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Review Article

Hyaluronic acid in the treatment of dry eye disease

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ABSTRACT.

Dry eye disease (DED) is a highly prevalent and debilitating condition affecting several hundred million people worldwide. Hyaluronic acid (HA) is a naturally occurring glycosaminoglycan commonly used in the treatment of DED. This review aims to critically evaluate the literature on the safety and efficacy of artificial tears containing HA used in DED treatment. Literature searches were conducted in PubMed, including MEDLINE, and in Embase *via* Ovid with the search term: "(hyaluronic acid OR hyaluronan OR hyaluronate) AND (dry eye OR sicca)". A total of 53 clinical trials are included in this review, including eight placebo-controlled trials. Hyaluronic acid concentrations ranged from 0.1% to 0.4%. Studies lasted up to 3 months. A broad spectrum of DED types and severities was represented in the reviewed literature. No major complications or adverse events were reported. Artificial tears containing 0.1% to 0.4% HA were effective at improving both signs and symptoms of DED. Two major gaps in the literature have been identified: 1. no study investigated the ideal drop frequency for HA-containing eyedrops, and 2. insufficient evidence was presented to recommend any specific HA formulation over another. Future investigations assessing the optimal drop frequency for different concentrations and molecular weights of HA, different drop formulations, including tonicity, and accounting for DED severity and aetiology are essential for an evidence-based, individualized approach to DED treatment.

Key words: artificial tears – dry eye disease – dry eye treatment – hyaluronate – hyaluronic acid

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For the sake of transparency, Tor Paaske Utheim is co-founder and co-owner of The Norwegian dry eye clinic and the Clinic of eye health, Oslo, Norway, which delivers talks for and/or receives financial support from: ABIGO, Alcon, Allergan, AMWO, Bausch&Lomb, Bayer, European school for advanced studies in ophthalmology, InnZ Medical, Medilens Nordic, Medistim, Novartis, Santen, Specsavers, Shire Pharmaceuticals and Thea Laboratories. He has served on the global scientific advisory board for Novartis and Alcon, and on the European advisory board for Shire Pharmaceuticals. Utheim is the Norwegian Global Ambassador for the Tear Film and Ocular Surface Society (TFOS), a Board Member of the International Ocular Surface Society, an international member of the Japanese Lid and Meibomian gland working group (LIME), a Consultant at the Norwegian Association for the Blind and Partially Sighted, the President of the Oslo Society of ophthalmology, and the Editor-in-Chief of the eye journal Oftalmolog which contains advertisements from pharmaceutical companies, companies selling ophthalmological equipment, and associations organizing conferences and events in ophthalmology.

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Introduction

Dry eye disease (DED) is a complex and debilitating inflammatory condition of the ocular surface. World-wide epidemiological studies find that DED prevalence ranges from 5–50% (Stapleton et al. 2017). When extrapolated, this would equate to between 400 million and 3.7 billion DED patients globally. Dry eye disease increases with age, with the prevalence of the condition rising steeply from around 50 years of age (Vehof et al. 2021). Through limiting participation in working life and daily activities and increasing healthcare costs, DED introduces a substantial financial burden for both the individual patient and society as a whole (Stapleton et al. 2017). The estimated yearly direct and indirect costs of DED to the US society alone is 56 billion USD (Yu et al. 2011). For patients, DED causes a considerable reduction in quality of life and quality of vision (Morthen et al. 2021, 2022).

Dry eye disease results in deterioration of the ocular surface including dysfunction of the tear film, lacrimal system, eyelids, conjunctiva, and cornea (Bron et al. 2017). The healthy tear film serves to protect and lubricate the ocular surface by providing a physical, chemical, and immunological barrier to the environment. The tear film consists of an inner muco-aqueous layer and an outer lipid layer that combined contribute to a stable ocular surface in a normal eye (Fig. 1; Craig et al. 2017). Aetiologically, DED is divided into aqueous deficient, evaporative, and mixed types. In DED, regardless of aetiology, ocular surface instability promotes a vicious circle of inflammation, exacerbating signs and symptoms of disease, and damage of the ocular surface (Bron et al. 2017). Breaking this vicious circle, plays an essential role in the treatment of DED.

Artificial tears are the first-line treatment for DED. They help restore and stabilize the tear film and protect the ocular surface (Jones et al. 2017). This aids in slowing or stopping DED progression which decreases signs and symptoms and prevents further damage (Nebbioso et al. 2016). There is a wide array of artificial tears on the market with various active ingredients. One clinically proven and commonly used component is hyaluronic acid (HA; Ang et al. 2017).

Hyaluronic acid is a naturally occurring, non-toxic (Debbasch et al. 2002) glycosaminoglycan disaccharide bi-polymer (Silvani et al. 2020; Fig. 2). Hyaluronic acid serves several crucial purposes in the human body, including joint and tendon lubrication (Lin et al. 2019) and cell-to-cell communication (Bayer 2020). Hyaluronic acid was first discovered in bovine vitreous humour in the 1930s and has since become an important addition in many fields of medicine (Kotla et al. 2021). It is a frequently used component of slow-release drug formulas (Bayer 2020), skin-care products, and fillers for cosmetic and reconstructive purposes (Vorvolakos et al. 2011). Due to HA's safety profile and physiological effects, it has become an important substance in ophthalmology (Higashide & Sugiyama 2008; Salwowska et al. 2016; Fig. 3).

Hyaluronic acid is found naturally in the tear film, outer cornea, and vitreous humour (Posarelli et al. 2019). However, the highest concentration of HA is found as a chief component of the extracellular matrix in soft connective tissues (Gudowska-Sawczuk et al. 2017; Mateo Orobia et al. 2018). Under physiological conditions, HA takes the form of a highly hydrophilic, negatively charged bipolymer. It is made of repeating units of N-acetyl-d-glucosamine and d-glucuronic acid. These units are linked together *via* alternating β-1,3 and β-1,4 glycosidic bonds (Fig. 2). The *in-vivo* structure of HA is largely homogeneous, with variable chain lengths and occasional deacetylated glucosamine residues (Mateo Orobia et al. 2018; Bayer 2020). Hyaluronic acid exhibits high pseudo-plasticity and introduces non-Newtonian mechanics in fluids (Chernos et al. 2017). This means that the viscosity of the liquid changes depending on the applied shear forces. At the ocular surface, the viscosity of an HA-containing tear film will decrease during a blink, allowing even distribution of the tear film. Once at rest, higher viscosity is restored which prolongs its residence time on the ocular surface (López-García et al. 2014; Fig. 3).

Hyaluronic acid is rich in hydroxyl-groups that attract water molecules, thus thickening and stabilizing the tear film (Kaya et al. 2015; Szegedi et al. 2018), and reducing the effects of mechanical trauma to the ocular surface by lubrication (van Setten 2020), and contributing to re-epithelialization (Carlson et al. 2018; Fig. 1). Hyaluronic acid also reduces evaporation from the ocular surface (Tsubota & Yamada 1992), the driving

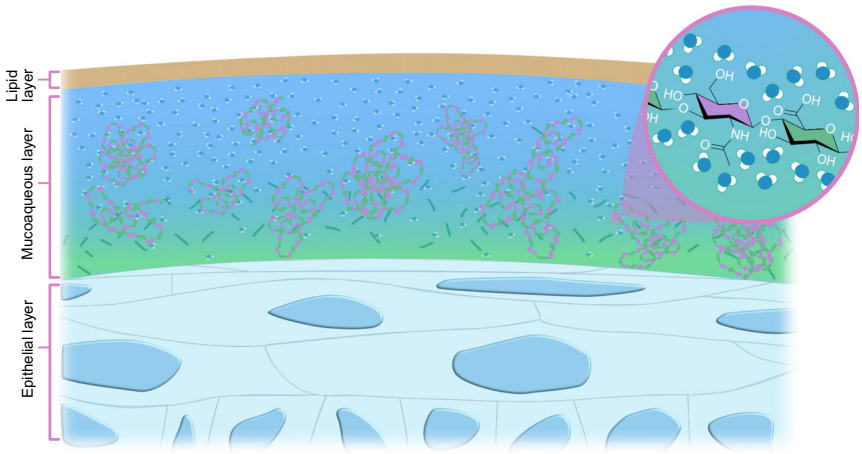

Fig. 1. Illustration of the pre-corneal tear film containing hyaluronic acid. Hyaluronic acid attracts surrounding water molecules with its numerous hydroxyl groups, thickening and stabilizing the mucoaqueous layer of the tear film. *Illustration by Emily Moschowits.*

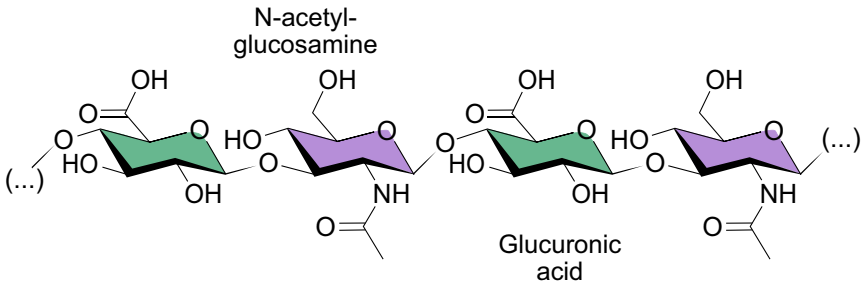

Fig. 2. The hyaluronic acid molecule made up of repeating N-acetyl-glucosamine and glucuronic acid linked together *via* alternating β-1,3 and β-1,4 glycosidic bonds. *Illustration by Emily Moschowits.*

