Eye (2003) 17, 923–926

www.nature.com/eye

Tears in health and disease

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Abstract

Purpose **A brief review of normal tear function and changes resulting from disease.**
Methods **The factors contributing to normal tear physiological function are considered, and the effect of changes in composition, as reported in the literature, is surveyed, with emphasis on the physical performance of the tears. Major classes of tear tests which would reveal functional changes are listed.**
Results **Where possible, changes of measurable functions in disease are described. Gaps in our current knowledge are indicated.**
Conclusions **Many techniques exist for examination and assessment of both normal and disease tears, but further development is needed to adapt some of these to clinical situations, and to make them more specific diagnostic tests.**
Eye (2003) **17,** 923–926. doi:10.1038/sj.eye.6700566

Keywords: tears; composition; physical measurements; functional tests; ageing; dry eye

Introduction

Ocular disease can occur in many forms, which can broadly be classified as arising from systemic or local causes. Systemic causes include inborn errors of metabolism such as Tay–Sachs disease, involving production of high levels of glycosidases detectable in the tears; infections or general pathological events that affect the lacrimal system and influence its ability to produce the tears; or drug-related conditions where damage may be caused to various structures affecting ocular wetting or tear production. In addition, local infections or injuries can give rise to products that are transported or that accumulate in the tears. Any leakage of the conjunctival capillaries will add a variable number and quantity of blood components into the tears, and many reports of changing tear composition may do no more than reflect this addition. There are also side effects of treatment of an underlying condition, such as tarsorraphy for severe dry eye, where retention of the small available volume of tears allows accumulation of toxic products and the development of so-called 'toxic tears'. These broad classifications have been well summarised by Seal[1] in an earlier symposium.

An extensive literature exists reporting various substances (inflammatory mediators, growth factors, invading white cells, remodelling enzymes such as collagenase, plasmin, or plasminogen activator, etc) that have been detected in tears in various disorders relative to normal controls. This presentation could list some of these, but one may question whether any very useful purpose would be served by doing so. It is perhaps more important to ask what changes in the physical or biochemical performance of the tears, if any, appear as a consequence of the disease. In so doing we shall recognise what are the characteristics most readily measurable, and how this can be done, but also see where our present knowledge is incomplete.

Functions of tears

Tears act as both a delivery and an excretory route for nutrients and metabolic products of the corneal epithelium and anterior stroma, since it has been shown that the diffusional route from the limbus into the avascular cornea is inadequate. During waking hours, delivery of aqueous tears from the lacrimal gland is continuous, but fresh fluid remains in the upper and lower marginal meniscus, and possibly under the upper lid, until after the next blink, when fluid is drawn from the menisci to form the film.[2] The action of blinking also squeezes or massages the meibomian glands within the tarsal plates, delivering fresh oil to the lid margin.[3] The presence of the tear film improves the quality of the retinal image[4] by smoothing out irregularities of the cellular surfaces. The action of blinking also spreads mucus over the epithelial surface and the tears keep this layer fully hydrated, to act both as a lubricant and protection for the epithelium. Hence the stability of the film is important to maintain

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Received: 28 February 2003
Accepted in revised form: 28 February 2003

Presented at the Cambridge Symposium on 'The Dry Eye', St John's College, Cambridge, 5–6 September 2002

these functions in the eye, and the factors influencing it, and the way they may change with disease, deserve attention.

Components of tears affecting performance

Table 1 summarises the major components of human tears. There are many minor components not included, some of which are known to change in various diseases (Table 2).

The electrolytes are principally Na^+, K^+, Cl^-, and HCO^-, with lower levels of Mg^{2+} and Ca^{2+}; as secreted, tears are isotonic with serum although the proportions of ions are somewhat different, especially K^+. Many of the small molecules, such as glucose, lactate, urea, etc., also occur in serum but at different levels. More proteins can be detected[5] (some estimates suggest as many as 80–100) but only four are present in large amounts, secreted from the lacrimal gland, its ductal epithelium and associated plasma cells (lysozyme, lactoferrin, lipocalin, and sIgA); lipids come both from the meibomian glands and lipocalin-associated, apparently delivered with the protein from the lacrimal gland; various mucins of both secreted and epithelial types have been reported.

