobre esta familia de substancias ha cristalizado en la formalización de una patente sobre dos compuestos que presentan una potente acción farmacológica sobre la hipertensión ocular, y mínimos efectos secundarios, titulada “Uso de un análogo de la melatonina para la reducción de la presión intraocular” (WO 2013060908 A).

En esta misma línea, se han estudiado diferentes estrategias para minimizar las concentraciones terapéuticas de los fármacos aplicados por vía tópica, y reducir así la citotoxicidad y los efectos no deseados. Los polímeros de celulosa son utilizados comúnmente en el diseño de las soluciones de lágrimas artificiales, debido a que reducen la velocidad de drenaje del fármaco y mejoran su eficacia terapéutica [278]. En este sentido, se ha realizado una combinación de 5-MCA-N AT con polímeros bioadhesivos para lograr potentes agentes hipotensores que posean una mayor seguridad ocular [278]. Además, se han realizado nuevas formulaciones de 5-MCA-N AT y polietilenglicol (aceptado en formulaciones de tratamientos oculares tópicos) [279] con similares objetivos.

A pesar de que en los últimos años se ha conseguido un notable progreso en el desarrollo de nuevos análogos de la melatonina, y de su uso potencial en el tratamiento de la hipertensión ocular y el glaucoma, han de resolverse aún una serie de cuestiones antes de que veamos un compuesto melatoninérgico unirse al grupo de los compuestos comerciales actuales. En este sentido, es necesaria la inversión de una gran cantidad de recursos económicos, intelectuales y humanos para el desarrollo de compuestos con solubilidad mejorada, con biodisponibilidad oral y protección contra la degradación metabólica del ligando. Por este motivo, en la actualidad, la melatonina y sus análogos pueden considerarse como un prometedor candidato como punto de arranque en la evolución de un fármaco con propiedades hipotensoras sobre la PIO y a la vez con un eminente papel neuroprotector; reduciendo además los efectos secundarios y mejorando la seguridad ocular de los fármacos actualmente comercializados.

5.1.2.2.2 RHO QUINASAS

La Rho-quinasa asociada (ROCK) es una enzima perteneciente a la familia de las quinasas AGC (quinasas asociadas a proteína G) de serina/treonina quinasas y es la primera efectora en la cascada de señalización a través de las "*small*" GTPasas (guanosina trifosfatasa). Hasta ahora, se han identificado dos isoformas: La ROCK-I (también llamada ROKβ y p160ROCK) y la ROCK-II (también conocida como ROCKα) [280]. Tanto las ROCK-I como las ROCK-II presentan una alta homología de secuencia, incluyendo un sitio de unión al ATP [281].

Las ROCK interactúan con la RhoA, proteína de unión a GTP, para generar la proteína activa. La Rho-quinasa activa, es capaz de fosfolizar la fosfatasa de la cadena ligera de miosina (MLC), inactivándola a través de la fosforilación de la subunidad de unión a miosina (MBS) [282]. La Rho-quinasa y la fosfatasa de miosina, coordinadamente regulan el estado de fosforilación de MLC, que se cree que induce la formación de fibras de contracción muscular y el estrés suave en células no musculares [283].

La RhoA y las ROCK, han sido implicadas directamente en fenómenos tales como la regulación de la contracción del músculo liso, la reorganización del citoesqueleto, la migración celular y la proliferación [284]. La inhibición de la actividad ROCK ha demostrado que induce alteraciones en la migración, adhesión, y cambios morfológicos a nivel celular [285]. De hecho, la Rho-quinasa está implicada en la regulación del tono vascular, la proliferación, la inflamación y estrés oxidativo [286]. Además, las Rho-quinasas juegan un papel en las vías de señalización que conducen a la formación de fibras de estrés de actina y adhesiones focales y ha demostrado que se expresa en los tejidos oculares, incluyendo la malla trabecular y músculo ciliar (MC) [287].

Se han identificado varias moléculas con la capacidad de inhibir las Rho-quinasas. La mayoría de ellos son compuestos heteroaromáticos que imitan la propia guanina [288]. Estos hallazgos abren la puerta a una nueva clase de fármacos que mejoran el drenaje del humor acuoso basados en las características de las ROCK. Esta nueva clase de fármacos basados en inhibidores de ROCK, actuarían a través de la modulación de cambios en el citoesqueleto de actina y la motilidad celular de la malla trabecular, el canal de Schlemm, y músculo ciliar [244].

Es probable que estos inhibidores disminuyen la resistencia a la salida del humor acuoso por la disminución en los niveles de fosforilación de la miosina de cadena ligera. Este fenómeno conllevaría la relajación celular en la malla trabecular humana y las células del canal de Schlemm [289, 290], propiciando así el aumento en el drenaje del humor acuoso y la disminución de la PIO [276, 291].

Varias compañías farmacéuticas ya están desarrollando inhibidores de ROCK como una nueva generación de agentes terapéuticos para enfermedades cardiovasculares y otras enfermedades, debido a las evidencias que sugieren la posible participación de ROCK en la hipertensión y la arteriosclerosis [292].

En la actualidad existe un amplio interés en el estudio de fármacos anti-glaucomatosos que tienen como diana esta enzima. La inhibición de las Rho-quinasas, como se ha demostrado *in vitro* e *in vivo,* produce una significativa reducción de la PIO, aumentando el flujo a través de la malla trabecular (MT). También existen datos que apuntan la capacidad de estos fármacos de aumentar el flujo sanguíneo en el nervio óptico, reportándose además retrasos en la apoptosis del nervio óptico [293].