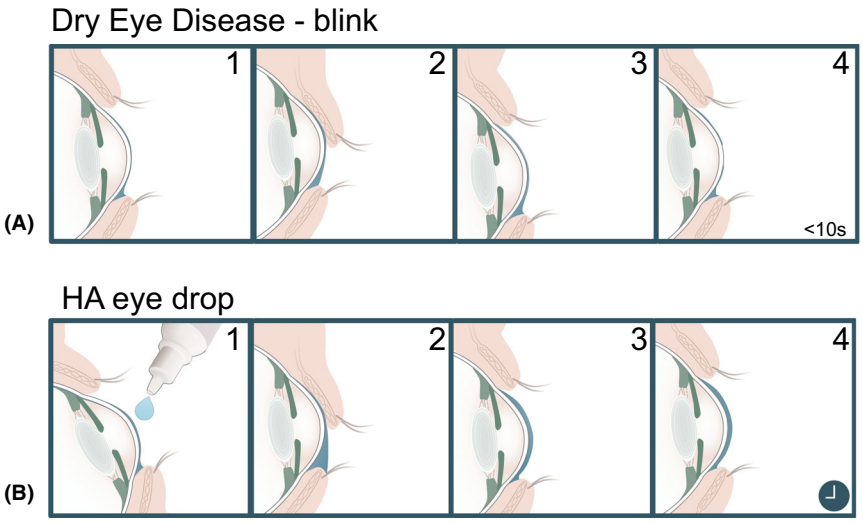

Fig. 3. Hyaluronic acid increases tear film stability. (A, 1–4) Cross section of a thin tear film in dry eye disease with short tear film break up time (TBUT). (B, 1–4) After application of hyaluronic acid, the tear film increases in viscosity and thickness and allows for even distribution across the ocular surface. Proportions are exaggerated for illustrative purposes. *Illustration by Emily Moschowits.*

force behind hyperosmolarity, which in turn is one of the main causes of inflammation and ocular surface damage in DED (Bron et al. 2017). Additionally, HA binds to hyaladherins, also known as hyaluronan-binding protein family receptors, which are expressed throughout the body including by the epithelial cells of the ocular surface (Lardner & van Setten 2020). Binding to these receptors activate various intracellular signalling pathways dependent on concentration, molecular weight and modifications of the HA molecule (Abatangelo et al. 2020), which can modulate inflammation, cellular migration and angiogenesis, which are the main phases of wound healing (Litwiniuk et al. 2016). Hyaluronic acid also protects damaged surfaces during wound healing (Debbasch et al. 2002; Pauloin et al. 2009b; Rah 2011; Carracedo et al. 2019; Lardner & van Setten 2020; Kotla et al. 2021). The specific biological effects and physical properties of the HA molecule vary with changing molecular weight, which ranges several orders of magnitude, from a few- to several thousand kilodaltons (Snetkov et al. 2020; Kotla et al. 2021). Generally, low molecular weight HA tends to have pro-inflammatory properties and lower viscosity while high molecular weight HA is anti-inflammatory and more viscous (Snetkov et al. 2020; Kotla et al. 2021).

Hyaluronic acid has been used as a viscoelastic for intraocular surgery since the 1970s (Higashide & Sugiyama 2008). The first study exploring the effects of HA on DED was published in 1982 (Polack & McNiece 1982). Four years later Mengher et al. (1986) showed that eye drops containing 0.1% HA could improve tear film break-up time (TBUT) in patients with DED. Since then, HA has become a key component in many artificial tear fluids, improving lubrication and tear film properties. The number of commercial options available on the market that use HA are ever growing (Salwowska et al. 2016). Figure 4 shows the increasingly important role of HA in ophthalmology over time.

Several meta-analyses and reviews have shown that HA is safe and effective in the treatment of DED (Doughty & Glavin 2009; Salwowska et al. 2016; Ang et al. 2017; Yang et al. 2021). This review will summarize and discuss the current literature on the treatment of DED with artificial tears containing HA, the safety and efficacy of HA in treating DED, explore how physiochemical properties of various HA formulations may influence treatment, and shed light on gaps in the literature.

Methods

A literature review was conducted in Embase using Ovid on the 24th of

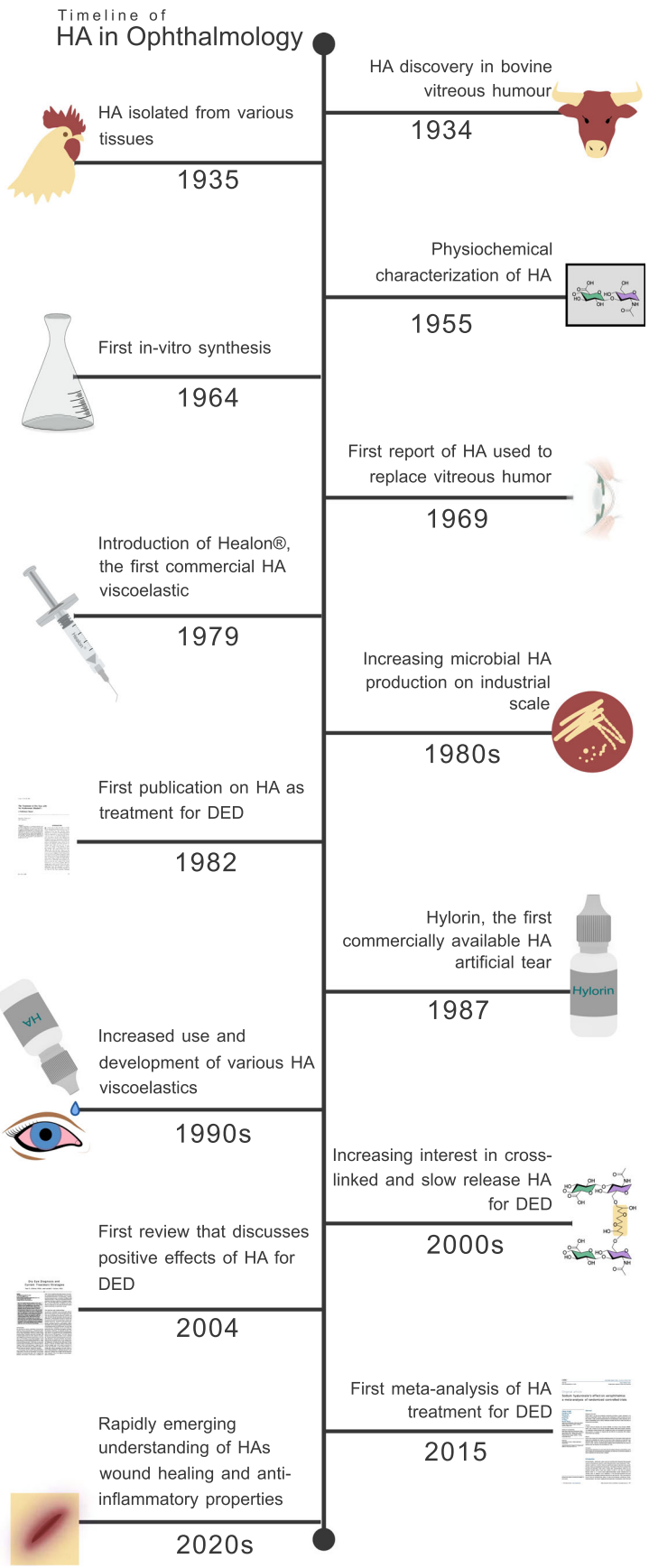

Fig. 4. Timeline of the discovery and development of HA in ophthalmology. DED = dry eye disease, HA = Hyaluronic acid. *Illustration by Emily Moschowits*. [Correction added on 14-May-2022, after first online publication: Figure 4 was corrected in this version.]

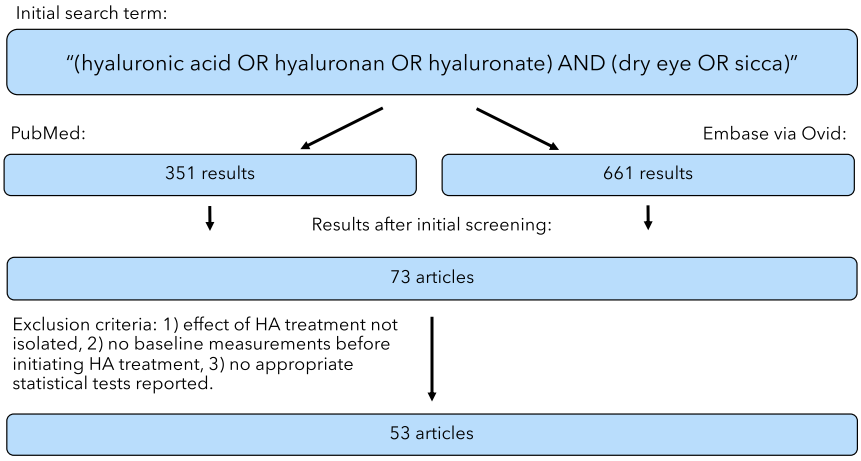

Fig. 5. Methodology for determining studies of relevance for the present review.

