Optimising tear replacement rheology in canine keratoconjunctivitis sicca

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Abstract

Dry eye is a substantial problem in a large number of human and canine patients. Numerous laboratory models for tear deficiency exist using genetically predisposed rodent models, animals treated with topical anti-muscarinics, or those kept in environments with increased air flow to produce the ocular surface changes seen in human patients. Canine keratoconjunctivitis sicca, seen in many thousands of dogs kept as companion animals, can provide a valuable spontaneous model for testing tear replacement medications that might better model disease in human patients, existing as it does in an outbred population that live in the same environments as their owners. Here the development of a crosslinked hyaluronic acid topical drop is described together with the results of trials on dogs with spontaneous keratoconjunctivitis sicca. Although hyaluronic acid in its native form in tear replacement drops shows a Newtonian rheology, the crosslinked product described here behaves in a non-Newtonian manner, with the same shear thinning shown by the tear film itself. The crosslinked product thus shows itself as a potentially valuable tear replacement medication for the human dry eye population as well as for dogs with the same condition.

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Introduction

It is a tough life being a tear drop. One moment you are adrift in the middle of a wide open cornea, clinging onto the ocular surface for grim death. Having as high a viscosity as possible in

this situation is vital to avoid simply running downwards into the tear lake inferiorly. And then the next moment, before you know it and as if without warning, the eyelid comes sweeping down and immediately you have to lose all that viscosity and become as fluid as possible, to avoid damage to the epithelial cells as the blink occurs. A drop of water increases its viscosity very slightly as it is moved. It has what we term Newtonian fluid dynamics as it was Sir Isaac Newton who first showed that the stresses arising from the fluid flow in a conventional fluid are proportional to the local rate of change of its deformation over time. The forces resulting from fluid flow are proportional to the rates of change of the fluid's velocity vector. As he says in his *Principia Mathematica* of 1687 'The resistance which arises from the lack of slipperiness originating in a fluid – other things being equal – is proportional to the velocity by which the parts of the fluid are being separated from each other'.¹ Newton's law of viscosity states that the shear stress on a fluid τ is equal to the shear rate γ multiplied by a proportionality constant QUOTE the viscosity of the fluid. However, biological fluids such as blood,² synovial fluid,³ and tears exhibit shear thinning, where their viscosity is not constant, but reduces with shear rate. This is related to the structural reorganisation of irregular moieties as velocity changes. In blood the interactions between erythrocytes are key in this behaviour whereas in synovial fluid it is hyaluronic acid existing as long chain anisotropic molecules that change the rheology of the fluid.⁴

If it is tricky being a tear drop, it is equally vexatious being a designer of a tear replacement drop. The very fact that we have so many from which to choose, carboxymethylcellulose, hydroxymethylcellulose, carbomer gel, hyaluronic acid,⁵ propylene glycol/hydroxypropyl-guar,⁶ and lipid emulsion⁷ among artificial lacrimomimetics⁸ in products

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ranging through Actimist, Advance Eye Relief, Akorn, Akwa, Blink, Clarymist, Clear Eyes, Freshkote, GenTeal, Hylogel, Isopto, Just Tears, Lacril, Lacrisert, Liposic, Lubrifresh, Murine Tears, Natural Balance, Nature's Tears, Nutratear, Oasis Tears, Paralube, Refresh, Rohto Hydra, Systane, Soothe, Tearisol, Tears Again, Tears Natural, Thera Tears, Ultra Tears, Visco Tears, and Vizulize Dry Eye Mist and even good old fashioned Lacrilube with products such as lanolin from sheep wool,⁹ not to forget of saliva in submandibular gland transplantation in human patients¹⁰ or parotid duct transposition in the dog,¹¹ suggests that none is 100% effective in every patient. And neither should we expect them to be, for the tear film is not one component alone but a number. The tear film is widely recognised now to be a good deal more complicated than the trilaminar structure first proposed by Wolff in 1946.¹² Mucins both attach to the conjunctival and corneal epithelium and float free in the tear film giving a more graded viscosity in the tear film, and a thicker tear layer to boot, as first demonstrated by Prydal and Campbell back in 1992.¹³ On top of this graded mucin/aqueous mix lies a layer of lipid that itself has complex rheological properties.¹⁴ The fact that we are dealing with several different structures, the epithelium and its glycocalyx, the mucin/aqueous gradation, and the lipid layer giving surface protection, means that dealing with the tear film as one single entity is bound to fail, both from a conceptual basis and at a practical level. How in one drop can we seek to replicate the complex structure and function of the tear film? And what model should we use to determine if we have got it right?

