Tear substitutes

Latest information on hyaluronic acid

Little attention has been paid so far on the biological function of hyaluronic acid (hyaluronan) in epithelia, with regard to dry eye therapy based on tear substitute solutions. Nevertheless, hyaluronan is the most important component of the extracellular matrix, which fills the intercellular space of multi-layered epithelia poorly supplied with blood. Its chain length (molar mass) is of vital importance.

Already in 1982, Polack und Mc-Niece described the successful use of a 0.1% solution of high-molecular hyaluronan in the treatment of severe dry eye cases (1). They had produced eye drops by diluting Healon® with BSS. It took other 13 years before the first hyaluronan eye drops were put on the market: In 1995, the product Hyalein®, developed by the Japanese ophthalmic manufacturer Santen, was approved by the Japanese drug regulatory agency. In 1998, the first hyaluronan eye drops arrived also on the German market. Since then, specialists and patients have had the possibility to choose between a large number of hyaluronan-based tear substitutes that are destined to be successful products also in the future.

Hyaluronan as film former

Lubricants and thickeners contained in tear substitute solutions are important, among other things, to improve eyelid lubrication properties in eyes with damaged tear film, in order to prevent epithelial damage caused by mechanical stress. Hyaluronic acid performs this task effectively not only as a natural element of synovial fluid, but also as tear substitute component.

Flow properties of tears and hyaluronic solutions: Viscosity η measures the resisting flow of a fluid. The unit of measurement for viscosity is mPa·s. Its reference value is water, that at 20 °C has by definition a viscosity η = 1 mPa·s. Water viscosity does not change under the influence of shear forces. Such fluids are referred to as Newtonian fluids. Tear film flow properties have been investigated and described in detail by Tiffany in the early 1990s (2-4).

Thanks to the mucins that are secreted by conjunctival goblet cells and dissolved in the tear film´s aqueous phase, the tear film has a high viscosity (with open eyes) in absence of shear forces, which contributes significantly to its stability. The shear rate generated when blinking and during eye movements is defined as the quotient of eyelid speed, measured in mm/s, divided by the distance between the edge of the eyelid and the corneal epithelium, measured in mm.

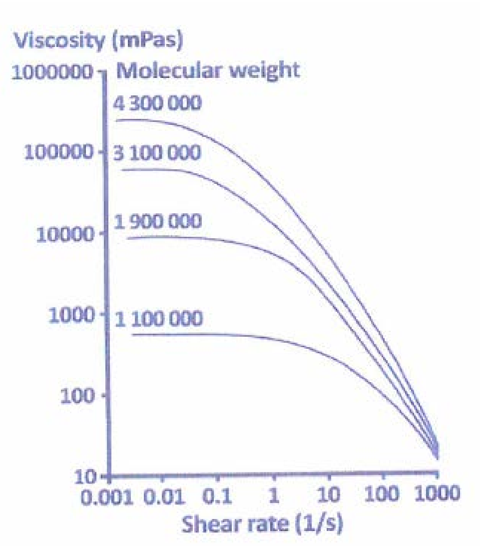

Figure 1: Dependence of viscosity on shear rates in 1% hyaluronic solutions with different molar masses (by Bothner & Wik 1987)

Therefore, the resulting unit of measure is s^{-1}. When blinking, the tear film is subject to a higher shear rate of about 20 000 s^{-1}. Due to this shear rate, there is a decrease in viscosity from around 65 to 10 mPa·s. On the one hand, this favours the even, streak-free distribution of tears on the cornea; on the other hand, it limits those shear forces acting on the epithelia. (3). Such a flow behaviour is called viscoelastic, or rather shear-thinning. A dysfunction in tear film flow properties can contribute to recurrent corneal erosions, as a result of corneal epithelium trivial injuries.

In order to achieve the best possible effectiveness and tolerance, tear substitutes should have viscoelastic properties as close as possible to those of natural tears. Hyaluronan offers the ideal conditions for this purpose. The works of Bothner & Wik revealed that the higher the molar mass of hyaluronic acids is, the more the viscosity of hyaluronic acid solutions depends on shear forces (5).

In order to ensure that a hyaluronic solution dwells efficiently on the eye as long as possible, and is at the same time evenly distributed with a low presence of shear forces when blinking, it is necessary to have the highest possible molar mass. The production of tear substitutes is mainly limited by two factors: firstly, the availability of hyaluronan with extremely high molar mass, and secondly, the possibility to filter these macromolecules through a 0.22 µm pore-size filter to achieve sterility. One single hyaluronan molecule dissolved in water with a molar mass of four million Daltons (in non-deformed conditions) has a diameter of around 0.5 µm! Only recently, it was possible to produce hyaluronan of extremely high molar mass by using bacterial fermentation.