La investigación realizada para este trabajo de los primeros informes de ensayos clínicos con humanos realizados por empresas farmacéuticas, muestra resultados muy prometedores [236]. El potencial de esta nueva clase de medicamentos se muestra prometedor en un campo donde los avances son escasos. Como consecuencia, los inhibidores de las Rho-quinasas están siendo activamente investigados y actualmente desarrollados por una amplia representación del sector farmacéutico. Compañías como Senju Pharmaceuticals, Novartis, Kowa, Santen, Aerie o Inspire Pharmaceutical entre otras, cuentan actualmente con programas activos en desarrollo de estas substancias. Hasta la fecha, dos inhibidores de ROCK han sido aprobados para uso clínico en Japón (*fasudilo* y *ripasudil*) y uno en China (*fasudilo*). En 1995 el *fasudilo* fue aprobado para el tratamiento del vasoespasmo cerebral, y más recientemente en 2014, *ripasudil* fue aprobado para el tratamiento del glaucoma [294].

Como era de esperar, la investigación de estos compuestos como potenciales fármacos también ha dejado al descubierto efectos no deseados asociados a las terapias con inhibidores de Rho-quinasas.

Podemos concluir que las principales desventajas asociadas a estas substancias están relacionadas con la alta homología estructural entre la variedad de las existentes quinasas, que limitan su especificidad [295]. Durante los últimos años, varios ensayos clínicos realizados con inhibidores de la Rho-quinasas no tuvieron éxito, a causa de los efectos secundarios sufridos por los pacientes. Se puede deducir de los informes presentados que existe una falta de tolerancia ante los inhibidores de la Rho-quinasa, algunos de ellos relacionados con la vasodilatación. Concretamente, como efecto secundario principal, se producen cuadros de hiperemia transitoria (enrojecimiento) detectado en un número elevado de pacientes [296].

Según las empresas farmacéuticas involucradas en estos proyectos, los efectos secundarios son leves por lo que existe una elevada expectativa sobre esta solución.

Sumado a este inconveniente, encontramos que los fármacos probados requieren de al menos dos aplicaciones diarias para alcanzar efectos terapéuticos; lo que además de incómodo para el seguimiento de la terapia por parte del paciente, aumenta además la posibilidad de aparición de efectos secundarios [297].

La solución aportada ante los problemas de selectividad y de tolerancia limitada por algunas compañías farmacéuticas pasa por la combinación de estos compuestos con otros fármacos ya existentes. Esta estrategia terapéutica ha sido ampliamente utilizada con otros grupos de fármacos de forma exitosa; y los resultados previos obtenidos para este nuevo grupo de compuestos parecen prometedores, especialmente cuando se utilizan análogos de prostaglandina o beta-bloqueantes [298-300 301, 302]. Los resultados muestran un aumento de la acción hipotensora de la terapia combinada frente a la acción de cada fármaco independiente. De igual forma se produce una dilatación en el tiempo de acción de la terapia combinada frente a la acción de los fármacos individuales.

5.1.3 REPERCUSIONES SOCIOECONÓMICAS

Dado que todos los medicamentos para el tratamiento de glaucoma estabilizan la enfermedad en lugar de curarla, es fundamental el desarrollo de fármacos con alto índice terapéutico y de bajo coste. En los últimos años, el creciente gasto de los fármacos oftalmológicos se ha convertido en una preocupación pública y el glaucoma en concreto constituye una carga financiera significativa para el sistema de salud. En los Estados Unidos actualmente 2,2 millones de personas que superan los 40 de edad, padecen POAG y el control de la enfermedad cuesta aproximadamente unos 3 mil millones de dólares anualmente, con 1,9 billones de dólares en costes directos y 1 mil millones de dólares en costes indirectos. Debemos tener en cuenta además como costes indirectos los derivados del absentismo laboral debidos a las bajas médicas producidas por esta patología [226].

Curiosamente los costes debidos a esta patología se ven agravados por tendencias inesperadas del mercado farmacéutico. El precio para las versiones genéricas de los medicamentos más antiguos, cuyo periodo de protección legal ha terminado, ha experimentado un aumento, agravando la frustración de pacientes y profesionales médicos. En algunos casos, medicamentos genéricos que se encontraban disponibles en el mercado con un precio barato, actualmente han incrementado su valor de venta al paciente más de 10 veces esa cantidad.

En general los gastos sociales y económicos del glaucoma son mayores en los países desarrollados debido a la mayor esperanza de vida, el perfil de la edad de la población que es mayor y el alto producto interno bruto per cápita. Asimismo, las bajas tasas de mortalidad y el elevado producto interno bruto per cápita, aumentan la relación coste-efectividad de la detección y el tratamiento de la enfermedad [303].

No existen datos económicos concretos para España, sin embargo, en la Tabla 3 analizamos el gasto medio anual por paciente, basado en los precios de los medicamentos en el año 2014. El Instituto Carlos III en su análisis sobre medicamentos y farmacoeconomía, concreta que para las gotas oftálmicas usadas en la terapia del glaucoma, se ha establecido una dosis fija en los diferentes subgrupos, no importando la concentración, basándose en la suposición de que por dosis dada, sólo se aplica una gota en cada ojo, no importando la concentración [247]. Esta misma tabla (Tabla 3) muestra además que de los medicamentos de primera elección para el glaucoma los beta-bloqueantes no selectivos son los de menos coste, con un gasto anual entre 68,95€ y 86,37€ excluyendo el *Timolol*© cuyo gasto anual asciende a los 227,06€. Los agonistas a_2 son la segunda opción de menor coste, con un gasto anual entre 105,08€ y 114,21€. Ha este

grupo de fármacos de menor coste económico debemos añadir el *Trusopt*© (inhibidor de anhidrasa carbónica), con un gasto anual cercano a los 76,80€.