Models of the tear film in health and disease

Laboratory animal models of dry eye are numerous and varied. There are rodent strains with inherited predisposition to immunologically mediated dry eye

similar to Sjogren's syndrome—the MRL mouse in particular.¹⁵ Pharmacological intervention using topical atropine or benzalkonium chloride has been widely used with markedly varying results even between different rodent strains in the case of benzalkonium chloride.¹⁶ The molecule causes corneal epithelial disruption, stromal neovascularisation, and infiltration of inflammatory cells that mimic those changes caused in dry eye but not through the same mechanisms. The muscarinic antagonist atropine sulphate does cause tear reduction in several laboratory animal models that have then been used to evaluate the efficacy of topical tear replacement.¹⁷ The same can be achieved by surgical removal of the lacrimal gland¹⁸ but not employing denervation by injection of the toxin saponin.¹⁹ All of these models however cause a panoply of signs that may be linked to the cause of the damage to the eye rather than to ocular surface dryness itself as shown by the toxic denervation model¹⁸ that does not reduce tear production as measured by the phenol thread test. Reducing tear production with atropine or scopolamine followed by exposure to a desiccating airflow for 5 to 10 days provokes the type of ocular surface pathology seen in naturally occurring dry eye and has been a valuable model for dissecting the processes happening in the development of keratoconjunctivitis sicca.²⁰ However, it has the disadvantage that it is very far from the spontaneous disease in human patients.

Spontaneous keratoconjunctivitis sicca seen in dogs kept as companion animals however has the advantage of being similar in pathogenesis to human dry eye.²¹ The condition occurs through autoimmune destruction of the lacrimal gland similar to that occurring in Sjogren's syndrome in the human patient and is particularly valuable in that it exists in an animal larger than the laboratory rodents and rabbits otherwise used (Figure 1a). It does have the disadvantage of being less readily controlled in its time and severity of onset than a rodent model²² and the fact that it occurs on a more varied

Figure 1 The eye of a 5-year-old English bull terrier with corneal oedema and ulceration, conjunctival hyperaemia, and ocular surface discharge classic of canine keratoconjunctivitis sicca before treatment (a) and after 3 weeks of topical CMHA-S treatment three times daily (b).

genetic background could be seen as a disadvantage in that it complicates matters by bringing into play a number of uncontrollable variables. On the other hand, this outbred genetic background could be seen as advantageous given that it more closely models the real world of human patients where disease occurs against different genetic and environmental backgrounds. The ocular disease in canine keratoconjunctivitis sicca is similar to human dry eye, although in many cases more severe often with complete absence of tear production to warrant consideration as an example of translation medicine in a 'one health' scenario.²³ Dry eye occurs very commonly in dogs kept as companion animals. The first survey of canine keratoconjunctivitis sicca was undertaken by Professor Lloyd Helper, a key player at the beginning of modern veterinary ophthalmology, who noted only 0.4% of the canine population to be affected with a deficiency in tear production in 1976.²⁴ After 20 years, Dr Renee Kaswan, a leader in canine dry eye research, reported a prevalence of up to 35% in the patients she surveyed.²⁵ The truth is that the number of animals affected is probably somewhere in between these two figures. Research we undertook in Cambridge to measure the Schirmer tear test (STT) in 1000 dogs demonstrated levels of tear production lower than 15 mm of tear strip wetting in 1 min in 131 dogs giving a prevalence of 13%. In a recent survey of the cases seen in the clinic of the Queen's Veterinary School Hospital, University of Cambridge, 181 of the last thousand cases seen were affected by keratoconjunctivitis sicca.²⁶ This high prevalence of the condition means that a population for evaluation of new products to be used in canine dry eye can readily be accessed.

Developing a non-Newtonian tear replacement

We noted above that synovial fluid is non-Newtonian—the lubricating liquid in the hip joint, to take one example, needs to exhibit shear thinning to be able to cope with standing still and then with moving rapidly. I often ask owners of dogs with dry eye whether they have ever considered the eye to be like a hip joint. Few, if any, have! Yet, as I explain to them, the eye is, in effect, a ball and socket joint. The sclera is like cartilage, the conjunctiva like synovial membrane, and tears are like joint fluid. Except that of course it is not quite so simple: such a viscous covering of the ocular surface would blur our vision quite inappropriately. We have seen that tears do behave in a non-Newtonian manner but in a very thin layer across the ocular surface, comprising a volume estimated to be $\sim 3 \mu\text{l}$.²⁷ A drop of a lacrimomimetic fluid from an ophthalmic dropper bottle is between 25 and $70 \mu\text{l}$.²⁸ Whichever product we use, a single drop will dramatically overload the tear film. A fluid behaving