The heat sterilization of hyaluronic solutions in final containers is not possible if plastic vials and single-dose containers are used; this would lead to a reduction of the hyaluronan chain length through thermal hydrolysis. This is why the only alternative is to filter the macro-molecule solution through a 0.22 µm pore-size filter. The higher the hyaluronan molecular mass, the bigger the technical challenge and the longer the experience required in careful sterile filtration of hyaluronic solutions.

Water binding by hyaluronan: The water binding capacity of hyaluronic acid (up to six litres per gram) is exceptionally high. Hyaluronic acid of average molar mass binds about 50 times more water than cellulose or carboxymethyl cellulose (Carmellose), and hyaluronic acid of higher molar mass even three times more than hyaluronic of average molar mass. As a result, tear substitutes containing hyaluronan can make an important contribution to reducing the evaporation rate, and thereby to stabilizing the osmolarity also in eyes with hyper-evaporative tear film.

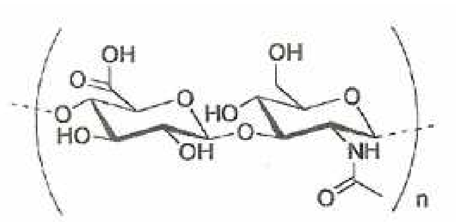

Figure 2: Building unit of a hyaluronan chain

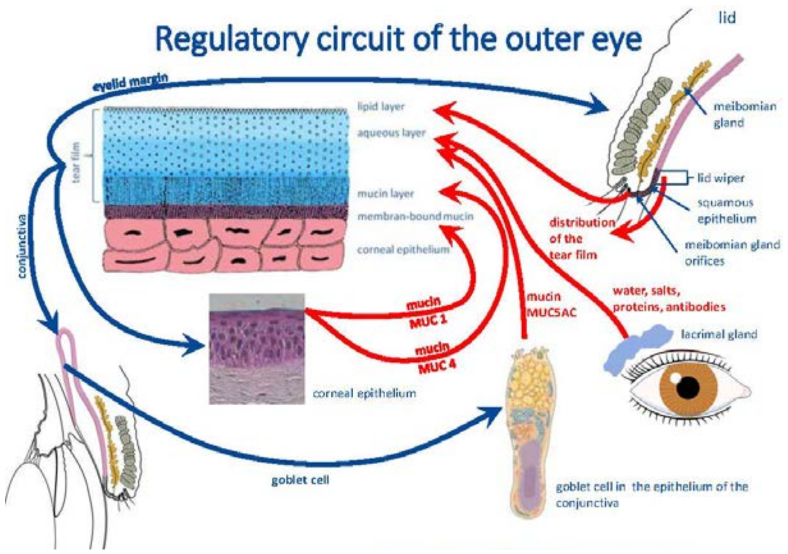

Figure 3: Regulatory circuit of the external eye*

The chemical relationship between hyaluronan and mucins: Hyaluronan is a Glycosaminoglycan (GAG). Glycosamino-glycans, also referred to as Mucopoly-saccharides, are long unbranched poly-saccharides (= sugar chains) consisting of a repeating disaccharide unit (= 2 sugar molecules). One single disaccharide unit consists of an uronic acid (most commonly glucuronic acid, rarely iduronic acid), which is 1-3 glycosidically linked with an amino sugar (e.g. N-acetylglucosamine). The disaccharide units of the chains are 1-4 glycosidically linked.

Mucins (from *mucus*) consist of a central protein molecule, with glycosaminoglycans alongside. Three kinds of mucins appear on the human eye:

MUC5AC: secreted by conjunctival goblet cells in tear film aqueous phase.

MUC4: built and secreted by conjunctival and corneal epithelial cells.

MUC1: built and membrane-bound by conjunctival and corneal epithelial cells.

While MUC1 protects epithelia from infections and ensures their wettability, MUCSAC and MUC4 positively contribute to tear fluidity and water binding capacity. Glycosaminoglycan molecules of mucin are much smaller than hyaluronic acid molecules and do not contain hyaluronan. By contrast, each hyaluronic molecule can bind several mucin molecules thanks to adhesive forces. This is why hyaluronan is also described as mucoadhesive. Hyaluronic acid mucoadhesive properties contribute directly to stabilizing damaged tear films.