Concluyendo hay que tener en cuenta que los betabloqueantes no selectivos siguen siendo la clase más barata de medicamentos para el glaucoma y los gastos de medicamentos para el glaucoma pueden afectar las decisiones en el tratamiento médico de la enfermedad [304].

5.2 SÍNDROME DE OJO SECO

5.2.1 NUEVOS TRATAMIENTOS DE OJO SECO

Durante los últimos años el desarrollo de nuevos fármacos para el tratamiento del ojo seco se ha convertido en un objetivo clave para la industria farmacéutica. A pesar de que el ojo seco es la enfermedad ocular más extendida, no es peligrosa para la vida y sus síntomas normalmente no son graves, por lo que hasta fechas recientes existía una oferta limitada de agentes terapéuticos eficaces. En el transcurso de la última década, numerosas empresas han iniciado el desarrollo de nuevos fármacos candidatos a cubrir las necesidades terapéuticas actuales del ojo seco, sin embargo, la complejidad global de la enfermedad, dificulta su gestión.

Un detalle interesante en esta carrera farmacológica, revelado por nuestro estudio, es el de que la mayoría de los fármacos actualmente en estudio para el tratamiento del ojo seco, ya están aprobados como tratamiento para otras enfermedades. Es un hecho bien documentado que las mismas vías biológicas, pueden estar activas en diferentes formas, en diversas enfermedades [305]. El descubrimiento de nuevos usos para estos medicamentos de dilatada vida comercial, puede asegurar importantes beneficios económicos, para los pacientes y las compañías de biotecnología, mediante la reducción de los costes y la deducción de los plazos de aprobación.

Los tratamientos utilizados actualmente, han demostrado ser claramente ineficaces en el abordaje de las causas del ojo seco; tratando simplemente de aliviar los síntomas asociados a la enfermedad. La mayoría de los compuestos en uso, son meros sustitutos de la lágrima; lo que convierte a las lágrimas artificiales en el pilar básico de la terapia del ojo seco [306].

En contra de la tendencia terapéutica actual, los nuevos compuestos en estudio o desarrollo recogidos en este trabajo, centran su acción principal en el control de la inflamación, como los corticoides (Hydroxychloroquine, Dexametasona Fosfato, Rimexolone), los antibióticos (Azitromicina) y los antinflamatorios no esteroideos (Ciclosporina A), o la restauración de los niveles normales de cantidad y calidad de lágrima y mucina endógena, como los secretagogos de mucinas y los agentes de reepitelización. Aun teniendo en cuenta este cambio de dirección en el abordaje terapéutico de los nuevos fármacos, éstos mantienen la tendencia actual de contrarrestar los síntomas, sin profundizar en los mecanismos causales del ojo seco. Este hecho responde probablemente a la complejidad en la etiología de la enfermedad. En el estudio de esta patología hemos de tener en cuenta que la fisiología lacrimal implica la intervención de un gran número de estructuras oculares como la córnea, conjuntiva, las

glándulas de Meibomio, las células caliciformes y las glándulas lagrimales. La afectación de una o varias de estas estructuras incide directamente en el deterioro de la función lacrimal, afectando negativamente al equilibrio en la producción de lágrima [307, 308]. De forma paralela, los fenómenos inflamatorios asociados al deterioro de la función lacrimal, conducen a la apoptosis de las células epiteliales en las glándulas lagrimales, retroalimentando de forma negativa el sistema y agravando la sintomatología del ojo seco [309].

Durante la realización de este trabajo hemos hallado una gran variedad de nuevos fármacos que responden a este abordaje terapéutico, pertenecientes al grupo de secretagogos o reepitelizantes, que funcionan como nuevos lubricantes polivalentes del ojo. De los datos de los lubricantes analizados se concluye que existe en desarrollo una variedad de sustitutos de lágrimas, cuya principal característica es su capacidad de abordar deficiencias específicas de los componentes de la lágrima. Aunque el uso de lágrimas artificiales puede mejorar los síntomas asociados al ojo seco, suprimiendo los síntomas negativos temporalmente, no hay evidencia de que pueden resolver las condiciones subyacentes que producen la inflamación de la córnea y de la conjuntiva [161]. En la mayoría de los pacientes, la aplicación únicamente de lágrimas artificiales, no es suficiente y se requiere una terapia complementaria anti-inflamatoria para corregir el componente inflamatorio de la patología [161]. En este sentido es necesaria una investigación más exhaustiva de los mecanismos de acción de los nuevos compuestos secretagogos y de su capacidad real como substancias anti-inflamatorias y su capacidad profiláctica frente a ésta. De igual forma, podemos resaltar en este sentido la necesidad de investigar los mecanismos inmunomoduladores e inflamatorios de la conjuntiva y aumentar nuestro conocimiento de los procesos implicados en esta enfermedad.