August 2021 and PubMed, including MEDLINE, on the 20th of September 2021 (Fig. 5). The search terms "(hyaluronic acid OR hyaluronan OR hyaluronate) AND (dry eye OR sicca)" were used in both searches. All original English language published articles available in full text were considered. Titles and abstracts were screened to ensure relevance to the topic. Reviews, meta-analyses, case studies, and papers on unrelated subjects were not considered. When duplicates were identified, the latest version was included. Studies were narrowed down by checking against the exclusion criteria: 1) effect of HA treatment not isolated, 2) no baseline measurements before initiating HA treatment, 3) no appropriate statistical tests reported. Only studies investigating treatment of DED with artificial tears containing HA with reported statistical tests for subjective or objective measurements against baseline or placebo were included.

Results

Review of existing literature

The search term "(hyaluronic acid OR hyaluronan OR hyaluronate) AND (dry eye OR sicca)" in Embase the 24th of August 2021 through Ovid produced 661 results. The same search term in PubMed on the 20th of September 2021 produced 351 results. Studies that investigated eye drops containing HA along with other active ingredients like steroids, cyclosporine, trehalose, or polyethylene glycol were excluded if the effect of HA could not be isolated (Versura et al. 2010; Montani 2013; Macri et al. 2015; Pinto-Bonilla et al. 2015; Kim et al. 2017; Rolando & Vagge 2017; Fariselli et al. 2018; Fondi et al. 2018; Wu et al. 2021). Studies where the effects of HA treatment could not be isolated for other reasons were excluded (Laflamme & Swieca 1988; Ibrahim et al. 2012; Kamiya et al. 2012; Çakır et al. 2018). Studies without statistical tests comparing the results after HA treatment to either baseline values or to a control group receiving placebo were excluded (Limberg et al. 1987; Nepp et al. 2001; Matsuo 2004; Brignole et al. 2005; Baudouin et al. 2012; Robert et al. 2016; Labetoulle et al. 2018; van Setten et al. 2020).

Finally, 53 clinical trials remained. A flow chart of the process is shown in Fig. 5. The final list of clinical treatment studies (with some overlap due to variations in combinations of study design) includes 8 randomized placebo-controlled trials (RCTs), 44 baseline controlled RCTs, 6 baseline controlled non-randomized prospective-longitudinal studies, and 10 cross-over studies (Tables 1 and 2). The studies recruited patients in 17 different countries across Europe, Asia, North America, and Africa, including 5 multicenter studies that recruited patients in more than one country (Condon et al. 1999; Baeyens et al. 2012; Gong et al. 2015; Chiambaretta et al. 2017; Labetoulle et al. 2017). The geographical spread of the studies is shown in Fig. 6. Tables 1 and 2 summarize the results of each article. Table 1 summarizes the 50 studies that provided HA treatment results compared to baseline, focusing on reported efficacy in subjective and objective measures. Table 2 summarizes results of HA against placebo in the 8 RCTs that had a double-blinded study design with a placebo group. Five studies are represented in both tables as they provided statistical results both against baseline and against placebo (Condon et al. 1999; Vogel et al. 2010; Baeyens et al. 2012; López-de la Rosa et al. 2017; Pinto-Fraga et al. 2017). Seven studies comparing HA treatment against baseline (Papa et al. 2001; Aragona et al. 2002a; Milafzzo et al. 2002; Troiano & Monaco 2008; Lee et al. 2014a; Groß et al. 2017; Park et al. 2017) and one study comparing against placebo (Sand et al. 1989) had more than one HA treatment arm, making a total of 58 treatment arms in Table 1 and 9 treatment arms in Table 2. Important factors examined in these tables includes the type of study, sample size, disease severity of the sampled population, HA concentration, drop frequency, patient outcomes at last follow up, and other key findings. The results are broken down into subjective and objective measures and compared across articles. Safety features are not represented in the tables as there were no serious adverse effects associated with HA use in any of the studies.

Changes in subjective scores against baseline in treatment studies

As seen in Table 1, 45 treatment studies provided subjective symptom data in 53 treatment arms on HA treatment compared to baseline (DeLuise & Peterson 1984; Nelson & Farris 1988; Condon et al. 1999; Iester et al. 2000; Papa et al. 2001; Aragona et al. 2002a; McDonald et al. 2002; Milafzzo et al. 2002; Lee et al. 2006; Rolando & Valente 2007; Johnson et al. 2008;

Table 1. Changes in commonly measured clinical signs and symptoms with HA treatment compared to baseline, sorted by publication date.

First Author (year)	Design	Population	Setup	Duration	Symp.	TBUT	OSS	Schi.	Other outcomes
Brar S. (Brar et al. 2021)	RCT	60 DED	0.1% HA x4/d	3 mo	↑	↑			↑ tear film osmolarity, ↑ TMH, ↑ MG loss, ↑ objective scatter index, ⟷ VA
Morya A. K. (Morya et al. 2021)	SB RCT	384 mild-to-severe DED	0.1% HA x4/d	2 mo	↑	⟷	↑	⟷	⟷ TMH
Balestrazzi A. (Balestrazzi et al. 2020)	OL RCT	19 DED with gastric reflux	0.2% HA x3/d	3 mo	↑	⟷		⟷	
Cai M. (Cai & Zhang 2020)	SB RCT	45 moderate-to-severe DED	HA x4/d	1mo	↑	⟷	↑	⟷	
Laihia J. (Laihia et al. 2020)	DB RCT	52 moderate-to-severe DED	0.2% x3/d	1 mo	↑	⟷	⟷		
Garcia-Conca V. (García-Conca et al. 2019)	SB RCT	84 mild-to-severe ADDE	0.18% HA x6/d	1 mo	↑		⟷	⟷	⟷ tear osmolarity, ⟷ hyperemia, ⟷ VA, ⟷ CIC measures
Kim Y. (Kim et al. 2019)	OL RCT	54 mild-to-moderate DED	0.15% HA x5-6/d	3 mo	↑	⟷	↑		
Essa L. (Essa et al. 2018)	SB RCT XO	50 DED	0.15% and 0.4% HA x3/d	1 mo	↑	⟷	↑/⟷	⟷[r]	↑ LIPCOF, ⟷ tear meniscus height,
Groß D. (Groß et al. 2018)	SB RCT	80 moderate DED	0.1% HA x3/d	3 mo	↑	⟷	↑		
Miháltz K. (Miháltz et al. 2018)	SB RCT	25 mild-to-moderate DED	0.2% HA x4/d	3 mo	↑	↑	↑	↑	
Postorino E. (Postorino et al. 2018)	SB RCT	40 mild-to-moderate DED	0.15% HA x4/d	3 mo	↑	⟷	↑		⟷ MG assessment, ⟷ corneal sensitivity, ⟷ VA
Roberti G. (Roberti et al. 2018)	SB RCT	39 DED using long term preserved glaucoma medication	0.2% HA x4/d	3 mo	⟷	⟷	⟷	⟷	⟷ goblet cell density by IVCM
Groß D. (Groß et al. 2017)	SB RCT	60 moderate-to-severe DED	0.2% HA x3/d	3 mo	↑	⟷	↑		
			0.18% HA x3/d		↑	⟷	↑		
Labetoulle M. (Labetoulle et al. 2017)	SB RCT	80 moderate-to-severe DED	0.18% HA hypotonic x2-6/d	3 mo	↑	⟷	↑	⟷	
Lambiase A. (Lambiase et al. 2017)	DB RCT	35 moderate DED	0.18% HA 2-6/d	2 w	↑		↑		
López-de la Rosa A. (López-de la Rosa et al. 2017)	DB RCT XO	16 moderate-to-severe DED	0.3% HA hypotonic x3-8/d	1 mo x 2	↑	↑	↑/⟷	⟷	↑ tarsal hyperemia, ⟷ bulbar hyperemia
Park Y. (Park et al. 2017)	SB RCT	176 moderate-to-severe DED	0.1% HA x5-6/d	3 mo	↑	↑	↑	⟷	⟷ MG parameters
			0.15% HA x5-6/d		↑	↑	↑	↑	
			0.3% HA x5-6/d		↑	↑	↑	⟷	
Pinto-Fraga J. (Pinto-Fraga et al. 2017)	DB RCT XO	16 mild DED	0.2% HA x3-8/d	1 mo x 2	↑	⟷	↑	⟷	↑ bulbar hyperemia, ⟷ tarsal hyperemia
Chiambaretta F. (Chiambaretta et al. 2017)	SB RCT	105 moderate-to-severe DED	0.18% HA x3-6/d	3 mo	↑	↑	↑	↑	↑ conjunctival hyperemia
Gong L. (Gong et al. 2015)	SB RCT	489 moderate DED	0.1% HA x6/d	1 mo	↑	↑	↑		
Lanzini M. (Lanzini et al. 2015)	PL	24 DED	0.2% HA x4/d	3 mo		⟷	↑	⟷	↑ IVCM, ⟷ CIC measures
Liu X. (Liu et al. 2015)	SB RCT	58 severe DED + glaucoma	0.3% HA x3/d	3 mo	↑	↑	↑	↑	↑ goblet cell density by CIC
Hwang H. S. (Hwang et al. 2014)	OL RCT	128 moderate ADDE	0.1% HA x4/d	3 mo	↑	↑	↑	↑	↑ goblet cell density and CIC grade
Lee H. S. (Lee et al. 2014a)	PL	30 mild DED	0.1% HA isotonic x4/d	3 mo	⟷	⟷	⟷	⟷	
			0.18% HA hypotonic x4/d		⟷	⟷	↑	↑	
Lee J. E. (Lee et al. 2014b)	OL RCT	86 moderate DED	0.1% HA x5/d	1 mo	↑	↑	⟷	⟷	↑/⟷ tear osmolarity
Aragona P. (Aragona et al. 2013)	DB RCT	40 moderate-to-severe DED, SS	0.15% HA x5/d	3 mo	↑	↑	⟷	⟷	↑ morphometric analysis by IVCM
Kinoshita S. (Kinoshita et al. 2013)	SB RCT	182 moderate DED	0.1% HA x6/d	1 mo	↑	↑	↑	↑	70% of patients reported symptomatic improvement with HA
Saeed N. (Saeed et al. 2013)	OL PL	240 DED	HA x2-4/d	2 mo	↑	↑		↑	
	DB RCT		0.18% HA x2-4/d	3 mo	↑	↑	↑	↑	