Synthesis, localization and function of hyaluronans

Hyaluronan is synthetized in the cellular membrane and directly extruded into the extracellular matrix that fills the intercellular space (7). The body of an adult contains about 15 grams of hyaluronan, of which half is located in the skin; in almost ten kilograms of adult's skin, this means a skin hyaluronic level of around 0,075% (8). Hyaluronan is the most important component in the extracellular matrix of multi-layered epithelia poorly supplied with blood. The extremely narrow intercellular spaces of epidermal keratinocytes have a high hyaluronic concentration of 2,5 mg/ml (9). In this tissue, the half-life is only about 24 hours.

Hyaluronan

- organizes the extracellular matrix (10)
- favours water storage and retention (11)
- facilitates the diffusion of nutrients and metabolites
- controls the keratinocyte proliferation and differentiation
- has an efficient radical scavenging capacity in case of exposure to UV light, infections, oxidative stress, tissue necrosis due to inflammatory processes (12-13)
- allows migration of epithelial cells during wound healing (14-15).

Hyaluronan, a messenger substance

The molar mass of hyaluronic acid in the intact extracellular matrix of epithelial cells is around three to four million Daltons (9,16).

In case of increased degradation caused by injuries or inflammations, hyaluronic acid has also a fundamental "signaling" role, which is strongly linked to the molar mass:

- Hyaluronan of high molar mass has an antiangiogenic and an immuno-suppressive action (17-20).
- Hyaluronan of medium molar mass has inflammatory and immuno-stimulating, as well as angiogenic functions (20-22)
- Small hyaluronan fragments counteract apoptosis and cause the synthesis of heat shock proteins (23)
- Proteolytic enzymes in inflammatory processes lead to an increase in hyaluronan decomposition (10, 21)
- Cellular coating, achieved through hyaluronic acid binding to receptors in the cell membrane, protects them from lymphocytes and macrophages attacks (10, 24). This coating can also be produced by supplying extrinsic hyaluronans (25).

Intrinsic epithelium-ageing

The hyaluronic acid amount contained in the extracellular matrix of multi-layered epithelia does only marginally depend on ageing; however, the amount of hyaluronic acid which is bound to cellular membranes through receptors, starts to increase after the fifth decade of life, while the amount of available, quickly extractable hyaluronan in intercellular spaces starts to decrease (20, 26, 27). This also involves the loss of hydration and thickness of the epidermis (parchment skin in the elderly). Only hyaluronic acid that is not bound to cellular surface receptors is active in the wound healing action of corneal epithelia (28). This suggests that hyaluronan plays also a key role in dry eye problems caused by old age.

Consequences

Many different factors take part in the regulatory circuit of the external eye. Tear composition depends mainly on the balanced production and distribution of different tear film components.

Any dysfunction can in the first place cause an increase in evaporation rates and, consequently, in tear osmolarity. Secondly, a long-lasting high osmolarity triggers a chronical inflammation process, during which all the organelles involved in the synthesis, secretion and distribution of tear components, are affected (29). This has been reported in literature as the vicious circle of dry eye (30). Traditional tear solutions were mainly used to preserve eyelid fluidity by avoiding mechanical epithelial irritations. Viscosity-increasing additives contributed to improving the dwell time on the eye. Since lubricants and thickeners had no or just poor viscoelastic properties, it was necessary to sacrifice high viscosity (with open eyes) during the day, in order to avoid blurry vision. Gels were then used during the night in worst dry eye cases, which however cause high shear forces on the epithelium and can be problematic when recurrent corneal erosion occurs.

The situation has improved dramatically since the introduction of hyaluronic acid. The viscoelastic properties of hyaluronan solutions increase the ratio between higher viscosity with open eyes and lower viscosity when blinking, hence also the dwell time of tear solutions on the eye. At the same time, hyaluronic acid contributes over a longer period to the water binding capacity, and thus to the stabilization of tear osmolarity.

The biochemical role of hyaluronan in epithelium has not been taken into account in ophthalmology yet. For the longest possible dwell time on the eye and for water retention, as well as for the physiological and "signaling" function of hyaluronic acid in the extracellular matrix of epithelial intercellular spaces, tear solutions with extremely high hyaluronic molar mass (at least > 2 MDa) should be preferred. Hyaluronan contained in Comfort Shield® (Hylan A) has a molar mass > 3 MDa, therefore it can substitute the hyaluronic acid of corneal and conjunctiva epithelia. Comfort Shield® was also successfully introduced in treatments for recurrent corneal erosion, thanks to low shear forces when blinking (private communication by Mark Tornalla).

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