The levels of the major tear proteins are known to decline with age,[1,6,7] at the same time the volume of tears also tends to decline, while levels of added serum proteins (albumin, caeruloplasmin, etc) remain constant or increase. Ageing therefore parallels to some extent the onset of a fairly mild dry eye, even if it cannot adequately be diagnosed as such. During sleep, secretion of all major proteins and water is inhibited, but sIgA release continues, producing a highly concentrated solution in the limited fluid. Polymorphonuclear leucocytes migrate into the conjunctival sac, bringing a variety of destructive and remodelling enzymes and effectively creating a subclinical inflammatory state.[8]

Lipid is delivered from the meibomian glands to the lid margin and spreads on the tear film in a thin layer whose thickness can be assessed by its interference colours. This is thought to promote tear film stability by reducing evaporation from the open eye. Increase in thickness of the layer by more forcible blinking has been shown to promote stability.[9]

Mucins are a class of very large glycoproteins containing more than 50% carbohydrate, consisting of a linear polypeptide core with regions containing many serine and threonine residues as attachment points for oligosaccharide side chains. Other regions of the polypeptide are unglycosylated but contain cysteine residues capable of forming linkages to other molecules to build up polymeric molecules and gels. The principal gel-forming conjunctival mucin (MUC5AC) is produced by goblet cells, but the products of other mucin genes are also found, including MUC1 and MUC4 bound to the free surface of corneal epithelium and identified as the glycocalyx. At one time, it was believed that mucin (largely MUC5AC) dissolved in the tears was responsible for both the surface tension and viscosity, on the grounds that similar behaviour was seen in mucin solutions of about 5 mg/ml. Recent reports show that the mucin levels in normal tears are both much lower than this and very variable, mostly in the range 0–100 μg/ml,[10] so it now seems unlikely that mucin is a major contributor to these physical properties, although some mucin/protein or mucin/lipid interactions cannot as yet be ruled out.

There is comparatively little information on changes in levels of these components in any eye diseases except for various forms of dry eye. Here, however, because of the frequency of the condition, and the similarities between its milder forms and the changes associated with ageing, we have some data, although with considerable gaps.

Table 1 Composition of human tears

Water
Electrolytes
Proteins (lysozyme, lactoferrin, lipocalin, secretory IgA)
– albumin, IgG (leakage from conjunctiva)
Lipids (meibomian glands, lipocalin-associated)
Mucins (epithelial membrane-anchored type, soluble goblet-cell type)
Defensins, collectins, other small molecules

Table 2 Additional components reported present in tears in various disease states

Inflammatory mediators
Cytokines
Growth factors
White blood cells
Antigens
Signalling molecules
Complement components
Remodelling enzymes

Tests on tears

Apart from specific biochemical assays to assess the quantities of normal or abnormal components in the tears resulting from disease, there are a number of other, largely physical, tests that can give useful information about the functional state of the lacrimal/conjunctival system.

Break-up time of the tear film is a direct measure of stability. The film thins if the eye is held open, until at certain points, apparently randomly distributed, some form of disruption takes place. If fluorescein is present, this may mean that locally the film has thinned to a point

where no significant fluorescence is visible; in the noninvasive method, disruption of one of the reflected lines may mean displacement of mucoid material and hence a break in film/cornea adhesion. It has been suggested that break-up is initiated at the points where a surface epithelial cell has recently been sloughed off, and the newly exposed epithelial cells have slightly lower wettability until they mature by expression of their surface glycocalyx; reduction of BUT in ocular surface disease may be indicative of greater sloughing. BUT is reduced in a high proportion of dry eye cases.[11,12]

Impression cytology can indicate both goblet cell density and the general well-being of surface cells. It might be assumed that thickness and integrity of the surface mucus gel layer would promote stability, but it is recently reported that there is no correlation between goblet cell density in the conjunctiva and BUT.[13]

Change in the osmolarity of the tears is invariably upwards, indicating greater evaporative loss. In evaporative dry eye, this may result from some diminution of the evaporative control of the meibomian lipid layer on the tear film, owing to blockage of the glands or change in the composition of the secretion.[14] In the aqueous-deficient form of dry eye, more rapid break-up of the film can encourage more rapid evaporation and hence concentration of the tears.[15]

The rate of production or available volume of tears is usually measured by the Schirmer test, or its variant the cotton thread test (which may be made more sensitive by surrounding the thread with a fine plastic tube to cut evaporative loss from the surface of the thread, and more visible by including the indicator dye phenol red).[16] If the ocular surface is anaesthetised prior to the test, there is no stimulated flow, and the wetting of the paper indicates the volume of tear fluid present in the menisci and tear film; however, if no anaesthetic is used, the test reveals the ability of the lacrimal gland to respond to stimulation. In aqueous-deficient dry eye, the gland may be operating almost continually at near-maximal output in response to persistent irritation, and hence be unable to produce at any higher rate in response to stimulation. Reductions in tear volume, flow rate, and turnover time are inevitable consequences of aqueous-deficient dry eye,[12] although opinions differ on the magnitude of the change in flow rate, using the fluorometric dilution method.[17]