Table 1 (Continued)

First Author (year)	Design	Population	Setup	Duration	Symp.	TBUT	OSS	Schi.	Other outcomes
Baeyens V. (Baeyens et al. 2012)		303 mild-to-moderate DED							Evaluated as moderately-very effective by >60% of evaluators and participants
Liu X. (Liu et al. 2012)	DB RCT	60 moderate-to-severe DED	0.1% HA x4/d	1 mo	⟷	⟷	⟷	⟷	⟷ CIC measures
McCann L. C. (McCann et al. 2012)	SB RCT	73 mild-to-moderate DED	0.15% HA x4/d	3 mo	↑	↑	⟷		↑ evaporation, ↑ tear film stability by interferometry, ⟷ osmolarity
Takamura E. (Takamura et al. 2012)	DB RCT	286 moderate DED	0.1% HA x6/d	1 mo		↑	↑		
Lee J. H. (Lee et al. 2011)	SB RCT	65 mild-to-moderate DED	0.1% HA x6/d	2 mo	↑	↑	↑		
Monaco G. (Monaco et al. 2011)	DB RCT XO	20 glaucoma patients with dry eye symptoms	0.2% HA x4/d	2 w x2	⟷		⟷		
Sanchez M. (Sanchez et al. 2010)	SB RCT	15 mild-to-moderate DED	0.15% HA x4/d	1 mo		⟷	⟷	⟷	↑ HLA-DR by CIC flow cytometry
Vogel R. (Vogel et al. 2010)	DB RCT	436 moderate DED	0.18% HA hypotonic x3-6/d	2 w	↑		↑		
Johnson M. E. (Johnson et al. 2008)	DB RCT	65 moderate DED	0.18% HA x2/d	1 mo	↑	⟷	↑		
Troiano P. (Troiano & Monaco 2008)	SB RCT XO	28 moderate DED	0.4% HA hypotonic x4/d	1 w x 2	↑↑		↑↑		↑ conjunctival hyperemia, 61% preferred hypotonic over isotonic drops
			0.4% HA isotonic x4/d		↑		↑		↑ conjunctival hyperemia
Rolando M. (Rolando & Valente 2007)	OL RCT	30 mild-to-moderate DED	0.2% HA x3-4/d	3 mo	↑	↑	↑		
Lee H. K. (Lee et al. 2006)	DB RCT	41 DED (severity NG)	0.1% HA x3/d	1 mo	↑	↑		⟷	⟷ NFG/TP, ⟷ CIC measures
Benitez-del-Castillo J. M. (Benitez-del-Castillo et al. 2002)	PL	6 moderate-to-severe DED	0.18% HA x4/d	2 w			↑[s]		
Aragona P. Aug. 2002 (Aragona et al. 2002a)	SB RCT	40 severe DED with SS	0.4% HA hypotonic x6/d	3 mo	↑	↑↑	↑↑	⟷	↑↑ CIC measures
			0.4% HA isotonic x6/d		↑	↑	↑	⟷	↑ CIC measures
McDonald C. C. (McDonald et al. 2002)	DB RCT XO	32 severe DED with SS	0.1% HA x3-4/d	2 mo	↑/⟷				
Milafzzo G. (Milafzzo et al. 2002)	DB RCT XO	139 moderate KCS	HA hypotonic	1 mo x 2	↑	↑↑	↑	↑	
			HA isotonic		↑	↑	↑	↑	
Papa V. (Papa et al. 2001)	DB RCT XO	139 moderate DED	HA hypotonic up to x 6/d	1 mo x 2	↑	↑↑	↑	↑	↑ conjunctival hyperemia
			HA isotonic up to x 6/d		↑	↑	↑	↑	↑ conjunctival hyperemia
Iester M. (Iester et al. 2000)	OL RCT	113 moderate-to-severe DED	0.4% HA hypotonic x6/d	3 mo	↑	↑	↑	↑	↑ tear osmolarity, ↑ CIC score
Condon P. I. (Condon et al. 1999)	DB RCT XO	70 KCS, SS	0.1% HA x3-4/d	1 mo x2	↑		↑	↑	
Yokoi N. (Yokoi et al. 1997)	PL	7 SS + 4 moderate KCS	0.1% HA x5/d	1 mo			↑		
Nelson J. D. (Nelson & Farris 1988)	DB RCT	35 moderate KCS	0.1% HA x8/d	2 mo	↑	↑	⟷	⟷	↑ tear film osmolality, ↑ conjunctival hyperemia, ↑/⟷ CIC measures
DeLuise V. P. (DeLuise & Peterson 1984)	PL	28 severe DED, SS	0.1% HA x4/d	2 mo	↑	↑	↑	⟷	↑ mucus strand formation, ⟷ TMH

⟷ = no statistically significant difference at $p > 0.05$ compared to baseline, ↑ = statistically significant improvement at $p < 0.05$ compared to baseline, ↑/⟷ = statistically significant improvement at $p < 0.05$ in some parameters and no difference in others compared to baseline, ↑↑ = statistically significant improvement at $p > 0.05$ compared to baseline and compared to other hyaluronic acid treatment arm (empty cell) = not described, ADDE = Aqueous-deficient dry eye, CIC = conjunctival impression cytology, DB = double-blinded, DED = dry eye disease/syndrome, IVCM = *in vivo* confocal microscopy, KCS = keratoconjunctivitis sicca, LIPCOF = lid parallel conjunctival folds, MG = meibomian gland, mo = month(s), NFG/TP = Nerve Growth Factor/total protein ratio, OL = open label, OSS = ocular surface staining, PL = prospective longitudinal study, [r] = phenol red test, RCT = randomized controlled trial, [s] = stromal fluorescein uptake by fluorophotometer, SB = single-blinded, Schi. = Schirmer's test, SS = Sjögren's syndrome, Symp. = subjective symptoms, TBUT = tear film break-up time, TMH = tear meniscus height, VA = visual acuity, w = weeks, XO = cross-over design.

Troiano & Monaco 2008; Vogel et al. 2010; Lee et al. 2011; Monaco et al. 2011; Baeyens et al. 2012; Liu et al. 2012; McCann et al. 2012; Aragona et al. 2013; Kinoshita et al. 2013; Saeed et al. 2013; Hwang et al. 2014; Lee et al. 2014a, b; Gong et al. 2015; Liu et al. 2015; Chiambaretta et al. 2017; Groß et al. 2017; Labetoulle et al. 2017; Lambiase et al. 2017; López-de la Rosa et al. 2017; Park et al. 2017; Pinto-Fraga et al. 2017; Essa et al. 2018; Groß et al. 2018; Miháltz et al. 2018; Postorino et al. 2018; Roberti et al. 2018; García-Conca et al. 2019; Kim et al. 2019; Balestrazzi et al. 2020; Cai & Zhang 2020; Laihia et al. 2020; Brar et al. 2021; Morya et al. 2021). The most frequently used validated questionnaire was the Ocular Surface Disease Index (OSDI), used in 20 treatment studies (Monaco et al. 2011; Liu et al. 2012, 2015; Hwang et al. 2014; Lee et al. 2014a,b; Chiambaretta et al. 2017; Labetoulle et al. 2017; López-de la Rosa et al. 2017; Park et al. 2017; Pinto-Fraga et al. 2017; Essa et al. 2018; Postorino et al. 2018; Roberti et al. 2018; García-Conca et al. 2019; Kim et al. 2019; Balestrazzi et al. 2020; Cai & Zhang 2020; Laihia et al. 2020; Morya et al. 2021). The second most common was the Visual Analogue Scale (VAS), used in eight treatment studies (Nelson & Farris 1988; Papa et al. 2001; McDonald et al. 2002; Rolando & Valente 2007; Vogel et al. 2010; Baeyens et al. 2012; Aragona et al. 2013; Lambiase et al. 2017). Other symptom assessments included the ocular comfort index (Johnson et al. 2008; Groß et al. 2017, 2018), Symptom Assessment in Dry Eye (SANDE) questionnaire (McCann et al. 2012; Lambiase et al. 2017), global discomfort index for ocular symptoms (Aragona et al. 2002a), Standardized Patient Evaluation of Eye Dryness (SPEED) questionnaire (Brar et al. 2021), and patient or researcher assessment or preference (Troiano & Monaco 2008; Baeyens et al. 2012; Kinoshita et al. 2013). Eleven studies reported results of dry eye symptom assessments without the use of validated questionnaires (DeLuise & Peterson 1984; Condon et al. 1999; Iester et al. 2000; Milafzzo et al. 2002; Lee et al. 2006, 2011; Troiano & Monaco 2008; Kinoshita et al. 2013; Saeed et al. 2013; Gong et al. 2015; Miháltz et al. 2018). Symptoms are presented in the "*Symp.*" column in Table 1 as a single group of results, while additional investigator and patient assessments and preferences are presented in the "*Other outcomes*" column of Table 1.