Los fenómenos inflamatorios asociados a esta patología no están exentos de controversia, existiendo opiniones enfrentadas, especialmente sobre si forman parte de la etiología desencadenante o de los síntomas desencadenados por la enfermedad. En cualquier caso, y fuera de cualquier controversia, la inflamación de las estructuras oculares forma parte de un proceso activo que intensifica los signos y síntomas; por lo que las estrategias terapéuticas actuales están dirigidas o deben contemplar el control de la inflamación [107, 108, 161].

Los compuestos anti-inflamatorios utilizados incluyen esteroides, inmunomoduladores no esteroideos y agentes antibióticos que también han mostrado resultados prometedores en el tratamiento del ojo seco. En general, la terapia actual anti-inflamatoria puede incluir corticosteroides tópicos, tetraciclinas orales y ciclosporina A [310].

La mayoría de los anti-inflamatorios en fase de desarrollo son los corticoides, debido a su gran capacidad como agentes anti-inflamatorias y su rápida acción. El uso de estos esteroides en la superficie ocular está relacionado con el aumento de la densidad de células caliciformes, reducción de células inflamatorias, regulación de los niveles del factor de necrosis tumoral TNFα, bloqueo de la síntesis de prostaglandinas, o de la activación de linfocitos T. Los tratamientos con este grupo de fármacos son muy eficaces y de duración muy limitada, normalmente de 2-4 semanas dependiendo de la gravedad del caso, sin embargo, la mejoría experimentada por los pacientes remite al finalizar el tratamiento, produciéndose una regresión a la condición inflamatoria inicial. Este fenómeno se produce incluso en los pacientes en los que se ha constatado un aumento en la densidad de células caliciformes en la conjuntiva [311]. De igual forma, es necesario tener en cuenta que los compuestos esteroides presentan efectos secundarios importantes, tales como aumento de la presión intraocular y la inducción de la aparición de cataratas subcapsular; que los hace inadecuados para el tratamiento crónico del ojo seco[312]. El uso prolongado de esteroides tópicos puede por lo tanto considerarse como factor desencadenante de patologías oculares graves como las cataratas y glaucoma [313]. En algunos casos está indicado el uso de esteroides más suaves con el fin de evitar estos efectos secundarios.

Otro factor fundamental que debemos tener en cuenta en el uso de estas sustancias es su capacidad de penetración en los tejidos oculares. Por ejemplo, la dexametasona es considerada como uno de los esteroides de acción más potente disponibles, sin embargo, su baja capacidad de penetración en los tejidos y los fuertes efectos secundarios que acompañan su utilización la desestiman para un uso eficaz como tratamiento tópico. En este caso, la utilización de técnicas como la iontoforesis (EGP-437) podría evitar los problemas de penetración y disminuir la aparición de efectos no deseados, por lo que ésta alternativa podría ser una solución prometedora [314].

También existen alternativas farmacológicas a la dexametasona. La prednisona presenta un perfil farmacológico parecido a la dexametasona, sin embargo su capacidad de penetración en las estructuras oculares es muy elevada [315]. Otros esteroides como el etabonato de loteprednol y el fluorometholone son menos potentes y tienen un mejor perfil de seguridad. Los efectos secundarios de los esteroides más preocupantes son la presión intraocular (PIO) y la formación de cataratas. En comparación con dexametasona y prednisolona, fluorometholone loteprednol tienen menores tasas de estas complicaciones [316] [184].

Como ya mencionamos anteriormente, existen otros compuestos anti-inflamatorios no esteroideos como los inmunomoduladores y los agentes antibióticos como las tetraciclinas que mejoran los signos y síntomas del ojo seco. Estos compuestos ofrecen además mejores garantías de utilización segura que los corticosteroides en su uso a largo plazo, a pesar de que su acción es mucho más lenta [317].

La Ciclosporina A (CsA) es el primer anti-inflamatorio aprobado por la FDA para el tratamiento de la enfermedad del ojo seco. A finales de 2002, la emulsión oftálmica tópica de ciclosporina 0.05% (Restasis de la empresa Allergan) fue aprobada en los EEUU para el tratamiento del ojo seco asociado a causas inflamatorias [310]. El uso tópico de las CsA en oftalmología se remonta al principio de la década de los ochenta. En base a la evidencia disponible, las gotas oftalmológicas de CsA parecen ser seguras y no producen efectos secundarios graves [318]. Algunas de las propiedades fisicoquímicas desfavorables de la CsA se han corregido con éxito mediante la mejora de la disponibilidad ocular y la mejora de la tolerancia [319]. Sin embargo, sólo unas pocas formulaciones de la CsA están disponibles comercialmente, aunque la extensa literatura sobre la entrega de la CsA refleja el gran interés médico sobre estos compuestos. Teniendo en cuenta el desarrollo de nuevas formulaciones para la administración tópica, la adicción de nuevas tecnologías basadas en nanopartículas, en emulsiones con carga positiva y la generación de pro-fármacos de CsA, este grupo de fármacos se han convertido en candidatos muy prometedores en el tratamiento del ojo seco. Sin embargo, ninguno de los sistemas tópicos descritos, realmente ha tenido éxito en extender el período de tiempo de la aplicación del compuesto en la superficie corneal. Por lo tanto, la frecuencia de administración persiste como uno de los problemas asociado a estos sistemas. En este sentido existen actualmente alternativas en evaluación, como la administración de estas sustancias a través de lentes de contacto cargadas con CsA, que podrían solventar estos problemas en el caso de que los ensayos clínicos en curso tuvieran éxito [320].