Figure 2 Chemical reactions used in formulation of crosslinked carboxymethyl hyaluronic acid (taken from Williams and Mann³³).

with a Newtonian rheology will provide some degree of ocular surface lubrication, but require a sizeable volume of product blurring vision and also needing frequent application. Perhaps a product behaving on the ocular surface in a non-Newtonian manner can provide a long-lasting lubricant covering for the ocular surface, with a prolonged residence time although whereas existing as a thin pauci-molecular layer

The plethora of reports on topical treatments for dry eye are often contradictory. To take one example, standard hyaluronic acid tear replacement drops have been shown by some researchers to have a beneficial protective effect on corneal epithelium in patient studies²⁹ and in laboratory studies using chick corneal explants,³⁰ whereas others have shown no effect.³¹ Yet, these papers have used 0.3% and 0.1% hyaluronic acid, respectively, with neither report documenting the molecular weight of the molecule used in either study. If the rheology of the medication is a key factor differing molecular weights may be key. Hyaluronic acid molecules in the synovial fluid of young healthy human knee joints have been shown to have a molecular weight of between 2.5 and 7 MDa.³² Their rheological behaviour is non-Newtonian with shear thinning as discussed above.⁴ The hyaluronic acid molecules in standard tear replacement drops, on the other hand, are much smaller with a molecular weight of ~ 1.5 – 1.8 MDa. (Dr Brenda Mann, University of Utah Bioengineering Department, personal communication, 2017) The rheological behaviour of such hyaluronic acid products generally containing relatively short molecules at relatively low concentrations is for the most part Newtonian with an increase in viscosity with shear rate.³³ Research at the University of Utah Department of Bioengineering has investigated crosslinking of hyaluronic acid to create molecules exhibiting shear thinning and thus forming a valuable addition to the panoply of tear replacement medications. To crosslink the molecules a reactive group is first introduced into the

Figure 3 Change in viscosity with differing shear rates of conventional hyaluronic acid and crosslinked thiolated carboxymethyl hyaluronic acid showing classical shear thinning in the crosslinked product (taken from Williams and Mann³³).

molecule by carboxylating hydroxyl groups. Next, a hydrazide is coupled to the carboxyl groups producing free thiols that can then be crosslinked, covalently joining the hyaluronic acid molecules (Figure 2). It is difficult to give a precise molecular weight for this crosslinked thiolated carboxymethyl hyaluronic acid (CMHA-S) as the carboxylation is random and a range of molecules are thus produced. But what can be evaluated is the rheology of the resulting gel. As shown in Figure 3 non-crosslinked hyaluronic acid has a low viscosity that increases with elevated shear rate. The crosslinked product, on the other hand, has a much higher viscosity at low shear rates, dropping substantially as the shear rate increases—a non-Newtonian rheology that is just what the ocular surface needs as we noted above.

Testing tear replacement products in the spontaneous canine model

In a randomised, double-masked, controlled study, the beneficial effects of the novel topical crosslinked carboxymethyl hyaluronic acid product on canine eyes with aqueous tear deficiency were compared with those of a standard hyaluronic acid tear replacement drop. In this research on 30 dogs affected by keratoconjunctivitis sicca,³⁴ conjunctival hyperaemia, adnexal discharge, and ocular discomfort were all reduced significantly more by the crosslinked hyaluronic acid product than by the standard tear replacement drop. Indeed, the product not only ameliorates canine dry eye but, as we have recently

shown in a group of dogs kept as companion animals and effected with stromal corneal ulceration, the crosslinked product also significantly increases the speed of corneal ulcer healing.³⁵ Whether this relates to the rheological behaviour of the product and its prolonged ocular surface residence time is at present unclear; it may be that hyaluronic acid itself, binding to the CD44 molecule on the cell surface of corneal epithelial cells, and potentially stromal keratocytes as well, increases their migration rate to heal the ulcer.

Conclusion

Producing a tear replacement drop that accurately replicates the non-Newtonian rheology of the normal tear film is not easy. Testing tear replacements drops on an animal model of human dry eye might also be said to be equally taxing. Here a crosslinked hyaluronic acid product is described, tested for beneficial effects on spontaneous dry eye in dogs kept as companion animals, thus showing the value of a lacrimomimetic agent exhibiting shear thinning and the advantages of using spontaneous dry eye in pet dogs as a model for evaluating tear replacement medication.

Conflict of interest

The author declares no conflict of interest.

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