Generally, there was a clear improvement in subjective scores against baseline in studies of both shorter and longer follow-up (Table 1). Forty-seven of the 53 treatment arms showed statistically significant improvement in subjective symptoms with HA treatment against baseline (DeLuise & Peterson 1984; Nelson & Farris 1988; Condon et al. 1999; Iester et al. 2000; Papa et al. 2001; Aragona et al. 2002a; Milafzzo et al. 2002; Lee et al. 2006; Rolando & Valente 2007; Johnson et al. 2008; Troiano & Monaco 2008; Vogel et al. 2010; Lee et al. 2011; Baeyens et al. 2012; McCann et al. 2012; Aragona et al. 2013; Kinoshita et al. 2013; Saeed et al. 2013; Hwang et al. 2014; Lee et al. 2014b; Gong et al. 2015; Liu et al. 2015; Chiambaretta et al. 2017; Groß et al. 2017; Labetoulle et al. 2017; Lambiase et al. 2017; López-de la Rosa et al. 2017; Park et al. 2017; Pinto-Fraga et al. 2017; Essa et al. 2018; Groß et al. 2018; Miháltz et al. 2018; Postorino et al. 2018; García-Conca et al. 2019; Kim et al. 2019; Balestrazzi et al. 2020; Cai & Zhang 2020; Laihia et al. 2020; Brar et al. 2021; Morya et al. 2021). One study arm found improvement in some but not all subjective parameters (McDonald et al. 2002). Only five treatment arms found no statistically significant change in symptoms compared to baseline (Monaco et al. 2011; Liu et al. 2012; Lee et al. 2014a; Roberti et al. 2018). No studies reported worsening of subjective scores with HA treatment.

Changes in objective measures against baseline in treatment studies

As seen in Table 1, the most commonly reported objective measure compared to baseline was ocular surface staining (OSS) reported in 53 treatment arms in 45 studies (DeLuise & Peterson 1984; Nelson & Farris 1988; Yokoi et al. 1997; Condon et al. 1999; Iester et al. 2000; Papa et al. 2001; Aragona et al. 2002a; Benitez-del-Castillo et al. 2002; Milafzzo et al. 2002; Rolando & Valente 2007; Johnson et al. 2008; Troiano & Monaco 2008; Sanchez et al. 2010; Vogel et al. 2010; Lee et al. 2011; Monaco et al. 2011; Baeyens et al. 2012; Liu et al. 2012; McCann et al. 2012; Takamura et al. 2012; Aragona et al. 2013; Kinoshita et al. 2013; Hwang et al. 2014; Lee et al. 2014a, b; Gong et al. 2015; Lanzini et al. 2015; Liu et al. 2015; Chiambaretta et al. 2017; Groß et al. 2017; Labetoulle et al. 2017; Lambiase et al. 2017; López-de la Rosa et al. 2017; Park et al. 2017; Pinto-Fraga et al. 2017; Essa et al. 2018; Groß et al. 2018; Miháltz et al. 2018; Postorino et al. 2018; Roberti et al. 2018; García-Conca et al. 2019; Kim et al. 2019; Cai & Zhang 2020; Laihia et al. 2020; Morya et al. 2021), followed by TBUT reported in 48 treatment arms from 41 studies (DeLuise & Peterson 1984; Nelson & Farris 1988; Iester et al. 2000; Papa et al. 2001; Aragona et al. 2002a; Milafzzo et al. 2002; Lee et al. 2006; Rolando & Valente 2007; Johnson et al. 2008; Sanchez et al. 2010; Lee et al. 2011; Baeyens et al. 2012; Liu et al. 2012; McCann et al. 2012; Takamura et al. 2012; Aragona et al. 2013; Kinoshita et al. 2013; Saeed et al. 2013; Hwang et al. 2014; Lee et al. 2014a, b; Gong et al. 2015; Lanzini et al. 2015; Liu et al. 2015; Chiambaretta et al. 2017; Groß et al. 2017; Labetoulle et al. 2017; López-de la Rosa et al. 2017; Park et al. 2017; Pinto-Fraga et al. 2017; Essa et al. 2018; Groß et al. 2018; Miháltz et al. 2018; Postorino et al. 2018; Roberti et al. 2018; Kim et al. 2019; Balestrazzi et al. 2020; Cai & Zhang 2020; Laihia et al. 2020; Brar et al. 2021; Morya et al. 2021), and Schirmer's test in 37 treatment arms from 31 studies (DeLuise & Peterson 1984; Nelson & Farris 1988; Condon et al. 1999; Iester et al. 2000; Papa et al. 2001; Aragona et al. 2002a; Milafzzo et al. 2002; Lee et al. 2006; Sanchez et al. 2010; Baeyens et al. 2012; Liu et al. 2012; Aragona et al. 2013; Kinoshita et al. 2013; Saeed et al. 2013; Hwang et al. 2014; Lee et al. 2014a, b; Lanzini et al. 2015; Liu et al. 2015; Chiambaretta et al. 2017; Labetoulle et al. 2017; López-de la Rosa et al. 2017; Park et al. 2017; Pinto-Fraga et al. 2017; Essa et al. 2018; Miháltz et al. 2018; Roberti et al. 2018; García-Conca et al. 2019; Balestrazzi et al. 2020; Cai & Zhang 2020; Morya et al. 2021). The most commonly used method of measuring OSS was the Oxford system (Bron et al. 2003) and variations of the Oxford system, or

comparable standardized OSS grading methods. One study measured stromal fluorescein uptake by fluorophotometry (Benitez-del-Castillo et al. 2002) as a measure of corneal epithelial barrier integrity which is listed as an OSS measure in Table 1. Twenty-three studies performed Schirmer's test without topical anaesthetics (DeLuise & Peterson 1984; Nelson & Farris 1988; Condon et al. 1999; Iester et al. 2000; Aragona et al. 2002a; Lee et al. 2006; Baeyens et al. 2012; Liu et al. 2012; Aragona et al. 2013; Kinoshita et al. 2013; Saeed et al. 2013; Hwang et al. 2014; Lanzini et al. 2015; Labetoulle etal. 2017; López-de la Rosa et al. 2017; Park et al. 2017; Pinto-Fraga et al. 2017; Mihăltz et al. 2018; Roberti et al. 2018; García-Conca et al. 2019; Balestrazzi et al. 2020; Cai & Zhang 2020; Morya et al. 2021) while six studies performed Schirmer's test with topical anaesthetics (Papa et al. 2001; Milafzzo et al. 2002; Sanchez et al. 2010; Lee et al. 2014a,b; Liu et al. 2015). One study did not clearly state whether anaesthetics were used (Chiambaretta et al. 2017), and one study used the Phenol Red test, which is comparable to Schirmer's test and is therefore listed with Schirmer in Table 1 (Essa et al. 2018). Only one of the 53 studies listed in Table 1 did not report any objective measures with HA treatment against baseline (McDonald et al. 2002).

Total OSS scores showed statistically significant improvement compared to baseline in 40 out of 53 treatment arms (DeLuise & Peterson 1984; Yokoi et al. 1997; Condon et al. 1999; Iester et al. 2000; Papa et al. 2001; Aragona et al. 2002a; Benitez-del-Castillo et al. 2002; Milafzzo et al. 2002; Rolando & Valente 2007; Johnson et al. 2008; Troiano & Monaco 2008; Vogel et al. 2010; Lee et al. 2011; Baeyens et al. 2012; Takamura et al. 2012; Kinoshita et al. 2013; Hwang et al. 2014; Lee et al. 2014a; Gong et al. 2015; Lanzini et al. 2015; Liu et al. 2015; Chiambaretta et al. 2017; Groß et al. 2017; Labetoulle et al. 2017; Lambiase et al. 2017; Park et al. 2017; Pinto-Fraga et al. 2017; Groß et al. 2018; Mihăltz et al. 2018; Postorino et al. 2018; Kim et al. 2019; Cai & Zhang 2020; Morya et al. 2021). Two treatment arms showed improvement in some but not all OSS parameters (López-de la Rosa et al. 2017; Essa et al. 2018). Eleven treatment arms showed no statistically significant change in OSS (Nelson & Farris 1988; Sanchez et al. 2010; Monaco et al. 2011; Liu et al. 2012; McCann et al. 2012; Aragona et al. 2013; Lee et al. 2014a,b; Roberti et al. 2018; García-Conca et al. 2019; Laihia et al. 2020). Tear film break-up time improved in 29 of 48 treatment arms compared to baseline (DeLuise & Peterson 1984; Nelson & Farris 1988; Iester et al. 2000; Papa et al. 2001; Aragona et al. 2002a; Milafzzo et al. 2002; Lee et al. 2006; Rolando & Valente 2007; Lee et al. 2011; Baeyens et al. 2012; McCann et al. 2012; Takamura et al. 2012; Aragona et al. 2013; Kinoshita et al. 2013; Saeed et al. 2013; Hwang et al. 2014; Lee et al. 2014b; Gong et al. 2015; Liu et al. 2015; Chiambaretta et al. 2017; López-de la Rosa et al. 2017; Park et al. 2017; Mihăltz et al. 2018; Brar et al. 2021). Schirmer's improved in 15 of 37 treatment arms (Condon et al. 1999; Iester et al. 2000; Papa et al. 2001; Milafzzo et al. 2002; Baeyens et al. 2012; Kinoshita et al. 2013; Saeed et al. 2013; Hwang et al. 2014; Liu et al. 2015; Chiambaretta et al. 2017; Mihăltz et al. 2018). No studies reported worsening of objective measures compared to baseline.