Otra alternativa terapéutica frente a los corticoides se presenta en forma de compuestos inmunomoduladores. Actualmente al menos dos anti-inflamatorios no esteroides están en desarrollo por las empresas ISTA Pharmaceuticals e InSite Vision, combinando una alta penetración y acción terapéutica a baja dosis. Los resultados obtenidos hasta la fecha resultan prometedores, destacando su eficacia en el tratamiento del ojo seco, y un nivel de tolerancia aceptable en los pacientes. No obstante, debemos reseñar que no existen estudios a largo plazo sobre estas substancias, lo que genera incertidumbre sobre los efectos adversos de una medicación prolongada. A esta incertidumbre hemos de añadir como inconveniente, los altos costos derivados de esta terapia[320].

Como ya se ha referido con anterioridad en este trabajo, la enfermedad del ojo seco presenta una gran variabilidad en cuanto a signos y síntomas clínicos, con frecuencia no correlacionados. A este respecto, podemos concluir que una comprensión más profunda de los mecanismos celulares implicados en el ojo seco es necesaria, no sólo para mejorar los tratamiento sintomatológicos, sino también para restaurar la homeostasis de la superficie ocular [321].

Además, los criterios de valoración utilizados en los diferentes ensayos clínicos varían, lo que dificulta la comparación entre los distintos tratamientos. En este sentido es necesario el desarrollo y la inclusión en estos ensayos de métodos objetivos, con criterios certeros y fijos que puedan evaluar la evolución de la enfermedad frente al tratamiento, y simplificar así el análisis de los resultados obtenidos a partir de diversos tratamientos.

La búsqueda de estrategias eficaces en el tratamiento del ojo seco se ve irremediablemente frenada por la ausencia de un conjunto aceptado de criterios definitivos de evaluación de gravedad de la enfermedad. La falta de consenso en la comunidad clínica y científica refleja de igual forma la ausencia de una prueba diagnóstica objetiva que resulte además efectiva en la evaluación de la gravedad de la enfermedad; ya que las evaluaciones actuales, basadas en los síntomas, resultan insuficientes como factor de medición. En este dirección se han propuesto diferentes marcadores moleculares [322, 323] o la evaluación de la osmolaridad de la película lagrimal [324, 325] como pruebas objetivas de evaluación de esta patología.

En esta misma dirección, nuestro equipo de trabajo ha iniciado una línea de investigación basada en los nucleótidos polifosfatos. Los resultados aportados por nuestro laboratorio identifican estas substancias como componentes esenciales de la lágrima, e implicados directamente en la regulación de ésta. De esta forma la instilación en el ojo de ciertos nucleótidos polifosfato, tiene como respuesta un aumento en la secreción lagrimal [326].

Los nucleótidos presentes en la lágrima se han relacionado con diversos procesos activos relacionados no sólo con la regulación de ésta, sino también con procesos de cicatrización y de protección frente a infecciones de la superficie ocular [327]. Estas substancias además intervienen en muy diversos procesos en otras estructuras oculares como la contracción pupilar, la producción de humor acuoso [326] y modificando la fisiología de las células de la retina, especialmente las ganglionares [328]. Por este motivo, la comprensión de los mecanismos y funciones de estas moléculas no sólo puede ayudar a entender de una forma más precisa el funcionamiento y regulación del ojo, sino también a buscar nuevas terapias frente al ojo seco [329].

Los estudios actuales han identificado al nucleótido Ap_4A como uno de los implicados en la regulación lagrimal, ya que su instilación produce un aumento significativo de la secreción lagrimal. Aún más, la instilación tópica del Ap_4A conjuntamente con la melatonina provoca un aumento más sustancial de la producción de lágrima, por lo que un tratamiento conjunto con ambas substancias podría ser una alternativa terapéutica a las terapias actuales [330].

Más importante aún que su funcionamiento como fármaco es su posible papel como biomarcador de esta patología. Los estudios realizados en nuestro laboratorio señalan a esta molécula como un claro candidato a biomarcador del ojo seco. Como ya hemos mencionado el Ap_4A se encuentra en la lágrima, sin embargo sus niveles son anormalmente elevados en pacientes diagnosticados de ojo seco o en aquellos que presentan signos o síntomas de esta enfermedad [331]. De esta forma nuestro grupo de trabajo en la actualidad está desarrollando un nuevo método de diagnóstico no invasivo, basado en un biomarcador objetivo que podría predecir la aparición de los síntomas antes de que aparezcan los signos de la enfermedad [331]. Este biomarcador se queda recogido en la patente "Método de diagnóstico molecular del ojo seco mediante la determinación de los niveles de diadenosina tetrafosfato" (ref: WO/2007/128851)[331]. En la actualidad la metodología desarrollada se encuentra en la fase inicial del estudio clínico, como parte de un proyecto financiado por la Unión Europea ("Validation of a Novel Diagnostic Biomarker for Dry Eye Syndrome based in nucleotides detection", convocatoria H2020, 2015), y cuyo objetivo final es validar la sensibilidad y especificidad de este nuevo biomarcador.

Finalmente, a pesar de la evidente incertidumbre sobre los progresos que se puedan producir en este campo en el futuro, podemos inferir, a partir de los datos actuales, que los futuros tratamientos frente al ojo seco seguirán obedeciendo y corrigiendo principalmente la sintomatología que acompaña a esta enfermedad; sin embargo, abordarán esta problemática desde una visión más completa (incluyendo los síntomas inflamatorios). De esta forma, el tratamiento de los síntomas leves o moderados incluiría la utilización de lubricantes oculares novedosos, ciclosporina A, nuevos secretagogos y estimulantes de la producción de mucinas, y esteroides tópicos suaves. En casos de sintomatología grave, el tratamiento incluirá terapias más agresivas como el uso de esteroides tópicos con ciclosporina oral o la inclusión de otros anti-inflamatorios.