Another recently developed noncontact method of assessing tear volume is by meniscometry. The lower tear meniscus can be used as a concave cylindrical mirror to reflect a striped target; the spacing of stripes in the image is directly converted into curvature. The smaller the radius of curvature, the smaller the volume of tears present and the greater the capillary suction of fluid back into the menisci from the tear film. A theoretical relationship showing how this can influence tear film thickness has been published by Creech *et al*.[2]

The oil film on the surface of the tears can be examined by its interference colours, and the relative proportions of marmoreal and flow patterns and average thickness measured. Meibomian gland dysfunction (MGD) leads to the evaporative form of dry eye, so reduction of output from the glands will thin the lipid layer (except in severe dry eye, where the lipid layer appears thicker[18]). This may perhaps enhance disruption so that evaporation increases. The amount of lipid available at the lid margin can be assessed by the blotting procedure and photometric assay of meibometry; as a direct test this only gives information about the amount of lipid actually present (the casual level), but cleaning of the lid margin and remeasuring after a prescribed number of blinks can give a measure of delivery or replacement rate.[19] The casual level is significantly lower in MGD than normal, but little changed in aqueous-deficient dry eye.[20]

The viscosity of tears has been shown to be non-Newtonian (ie dependent on the shear rate at which it is measured) and relatively low (in the range 1–10 mPa s, compared to 1 mPa s for water at 20°C). Until recently, commercial rheometers with the required sensitivity in controlled-shear mode needed sample sizes of 70–100 µl, which effectively ruled out measurement on tears from individual donors or from patients with moderate to severe dry eye. Instruments are now available capable of using samples as small as 5 µl, but no study on individual tears, either normal or dry eye, has yet been performed. The only published comparison is of normals and mildly dry eyes,[21] where there is little obvious difference. The form of shear-thinning seen for tears is characteristic of solutions of linear charged polymers such as carboxymethyl cellulose or hyaluronan, which form the basis of many artificial tear preparations. Similar shear-thinning can be seen in highly concentrated solutions of globular proteins; neither of these models is appropriate in tears. At present, tear viscosity appears to depend partly on the binding of lacrimal lipids to tear-specific lipocalin, and partly on association between the major tear proteins, especially combinations including the net positively charged lysozyme. It seems possible that there is a loose aggregation of the lipocalin–lipid complex and other proteins at low shear rates, which is progressively but reversibly pulled apart by higher shearing forces (JM Tiffany, unpublished). Variation in levels of any of these components owing to disease, ageing, or the influx of serum components via capillary leakage could be reflected in viscosity changes.

Like viscosity, tear surface tension has also been shown to depend on the binding of lacrimal lipids to tear lipocalin,[22] and not on the presence of dissolved mucins.

A survey of surface tension in normals and dry eyes, using a horizontal-capillary micromethod, showed a broad spread of values in both groups, with considerable overlap between the groups; in general, normal tears were more strongly surface-active (ie lower values of surface tension).[23] Reduction of surface activity could reflect diminution of the lipocalin or lipid components, or competitive binding by other types of lipids. Whatever the mechanism, the effect would be to reduce the ability of the tears to be spread out following a blink and form a stable film. Holly *et al*[24] used a contact-angle method to measure tear surface tension; they found only a small decrease in surface activity in keratoconjunctivitis sicca and ocular pemphigoid, which they ascribed to reduction in mucus availability, and a rise in Stevens–Johnson syndrome which was ascribed to infiltration of other components with surfactant properties. Schoenwald *et al*,[25] using the horizontal-capillary method, showed that elevated surface tension in dry eye was at least partially corrected by boosting the output of tear proteins. It was shown that the improvement of surface activity was related to improved levels of one particular tear protein; although not named, this was identifiable as lipocalin.

Conclusions

At present there are many tests that are only practicable under laboratory conditions, requiring the collection of samples for assay. Much work still needs to be done on the best ways of taking the samples, on storing and transporting them, and in many cases on the actual techniques of testing. Some tests could be adapted for use in the clinical environment, which would have the advantage of promoting earlier diagnosis and treatment of ocular disease. Ultimately, changes in tear composition are of importance largely to the extent that they affect the physical properties and performance of the lacrimal system and help or hinder the action of its inbuilt protective mechanisms.

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