GLAUCOMA / BASICS, MORPHOLOGY, PHARMACOKINETICS, GENETICS

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HYALURONIC ACID CAN ENHANCE THE PROLIFERATION OF FIBROBLASTS IN VITRO

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Purpose Hyaluronic acid (HA) is a major component of viscoelastic substances which are widely used for intraocular surgery. HA is also naturally occurring in various parts of the eye and can be synthesized e.g. by stromal keratinocytes. Results from organ culture experiments indicate that HA has a mitogenic effect on the corneal epithelium (Inoue, IOVS 1993;34:2313). We investigated the effect of HA on the proliferation of fibroblasts.

Methods 3T3 cells and explants from human conjunctiva or tenon were grown in RPMI or DMEM with 10% calf serum (FBS) and antibiotics. Preconfluent cells were grown at the low seeding density of 1.4×10^4 cells/60 mm dish in medium with and without 1 % FBS. HA was added to the medium in concentrations between 10 µg/ml and 1000 µg/ml. The number of cells and BrdU labeling was determined on day 3 and 6.

Results Control cultures without FBS and HA showed a very slow increase of the total cell number. Addition of HA to these "quiescent" cultures led to a dose-dependent, statistically significant increase of the cell number ($p<0.001$).
In the presence of FBS morphology changed to a wider cytoplasm and cells formed small colonies. Addition of HA resulted in an dose-dependent increase of proliferation ($p<0.001$).

Conclusion Our results indicate that HA can be a mitogen for quiescent and actively dividing cells of fibroblastic origin in vitro. Although some caution has to be exercised when interpreting of data from culture experiments in respect to a clinical situation HA might also enhance proliferation in vivo. To pay attention to this possibility, HA containing visco-elastic substances should be carefully removed when used for ocular filtration surgery.

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INNERVATION OF MYOFIBROBLAST-LIKE SCLERAL SPUR CELLS IN HUMAN EYES

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Purpose. To study the innervation of the presumably contractile, myofibroblast-like scleral spur cells (SSC) in human eyes.
Methods. Serial tangential sections of the scleral spur region of the eyes of 16 human donors were investigated with immunocytochemical methods. Antibodies against acetylcholinesterase, synaptophysin, α-smooth muscle actin, calcitonin gene-related peptide (CGRP), neuropeptide Y (NPY), nitric oxide synthase (NOS), substance P (SP), tyrosine hydroxylase (TH), and vasoactive intestinal peptide (VIP) were used. In addition, sections were processed for glyoxylic acid-induced catecholamine fluorescence (CF), and for NADPH-diaphorase (NADPH-d).
Results. In all eyes examined, circumferentially oriented varicose axons were observed in the scleral spur region of all quadrants. Double labelling showed that most of these scleral spur axons were in close contact with the α-smooth muscle actin-positive, myofibroblast-like scleral spur cells. The axons showed like-immunoreactivity (LI) for SP, CGRP, NPY, VIP and NOS. In addition, numerous scleral spur axons stained for NADPH-d. Most SP-LI scleral spur axons were double-labelled for CGRP-LI, none for VIP-LI. All NPY-LI scleral spur axons were double labelled for VIP-LI, but lacked immunoreactivity to TH. Some VIP-LI axons were not labelled for NPY-LI. Nerve fibers immunoreactive (IR) for TH or positively stained for CF were not observed in association with scleral spur cells. Positive staining for acetylcholinesterase was seen only in the ciliary muscle, not in the scleral spur region.
Conclusions. The close association of varicose axons with the myofibroblast-like SSC indicates that nervous signals modulate SSC tone. Since SSC form tendon-like connections with the trabecular meshwork, changes in SSC tone might modulate outflow resistance. In addition to efferent scleral spur axons, we recently described afferent mechanoreceptors in the human scleral spur region. These data suggest that nervous elements in the scleral spur region might form a feedback system that controls SSC tone and indirectly modifies architecture and size of the aqueous humor outflow pathways.
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ALPHA-TOCOPHEROL INHIBITS PROLIFERATION OF TENON FIBROBLASTS IN VITRO

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PURPOSE Failure of glaucoma surgery is mostly due to a fibrocellular scar formation where Tenon fibroblasts play a major role. Fibroblast proliferation is inhibited clinically mostly by the antimetabolite Mitomycin-C which has major disadvantages like being toxic to other cells and often producing ocular hypotony.
METHODS Primary human fibroblast cultures of 7 different donors were seeded in multiwell plates. After serum deprivation of the subconfluent cells, a single dose of 50 and 100 uM alpha-Tocopherol was added, and the cell number was determined at different days.
RESULTS In the presence of Tocopherol a dose-dependent clear inhibition of proliferation compared to controls was observed for all donors. At day 2 we found an inhibition of cell proliferation of 30-78% (mean 60%) for 50 uM Tocopherol and 46-97% (mean 77%) for 100 uM Tocopherol. This inhibition was statistically significant. Maximal inhibition of proliferation was found on day 2, then the inhibition was slowly decreasing up to day 8.
CONCLUSIONS alpha-Tocopherol showed a clear inhibition of proliferation of human Tenon fibroblasts. If planned clinical studies will show similar effects, Tocopherol could be a promising drug to prevent glaucoma surgery failure with additional advantages of being non toxic and easy to applicate.

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Influence of Laser Induced Glaucoma on Coroidal Ganglion Cells

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Purpose. The purpose of this study was to investigated the influence of increased intraocular pressure (IOP) on choroidal thickness and the number of choroidal ganglion cells.
Methods. The eyes of 11 rhesus monkeys, in which IOP was increased due to laser coagulation of the trabecular meshwork in one eye, were obtained from the University of Iowa (USA). Duration of glaucoma ranged from 14 to 47 months with IOP peaks of 49-80 mmHg, but medians of 18-38 mmHg controlled by treatment with antiglaucomatous drugs (IOP median of controls: 17-19 mmHg). In addition, in five of the animals arterial hypertension had been induced. Thickness of the choroid was investigated with ultrasonic methods in six living non-hypertensive monkeys. For investigation of the choroidal ganglion cells whole mounts of the choroid were depigmentated and stained with panneuronal antibodies (PGP; Ultra Clone Limited, England). The nervous network was visualised with a fluoreszence microscope and the total number of ganglion cells counted. The data were compared using the U-test.
Results. In all glaucomatous eyes thickness of the choroid was significantly reduced compared with the contralateral control eyes.
In the whole mounts a perivascular network of PGP-positive stained nerve fibers was found in all choroids, showing varicose terminals near the arteries and arterioles of the choroidal stroma. In the control eyes the total number of ganglion cells in the choroid was 572 ±21. Most of them formed smaller groups of up to 10 cells. In the glaucomateous eyes the number of ganglion cells was significantly reduced to a number of 101 ±50. These findings could not be correlated to any clinical data; in detail there was no correlation with neither the median or maximal IOP, disc cupping nor duration of glaucoma. The monkeys treated additionally with arterial hypertension showed no differences compared with the non-hypertensive animals.
Conclusion. We could clearly show that long lasting increase in IOP leads to reduction in numbers of ganglion cells in the choroid. The reason for lack of correlation between number of ganglion cells and any clinical data might be, that the glaucomatous eyes were end staged damaged. The systemic hypertension had no additive effect. The reduced thickness of the choroid could be due to either the increased IOP per se or to loss of vasodilative innervation of the choroidal vasculature.