Por lo tanto, el desarrollo de nuevos lubricantes oculares de acción múltiple, generados por la adición de moléculas activas a éste, parece ser una solución atractiva para lograr resultados efectivos con rapidez y prolongados.

5.2.2 REPERCUSIONES SOCIOECONÓMICAS

El síndrome de ojo seco afecta a millones de personas alrededor del mundo con consecuencias socioeconómicas significativas, especialmente cuando se incluyen los gastos asociados a la atención de la salud (por ejemplo, medicamentos y visitas al médico), bajas laborales asociadas y con un impacto importante en el funcionamiento social y de calidad de vida [332-335].

Hasta fechas recientes el tratamiento tradicional para el ojo seco ha sido en gran medida paliativo, incluyendo gotas para los ojos, lubricantes de venta libre o lágrimas artificiales. El aumento en el conocimiento de la fisiopatología de ojo seco ha dado lugar a grandes avances en el tratamiento en las dos últimas décadas. Últimamente se han introducido nuevas modalidades farmacéuticas y actualmente se encuentran en fase de ensayos clínicos 49 moléculas diferentes, para el tratamiento del síndrome del ojo seco. En particular, en la actualidad existen tres productos en fase final de los ensayos clínicos, cuyo novedoso abordaje terapéutico incluye como diana el tratamiento de la inflamación asociada a esta patología.

A pesar de esta novedosa aproximación terapéutica, los oftalmólogos sugieren que la mayor necesidad no satisfecha en el tratamiento del ojo seco es su diagnóstico. El seguimiento del coste/efectividad del tratamiento de ojo seco, es bastante difícil debido a la naturaleza multifactorial de la enfermedad y las posibles limitaciones de las técnicas disponibles para evaluar los resultados terapéuticos de las modalidades de tratamiento multi-paliativo utilizado.

Con la introducción de nuevas modalidades farmacéuticas como la emulsión de ciclosporina tópica [336, 337], las drogas mucomiméticas y los suplementos orales [333, 338], que tienen un precio de adquisición sustancial, la carga en el presupuesto de los pacientes ha aumentado significativamente. El “pipeline” del síndrome del ojo seco es fuerte, con 49 moléculas en diferentes fases de desarrollo en el año 2015 [339] como se ha comentado. Debemos tener en cuenta que el aumento en el coste asociado al tratamiento del ojo seco puede afectar al cumplimiento del mismo, la elección del tratamiento por los médicos y el almacenamiento de los medicamentos hospitalarios.

Está previsto que el mercado global asociado a esta patología ocular alcance los 5.000€ millones de euros en el año 2022 [339]. Este crecimiento en el volumen de negocio esperado responde principalmente a factores como el lanzamiento de nuevos fármacos en tramitación y al envejecimiento progresivo de la población mundial. A pesar de que existe una falta generalizada de datos publicados sobre la utilización de recursos sanitarios en la gestión de ojo

seco [340], sólo en los Estados Unidos el gasto de la gestión de los pacientes en las organizaciones de atención de salud se estima en 600.000€ por cada millón de pacientes [341]. Los costes absolutos pueden ser mucho más altos para las poblaciones asiáticas, ya que la prevalencia de esta enfermedad es mucho mayor en estas poblaciones (prevalencia del 30%) en comparación con poblaciones caucásicas predominantes (prevalencia del 15%) (Tabla 4) [341].

En el caso de los países pertenecientes al continente europeo existen grandes variaciones en los costes asociados al tratamiento del ojo seco. Los gastos derivados de esta enfermedad por cada 1000 pacientes (tratados en consulta oftalmológica) oscilan entre los 200.000€ destinados en Francia, al 1.000.000 € destinado en Alemania o el 1.300.000€ destinado en el Reino Unido [342, 343].

Dada la limitación de los datos económicos disponibles, el síndrome de ojo seco escapa a la cuantificación real de la carga directa del gasto sanitario en los países investigados. Este fenómeno está muy relacionado con los hábitos poblacionales, en los que una gran proporción de los pacientes de este síndrome, recurre al auto-tratamiento sintomatológico, mediante la utilización de lágrimas artificiales *over-the-counter* y otros medicamentos. A esta situación se añaden los casos en los que esta dolencia y su tratamiento farmacológico son administrados por el médico de atención primaria. Por consiguiente, los verdaderos costes sociales del síndrome de ojo seco han sido repetidamente infravalorados y las cuantías y carga fiscal para los sistemas de salud nacionales son mucho mayores a los calculados teóricamente [341].

Predecimos que, en un futuro próximo, el mercado farmacológico del ojo seco se volverá significativamente más competitivo, sin embargo, la actual escasez de opciones en el tratamiento de este síndrome deja mucho espacio para nuevas terapias innovadoras y es por lo tanto un mercado factible en expansión.