Additional objective parameters were measured in some studies and compared to baseline (as seen in Table 1's "*Other outcomes*" column). Six treatment arms found improvement in conjunctival impression cytology (CIC) measures (Iester et al. 2000; Aragona et al. 2002a; Sanchez et al. 2010; Hwang et al. 2014; Liu et al. 2015), while one found some CIC measures to be improved and others not (Nelson & Farris 1988), and five treatment arms found no change (Nelson & Farris 1988; Lee et al. 2006; Liu et al. 2012; Lanzini et al. 2015; García-Conca et al. 2019). Three treatment arms showed improved tear osmolality or osmolarity (Nelson & Farris 1988; Iester et al. 2000; Brar et al. 2021), one showed improved tear osmolarity in one eye and no change in the other (Lee et al. 2014b), while two did not find any change in tear osmolarity from baseline (McCann et al. 2012; García-Conca et al. 2019). Six treatment arms found improvement in conjunctival hyperemia (Nelson & Farris 1988; Papa et al. 2001; Troiano & Monaco 2008; Chiambaretta et al. 2017), while two found improvement in either tarsal or bulbar hyperemia but not in both (López-de la Rosa et al. 2017; Pinto-Fraga et al. 2017), and one found no change in hyperemia (García-Conca et al. 2019). Tear meniscus height was improved in one (Brar et al. 2021) and unchanged in two (DeLuise & Peterson 1984; Morya et al. 2021) treatment arms. Meibomian gland measurements improved in one (Brar et al. 2021) and were unchanged in two (Park et al. 2017; Postorino et al. 2018) treatment arms.

Changes against control in placebo controlled clinical trials

The nine HA treatment arms in the eight placebo-controlled trials comparing HA treatment with either saline or vehicle are presented in Table 2. All but one treatment arm found improvement in at least one subjective or objective measure (Sand et al. 1989; Shimmura et al. 1995; Condon et al. 1999; Aragona et al. 2002b; Vogel et al. 2010; Baeyens et al. 2012; López-de la Rosa et al. 2017; Pinto-Fraga et al. 2017). Three of nine treatment arms showed statistically significant subjective improvement (Condon et al. 1999; Vogel et al. 2010; Pinto-Fraga et al. 2017), one of nine found improvement in some and no change in other subjective measures (Baeyens et al. 2012), and five of nine arms found no change in subjective measures (Sand et al. 1989; Shimmura et al. 1995; Aragona et al. 2002b; López-de la Rosa et al. 2017). Three of seven treatment arms found improvement in TBUT (Sand et al. 1989; Baeyens et al. 2012; López-de la Rosa et al. 2017). Five of nine treatment arms found improvement in OSS (Sand et al. 1989; Condon et al. 1999; Vogel et al. 2010; Baeyens et al. 2012; Pinto-Fraga et al. 2017). One of nine treatment arm found improvement in some but not all OSS measures (Shimmura et al. 1995). Only two of eight treatment arms found improvement in Schirmer's test (Condon et al. 1999; Baeyens et al. 2012). No studies showed worsening in HA treatment compared to placebo.

Preservatives

Four studies used HA formulations with benzalkonium chloride as a preservative (Lee et al. 2006; Liu et al. 2012; Takamura et al. 2012; Hwang et al. 2014). Two of these studies found improvement in all measures compared to baseline (Takamura et al. 2012;

Table 2. Changes in HA treatment versus placebo in randomized double-blinded placebo-controlled trials, sorted by publication date.

First Author (year)	Design	Setup	Placebo arm	Duration	Symp.	TBUT	OSS	Schi.	Other outcomes
López-de la Rosa A.[xo] (López-de la Rosa et al. 2017)	16 moderate-to-severe DED	0.3% HA x3-8/d	saline	1 mo x2	⟷	↑	⟷	⟷	
Pinto-Fraga J.[xo] (Pinto-Fraga et al. 2017)	16 mild DED	0.2% HA x3-8/d	saline	1 mo x2	↑	⟷	↑	⟷	↑ conjunctival hyperemia, ↑ subjective satisfaction
Baeyens V. (Baeyens et al. 2012)	303 mild-to-moderate DED	0.18% HA x2-4/d	saline	3 mo	↑/⟷	↑	↑	↑	↑ patient and investigator efficacy evaluation, ⟷ VA, ↑ blurry vision, less average drop instillations
Vogel R. (Vogel et al. 2010)	436 moderate DED	0.18% HA x6/d	vehicle	2 w	↑		↑		
Aragona P. Feb. 2002 (Aragona et al. 2002b)	44 moderate-to-severe DED	0.15% HA x4-8/d	saline	3 mo	⟷	⟷	⟷	⟷	↑ CIC
Condon P. I.[xo] (Condon et al. 1999)	70 KCS or SS	0.1% HA x3-4/d	saline	1 mo x2	↑		↑	↑	3:1 patient preference for HA over saline
Shimmura S. (Shimmura et al. 1995)	91 DED, SS	0.1% HA x6/d	vehicle	1 mo	⟷	⟷	↑/⟷	⟷[t]	⟷ patient preference
Sand B. B.[xo] (Sand et al. 1989)	18 severe KCS	0.1% HA x6/d 0.2% HA x6/d	vehicle	2 w x4	⟷ ⟷	⟷ ↑	⟷ ↑	⟷ ⟷	4/14 preferred 0.1% 8/14 preferred 0.2% 2/14 preferred placebo

⟷ = no statistically significant difference compared to placebo with $p > 0.05$, ↑ = statistically significant improvement compared to placebo with $p < 0.05$, ↑/⟷ = statistically significant improvement with $p < 0.05$ compared to placebo in some but not all measures of this category, CIC = conjunctival impression cytology, d = day, DED = dry eye disease, HA = hyaluronic acid, KCS = keratoconjunctivitis sicca, mo = months, SS = Sjögren's syndrome, [t] = Schirmer's and tear clearance test, VA = visual acuity, w = weeks, [xo] = cross-over study.

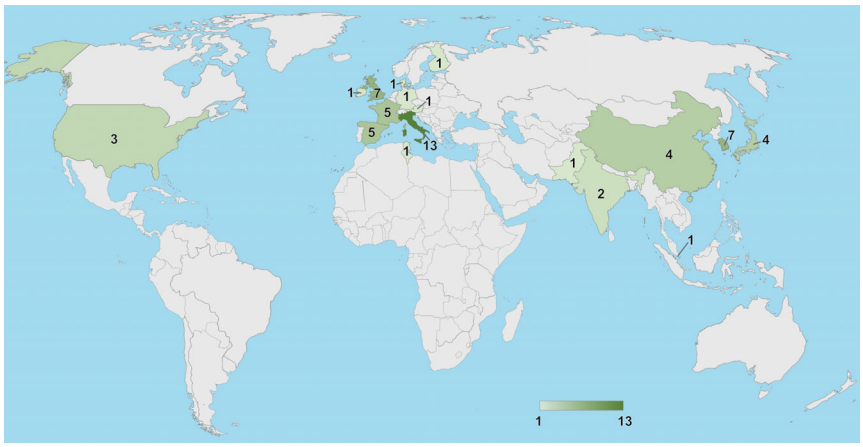

Fig. 6. Number of included studies conducted in each country. *Generated by Bing in Excel by Emily Moschowits.*

Hwang et al. 2014), one found improvement in some measures compared to baseline (Lee et al. 2006), and one found no improvement compared to baseline (Liu et al. 2012). 34 studies used preservative-free HA formulations (DeLuise & Peterson 1984; Nelson & Farris 1988; Sand et al. 1989; Shimmura et al. 1995; Yokoi et al. 1997; Condon