5.3 TABLAS

Tabla 1. Datos de los fármacos actuales de Glaucoma
β-Antagonistas adrenérgicos (no-selectivos)-disminución de la producción de humor acuoso
Carteolol (dosis 2 veces al día)
Levobunolol (dosis 1-2 veces al día)
Metipranolol (dosis 2 veces al día)
Timolol (dosis 1-2 veces al día)
Contraindicaciones: Asma, enfermedad pulmonar obstructiva, bradicardia sinusal, bloqueo cardíaco.
Efectos adversos: alergia, irritación, visión borrosa, anestesia corneal, queratitis, alergia, bradicardia, bloqueo cardíaco, broncoespasmo, disminución de la libido, depresión del SNC.
β-Antagonistas adrenérgicos (selectivos)-disminución de la producción de humor acuoso
Betaxolol (dosis 1-2 veces al día)
Contraindicaciones: bradicardia sinusal, bloqueo cardíaco o asma insuficiencia cardíaca (contraindicación relativa), la enfermedad pulmonar obstructiva.
Efectos adversos: Vista borrosa, irritación, anestesia corneal, queratitis punctata, alergia, bradicardia, bloqueo cardíaco, broncoespasmo, disminución de la libido, depresión del SNC.
Inhibidores de la anhidrasa carbónica-disminución de la producción de humor acuoso
Brinzolamida (dosis 1-2 veces al día)
Dorzolamida (dosis 1-2 veces al día)
Contraindicaciones: Alergia grave enfermedad renal y sulfonamida; se debe tener precaución en pacientes con comprometido endotelio corneal.
Efectos adversos: Irritación, miopía inducida, borrosos, queratitis, escozor, ardor, conjuntivitis, dermatitis, dolor de cabeza, náuseas, fatiga, sabor amargo.
Agonistas adrenérgicos (no selectivos)-aumento del flujo del humor acuoso
Epinefrina (adrenalina) (dosis 2 veces al día)
Dipivefrine (dosis 2 veces al día)
Contraindicaciones: ángulo de la cámara anterior estrecha y afaquia
Efectos adversos: Irritación, alergia, hiperemia conjuntival, hipertensión, dolor de cabeza, taquicardia (menos probable para pro fármaco).
Agonistas adrenérgicos (α2-selectivos)-disminución de la producción de humor acuoso, aumento del flujo del humor acuoso
Apraclonidine (dosis 2-3 veces al día)
Brimonidina (dosis 2-3 veces al día)
Contraindicaciones: la edad, el uso de monoaminooxidasa, el uso de fármacos antidepresivos, el ángulo de la cámara anterior estrecha y afaquia.
Efectos adversos: Irritación, alergia, visión borrosa, sensación de cuerpo extraño, edema de párpados, sequedad, dolor de cabeza, fatiga, mareos, taquicardia, depresión, sequedad en la boca.

Lípidos hipotensores (análogos de la prostaglandina)-aumento de flujo de salida del humor acuoso uveoescleral
Latanoprost (dosis 1 vez al día) Travoprost (dosis 1 vez al día) Contraindicaciones: Historia o el riesgo de edema macular quístico o uveítis (contraindicaciones relativas). Efectos adversos: Aumento de la pigmentación del iris y de las pestañas, hypertrichiasis, hiperemia conjuntival, blefaritis, síntomas similares a la gripe, dolor en las articulaciones/músculos, dolor de cabeza, aumento de la presión arterial.
Lípidos hipotensores (prostamidas)-aumento de flujo de salida del humor acuoso uveoescleral
Bimatoprost (dosis 1 vez al día) Contraindicaciones: Historia o el riesgo de edema macular quístico o uveítis (contraindicaciones relativas). Efectos adversos: aumento de la pigmentación del iris y de las pestañas, hypertrichiasis, hiperemia conjuntival, blefaritis, síntomas similares a la gripe, dolor en las articulaciones/músculos, dolor de cabeza, aumento de la presión arterial.
Lípidos hipotensores (decosanoidos)-aumento de flujo de salida del humor acuoso uveoescleral
Unoprostone (dosis 2 veces al día) Contraindicaciones: Historia o el riesgo de edema macular quístico o uveítis (contraindicaciones relativas). Efectos adversos: aumento de la pigmentación del iris y de las pestañas, hypertrichiasis, hiperemia conjuntival, blefaritis, síntomas similares a la gripe, dolor en las articulaciones/músculos, dolor de cabeza, aumento de la presión arterial.

Tabla 2. Efectos sobre la PIO de los análogos de la melatonina		
Compuesto	**pD_2**	**Max.% IOP**
Melatonina	9.3±0.24	22.0±1.6
5-MCA-NAT	8.9±0.07	42.5±1.6
Agomelatina	9.7±0.28	20.8±1.4
IIK7	7.1±0.31	38.5±3.2
INS48848	5.5±0.21	36.0±2.0
INS48862	5.7±0.33	26.0±1.3
INS48852	5.5±0.20	33.1±1.4