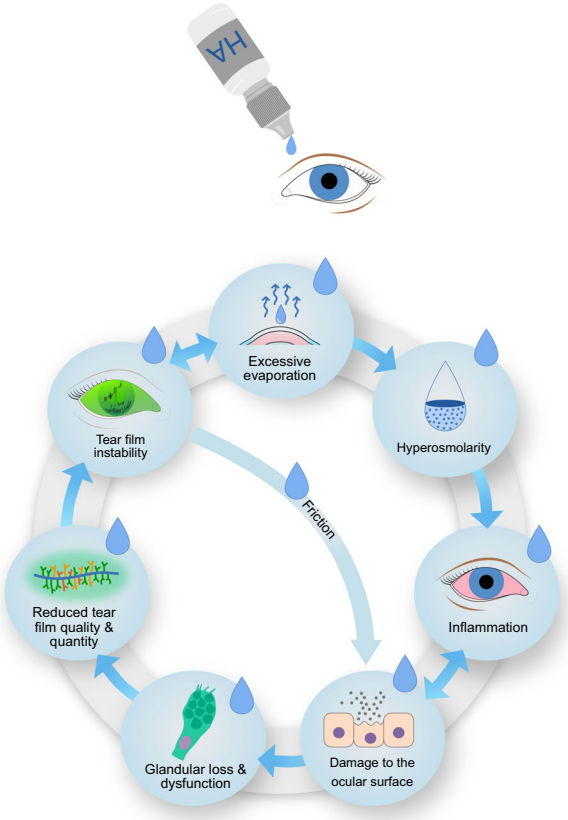

Fig. 7. Modified Vicious Circle of Dry Eye Disease inspired by Bron et al. Hyaluronic acid treatment attempts to break the circle. Blue drops illustrate action-points where hyaluronic acid contributes. *Illustration by Emily Moschowits, using elements from Sara Nøland with permission.*

et al. 1999; Iester et al. 2000; Benitez-del-Castillo et al. 2002; McDonald et al. 2002; Aragona et al. 2002a,b; Rolando & Valente 2007; Johnson et al. 2008; Troiano & Monaco 2008; Sanchez et al. 2010; Vogel et al. 2010; Lee et al. 2011, 2014a,b; Monaco et al. 2011; McCann et al. 2012; Kinoshita et al. 2013; Liu et al. 2015; Chiambaretta et al. 2017; Groß et al. 2017, 2018; Labetoulle et al. 2017; Lambiase et al. 2017; López-de la Rosa et al. 2017; Essa et al. 2018; Mihältz et al. 2018; Postorino et al. 2018; Roberti et al. 2018; Laihia et al. 2020). No studies compared clinical effects of HA treatment with preservatives compared to without preservatives.

Safety and complications

Hyaluronic acid was found safe in all reviewed literature. There were no serious adverse events associated with the use of HA in any of the included studies. However, some studies mentioned cases of conjunctival hyperemia, conjunctivitis, burning sensation, and/or discomfort with HA use (Sand et al. 1989; Vogel et al. 2010; Chen et al. 2014; Lee et al. 2014b; Labetoulle et al. 2017). One study discussed in general the very low adverse effects reporting from consumers of artificial tears with HA (Vogel et al. 2010). None of the double-blinded controlled treatment studies found clinically relevant differences in adverse events or tolerability between groups (Sand et al. 1989; Condon et al. 1999; Aragona et al. 2002b; Vogel et al. 2010; López-de la Rosa et al. 2017; Pinto-Fraga et al. 2017), with the exception of more blurry vision after instillation of 0.18% HA than saline in one study (Baeyens et al. 2012). Two studies using 0.15% and 0.18% HA respectively reported minor and tolerable temporary visual changes after instillation (Johnson et al. 2008; Sanchez et al. 2010).

Discussion

Summary

With over 30 years of clinical use, HA has proven to be an enduring element of DED treatment. This review summarizes the current knowledge on the safety and efficacy of HA in the treatment of DED against baseline measures and against placebo. Hyaluronic acid is shown to be effective at improving symptoms and objective measures of dry eye, such as TBUT and OSS compared to baseline and to placebo.

Change in Schirmer's test

Interestingly, despite improvements in symptoms, TBUT, and OSS in most studies that included these measures, Schirmer's scores improved in less than half. Schirmer's test mainly measures the lacrimal gland's aqueous production (Willcox et al. 2017). Only some DED patients have decreased Schirmer's values (Bron et al. 2017). Reduction in aqueous production in DED can be due to decreased function, destruction, fibrosis or atrophy of the lacrimal gland or adjacent conjunctiva (Conrady et al. 2016; Bron et al. 2017). Hyaluronic acid's mechanism for improving Schirmer's score may be through resolving the ocular inflammation of DED (Pauloin et al. 2009a), which may improve lacrimal gland function (McMonnies 2020), though restoration of an auto-immunologically damaged lacrimal gland as in the course of Sjögren's syndrome (Bjordal et al. 2020) is less likely.

Study populations

A full range of DED severities and types was covered in the reviewed literature (Tables 1 and 2). Only one study looked at mild and moderate DED separately, however the participants with moderate DED also received topical cyclosporine and steroid treatment, so the HA effect could not be isolated in the moderate DED groups (Lee et al. 2014a). The authors of that study suggested the small sample size and low drop frequency to contribute to the absence of significant improvements in the mild DED groups. Studies with across-the-board improvement were found among both smaller (DeLuise & Peterson 1984; Nelson & Farris 1988; Rolando & Valente 2007; Troiano & Monaco 2008; Mihältz et al. 2018; Brar et al. 2021), and larger (Iester et al. 2000; Papa et al. 2001; Milafzzo et al. 2002; Baeyens et al. 2012; Kinoshita et al. 2013; Saeed et al. 2013; Hwang et al. 2014; Gong et al. 2015; Chiambaretta et al. 2017; Park et al. 2017) study sizes. Non-improvement or

improvement in only one measure was only found in studies with less than 100 participants (Shimmura et al. 1995; Aragona et al. 2002b; Sanchez et al. 2010; Monaco et al. 2011; Liu et al. 2012; Lee et al. 2014a; Roberti et al. 2018; Balestrazzi et al. 2020). All studies with more than 100 participants found improvements in two or more measures (Iester et al. 2000; Papa et al. 2001; Milafzzo et al. 2002; Vogel et al. 2010; Baeyens et al. 2012; Takamura et al. 2012; Kinoshita et al. 2013; Saeed et al. 2013; Hwang et al. 2014; Gong et al. 2015; Chiambaretta et al. 2017; Park et al. 2017; Morya et al. 2021). This could indicate that some studies with little to no improvement were underpowered. The 10 studies that recruited patients with aqueous deficient dry eye, Sjögren's syndrome and keratoconjunctivitis sicca found improvement in most or all reported subjective and objective measures (DeLuise & Peterson 1984; Nelson & Farris 1988; Yokoi et al. 1997; Condon et al. 1999; Aragona et al. 2002a; McDonald et al. 2002; Milafzzo et al. 2002; Aragona et al. 2013; Hwang et al. 2014; García-Conca et al. 2019).

HA concentration

Concentrations of HA used in HA treatment studies ranged from 0.1% to 0.4% (Tables 1 and 2). Comparisons of treatment with different HA concentrations was limited. Only two baseline-controlled studies isolated the effects of different HA concentrations in parallel treatment arms (Groß et al. 2017; Park et al. 2017), both with a non-inferiority study design, thus not powered to find superiority of one treatment over the other. One placebo controlled study found no difference in any measures in the 0.1% HA treatment arm against placebo but found improvement in TBUT and OSS against placebo with 0.2% HA (Sand et al. 1989). In the same study, 0.2% concentration also won patient preference compared to both placebo and 0.1% HA (Sand et al. 1989). Increasing HA concentration from 0.1% to 0.3% has been found to cause further improvements in experimental DED in mice, including decreased goblet cell and corneal epithelial cell damage and increased tear film stability (You et al. 2018). Generally, HA in concentrations between 0.1% and 0.2% appear to provide objective improvement, symptom-relief, and patient comfort without substantial blurring of vision (Johnson et al. 2008; Sanchez et al. 2010; Carracedo et al. 2019). 94% of patients in the 2008 Johnson et al. study reported less than one minute of visual disturbance after drop installation with 0.18% HA (Johnson et al. 2008). Higher concentrations of HA (>0.2%) provide longer tear film stability, but also show increased complaints of blurry vision (Aragona et al. 2019; Carracedo et al. 2019). Ishioka et al. showed that 0.3% HA caused significantly more visual acuity loss compared to 0.1% HA immediately after drop instillation, but that this difference disappeared within 5 minutes of administering drops (Ishioka et al. 2009). This is supported by a study that found increased optical higher order aberrations and forward light scatter of the cornea for five minutes after instilling 0.3% HA drops (Koh et al. 2013). Future studies should investigate differences in treatment effects and tolerance with varying HA concentrations.

Drop frequency

Per-protocol drop frequency in treatment studies, as seen in Tables 1 and 2, ranged from 2 to 8 drops per day across studies. There was no clear pathophysiological or evidence-based reasoning behind the choice of drop frequency in any of the studies. No study aimed to find the ideal drop frequency for HA treatment. The three studies that compared different concentrations of HA used the same drop frequency for all HA treatment arms (Lee et al. 2014a; Groß et al. 2017; Park et al. 2017). One study found patients to use a significantly lower average drop frequency in the HA group compared to the placebo group (Baeyens et al. 2012). Six studies reported on compliance with protocol instructions (Aragona et al. 2002b; Rolando & Valente 2007; Johnson et al. 2008; Baeyens et al. 2012; Essa et al. 2018; Laihia et al. 2020). Five of those studies reported good compliance. One of those studies found the mean daily drop frequency of participants to be 9 drops per day (Aragona et al. 2002b). Another study, instead of specific instructions, asked severe DED patients to administer drops whenever they experienced DED symptoms (van Setten et al. 2020). They reported an average drop frequency among patients ranging from 2 to 23.8 drops per day with an average of 7.1 drops. Dry eye disease symptoms often do not correspond with objective findings in the office (Craig et al. 2017), and symptom relief does not necessarily correspond to objective improvement. It would be useful for clinicians and patients to have evidence-based drop frequency recommendations, ideally for any given formulation and DED severity or type. This would require a study design with several treatment-arms of varying strict drop frequency protocols with robust methods of measuring compliance. Such studies are currently missing from the literature. There is also the potential risk of patients over- or under-treating when self-administering, either not achieving the full clinical effect, or washing away the many trophic, anti-inflammatory, and antioxidating proteins, lipids, and mucins that are naturally present in the tear film (Willcox et al. 2017). Few prescribed medical interventions have such a wide range of treatment regimens across studies or allow patients to self-determine their own treatment based on symptoms. Future clinical studies need to investigate optimal drop frequency of HA in dry eye treatment and explore the possibility that different patient groups may need different drop frequencies.

Hypotonic versus isotonic HA drops

Five treatment studies compared hypotonic and isotonic HA treatment for DED (Papa et al. 2001; Aragona et al. 2002a; Milafzzo et al. 2002; Troiano & Monaco 2008; Lee et al. 2014a; Table 1). One treatment study using 0.4% HA found significantly more subjective and objective improvements in the hypotonic group compared to the isotonic group (Troiano & Monaco 2008). In the same study, the hypotonic group also won patient preference (Troiano & Monaco 2008). Three of the studies found significantly greater objective but not subjective improvement in the hypotonic group compared to the isotonic group (Papa et al. 2001; Aragona et al. 2002a; Milafzzo et al. 2002). One of the studies looking at hypotonic and isotonic HA drops also used different HA concentrations and thus did not separate the effect of different osmolarity or

concentration (Lee et al. 2014a). More studies are needed to determine if hypotonic HA formulations are better than isotonic formulations in DED treatment.

Molecular weight of HA

The molecular weights of the HA used was provided in only 10 of the included studies (Shimmura et al. 1995; Yokoi et al. 1997; Aragona et al. 2002a,b; Johnson et al. 2008; Groß et al. 2017, 2018; Roberti et al. 2018; García-Conca et al. 2019; Laihia et al. 2020) of which one study only provided molecular weights for one of two treatment arms (Groß et al. 2017). The molecular weight of HA ranged from 60 kilo-Daltons (Yokoi et al. 1997) to 3000 kilo-Daltons (Aragona et al. 2002b), which is in the low to high molecular weight range (Aragona et al. 2019; Lee et al. 2021). No studies provided comparative results between HA of different molecular weights. High molecular weight HA, especially above 1000 kiloDaltons, has known anti-inflammatory, immunosuppressive and antiangiogenic activity, including pro-inflammatory cytokine and chemokine suppression through cell-surface receptor-binding (Altman et al. 2019), while low molecular weight, and even medium molecular weight HA, may produce pro-inflammatory responses (Altman et al. 2019; Lee et al. 2021). Given the changing and even opposing physical characteristics and immunological effects of HA depending on its molecular weight (Litwiniuk et al. 2016; Snetkov et al. 2020), investigation into the effects of varying molecular weights in the treatment of DED is warranted.

Pathophysiology of DED and HA drop formulation

Hyaluronic acid works through breaking the vicious circle of dry eye disease that self-perpetuates the DED pathophysiology (Jones et al. 2017) as illustrated in our modified version of The Vicious Circle of Dry Eye Disease (Bron et al. 2017; Fig. 7). Across the reviewed literature it is shown that DED treatment with HA improves tear film stability, evaporation, osmolarity, inflammation, ocular surface damage, as well as goblet-cell, epithelial, and meibomian gland health (Tables 1 and 2). Some of these improvements can be explained through the direct or indirect effect of HA on the osmolarity of the tear film, which in turn will reduce the osmotic stress at the ocular surface (Yu et al. 2021). Osmotic stress results from hyperosmolarity of the tear film and is one of the main mechanisms behind the vicious circle of DED, leading to inflammation and ocular surface damage (Bron et al. 2017). The frictional damage to the ocular surface, conjunctival thinning, pain, and inflammation of DED (Bron et al. 2017; van Setten 2020; Aragona et al. 2021) is improved by HA through lubrication of the ocular surface by its viscous, mucoadhesive and non-Newtonian properties, which reduces shear-forces (van Setten 2020) as well as through HA's established anti-inflammatory effects (Kotla et al. 2021). Hyaluronic acid treatment appears to address the pathophysiology of both evaporative and aqueous deficient DED at multiple action points.

The variation in the physiochemical properties of HA and its effects in DED treatment is dependent on its concentration, molecular weight, chemical modifications, and the drop formulation (Abatangelo et al. 2020; van Setten 2020). Guillaumie et al. found that higher molecular weights of HA at equal concentrations provide prolonged residence time in the tear film of rabbits (Guillaumie et al. 2010). These actions were also found in human cells and tissues, including the cornea (Jiang et al. 2007; Pauloin et al. 2009a,b; Wu et al. 2013). One study found improved tear film evaporation rates after 90 days of HA treatment which suggests that the lipid layer of the tear film may also be improved with long term HA treatment (McCann et al. 2012). In a study published in 2019, Aragona et al. assessed the physiochemical properties of 18 commercially available HA-based artificial tears and concluded that the ideal HA-based artificial tear should include high molecular weight HA, and discussed the possibility of unique formulations that could target specific ocular surface conditions (Aragona et al. 2019).

During the data collection process, details on product used, exact ingredients, concentrations, molecular weights, osmolarity, and pH of HA containing artificial tear formulations were often unavailable. Only eight of the included studies provided complete, or nearly complete information on the HA formulation used (Yokoi et al. 1997; Aragona et al. 2002a,b; Johnson et al. 2008; Troiano & Monaco 2008; Vogel et al. 2010; Chiambaretta et al. 2017; Laihia et al. 2020). Commercially available artificial tear formulations containing HA have limited details listed online. A greater availability of information regarding drop formulation, osmolarity, HA concentration, molecular weight, chemical modifications, and preservatives, could help strengthen the available evidence for DED treatment, aid in the planning of future clinical trials, and enable the development of better treatments for DED.

Future directions for HA in DED

There are promising future directions for HA in the treatment of DED. Other modes of HA delivery have been tested, including oral administration (Kim et al. 2019), slow-release HA in contact lenses (Ali & Byrne 2009; Maulvi et al. 2015, 2017; Scheuer et al. 2016; Desai et al. 2018, 2020; Wei et al. 2020; Huang et al. 2021), and canaliculus HA gel-occlusion (Fezza 2018). Hyaluronic acid shows promise as part of an ocular surface delivery device for other drugs, such as dexamethasone and cyclosporine (Soiberman et al. 2017; Liu et al. 2019). The HA molecule allows for chemical manipulation, including elongation, cross-linking (Williams & Mann 2014), and for use as a derivative of new muco-adhesives (Laffleur & Dachs 2015; Posarelli et al. 2019). A 2019 review concluded that HA is superior to other artificial tears and that the HA molecule still has potential for further improvement including cross-linking to increase bioavailability and resistance to degradation (Posarelli et al. 2019). Hyaluronic acid lends itself to combination treatments and has been combined successfully with multiple other dry eye treatments, both experimentally and clinically, with promising results. A few examples of combination treatments include taurine (Roberti et al. 2018), which has osmoprotective and antioxidant properties, trehalose with similar properties as HA (Matsuo 2001; Schmidl et al. 2015; Bucolo et al. 2018; Fariselli et al. 2018; Fondi et al. 2018; Laihia & Kaarniranta 2020), omega-3 fatty acids (Li et al. 2014), and glycerol (Kiss & Németh 2015).

Limitations

A limitation of this review was that only articles available with English full text were included. Exclusion of non-English and non-full-text articles was a necessary step to ensure that thorough and correct information was available for review. None of the reviewed studies lasted more than three months. Longer studies could potentially reveal long-term beneficial aspects of HA treatment, considering HA's known anti-inflammatory and antioxidant properties. There were only eight placebo-controlled trials among the reviewed literature (Sand et al. 1989; Shimmura et al. 1995; Condon et al. 1999; Aragona et al. 2002b; Vogel et al. 2010; Baeyens et al. 2012; López-de la Rosa et al. 2017; Pinto-Fraga et al. 2017). Placebo brings a unique challenge in DED trials as both saline and vehicle control groups tend to improve signs and symptoms of DED. This is a recurring problem in artificial tear research (Vogel et al. 2010). Lubrication effects of placebo drops as well as regression to the mean are likely explanations for this phenomenon.

Conclusion

Hyaluronic acid as an active ingredient in artificial tears in concentrations between 0.1% and 0.4% is a safe and effective treatment for DED. Hyaluronic acid has lubricating, anti-inflammatory, antioxidant, and anti-toxic effects at the ocular surface. Hyaluronic acid treatment improved both signs and symptoms of DED in most of the reviewed literature. Hypotonic HA drops appear to have some clinical benefit over isotonic drops but more studies isolating the effects of tonicity are needed. There is a literature gap in determining optimal HA concentration, drop frequency, molecular weight of HA, and potential differences in optimal treatment for different DED severities and sub-types. Researchers should aim to isolate and investigate these variables in future studies of HA treatment for DED.

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