Tabla 3. Datos económicos de los medicamentos de Glaucoma en España en el año 2014							
Nombre de la medicina en el mercado	**Tamaño mg/ml**	**Contenido por frasco ml**	**Nº de gotas/ml**	**Régimen dosis/día = gotas/día por ojo**	**Nº días/frasco**	**Precio 2014 en €**	**Precio anual en €**
Prostaglandinas							
LUMIGAN	0.1	3	32	2=2	24	16,69	400,56
TAFLUPROST	0.3	3	32	2=2	24	20,51	492,24
TRAVATAN	0,004	2,5	40	2=2	25	20,01	500,25
XALATAN	0,005	2,5	30	2=2	18,75	11,22	210,37
Agonistas a_2-adrenérgicos							
ALPHAGAN	0,2	5	23	4=4	14,37	7,31	105,08
BRIMONIDINA-genérica	0,2	5	25	4=4	15,62	7,31	114,21
Inhibidores de anhidrasa carbónica							
AZOPT	1	5	37	4=4	23,12	12,75	294,84
TRUSOPT	20	5	24	4=4	15	5,12	76,80
Combinaciones							
COSOPT	20/5	5	22	4=4	13,75	12,11	166,51
Beta-bloqueantes							
BETOPTIC	0,25	5	24	2=2	30	3,67	110,10
Beta-bloqueantes genéricos							
BETAXOLOL	0,5	5	31	4=4	19,37	3,56	68,95
DORZOLAMIDA COLIRTEVA	20	5	27	4=4	16,87	5,12	86,37
DORZOLAMIDA FDC PHARMA	20	5	27	4=4	16,87	5,12	86,37
DORZOLAMIDA KERN PHARMA	20	5	27	4=4	16,87	5,12	86,37
DORZOLAMIDA PHARMATEN	20	5	27	4=4	16,87	5,12	86,37
DORZOLAMIDA TIMOLOL	20/5	5	30	4=4	18,75	12,11	227,06

Tabla 4. Compuestos recetados según la severidad de la enfermedad en España		
Ojo Seco Leve	**Ojo Seco Moderado**	**Ojo Seco Severo**
Ácido hialurónico (50% de los pacientes) Hipromelosa (25% de los pacientes) Alcohol de polivinilo (17% de los pacientes) Duración del tratamiento de 52 semanas	Carmelosa de sodio (25% de pacientes) Parafina (17% de los pacientes) Cloruro de sodio (17% de los pacientes) Povidona (15% de los pacientes) hipromelosa (15% de los pacientes) Alcohol polivinílico (12% de los pacientes) Duración del tratamiento de 52 semanas	Carmelosa de sodio (34% de los pacientes) Duración del tratamiento de 52 semanas

6. CONCLUSIONES

1. El acceso a nuevas técnicas de genómica, metabolómica y proteómica ha generado varios candidatos a biomarcadores moleculares en el diagnóstico del glaucoma, con un potencial valor clínico. Pese a estos descubrimientos, en la actualidad ninguna de las moléculas estudiadas puede considerarse como un biomarcador definitivo en la prevención y diagnóstico precoz, ya que ninguna de estas moléculas ha superado los ensayos clínicos.

2. En el ámbito del tratamiento de glaucoma, se puede constatar la existencia de tres líneas fundamentales o estrategias básicas encaminadas a reducir las desventajas asociadas a las terapias farmacológicas. La mejora de los medicamentos existentes, el desarrollo de nuevas combinaciones de fármacos y el diseño de nuevos fármacos con mecanismo de acción innovadores.

3. Los nuevos fármacos en fase de desarrollo pretenden mejorar el flujo sanguíneo ocular, particularmente en la retina y la cabeza del nervio óptico y tienen como dianas, la contracción de células de la MT, la inhibición de la producción de humor acuoso y la neuroprotección.

4. Una de las líneas más prometedoras dentro de los nuevos tratamientos antiglaucomatosos es el estudio de la melatonina y sus derivados. Los datos hallados demuestran un interés considerable en el diseño y la síntesis de nuevos análogos, no sólo más estables metabólicamente, sino también más selectivos.

5. En la actualidad existe un amplio interés en el estudio de fármacos anti-glaucomatosos que tienen como diana la inhibición de las Rho-quinasas. Éstos, producen un aumento en el drenaje del humor acuoso a la par que un aumento del flujo sanguíneo en el nervio óptico, reportándose además retrasos en la apoptosis del nervio óptico.

6. A pesar de los esfuerzos realizados, la falta de conocimiento de los desencadenantes y los factores subyacentes a esta enfermedad, dificultan el avance en la incorporación

de biomarcadores y fármacos que cumplan con las expectativas de especificidad y efectividad requeridos.

7. Esta situación responde en última instancia a la gran complejidad de esta enfermedad, por lo que estimamos que son necesarias nuevas redes médicas capaces de compartir recursos, crear nuevas bases de datos y establecer directrices comunes para la investigación, con el fin de definir, con precisión, el perfil molecular del paciente con glaucoma, las causas subyacentes de esta enfermedad y la validación objetiva de nuevos tratamientos y biomarcadores.

8. Durante los últimos años el desarrollo de nuevos fármacos para el tratamiento del ojo seco se ha convertido en un objetivo clave para la industria farmacéutica. En un futuro próximo, el mercado farmacológico del ojo seco se volverá significativamente más competitivo, sin embargo, la actual escasez de opciones en el tratamiento de este síndrome deja mucho espacio para nuevas terapias innovadoras y es por lo tanto un mercado factible en expansión.

9. A diferencia de los fármacos anteriores, meros sustitutos artificiales de la lágrima, los nuevos compuestos centran su acción principal en el control de la inflamación o la restauración de los niveles normales de cantidad y calidad de lágrima y mucina endógena.

10. Aun teniendo en cuenta este cambio de dirección en el abordaje terapéutico de los nuevos fármacos, éstos mantienen la tendencia actual de contrarrestar los síntomas, sin profundizar en los mecanismos causales del ojo seco.

11. La aparición de nuevos estudios sobre moléculas como el Ap_4A, revela la existencia de potenciales biomarcadores, disponibles para el diagnóstico objetivo, no invasivo y con capacidad para ser trasladado al uso clínico; aumentando las expectativas sobre la mejora en el desarrollo de los futuros tratamientos.

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