Improving tear ferning patterns collected from goats and camels after adding various electrolyte solutions

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Abstract

Background. Good quality of tear film is essential for healthy vision in both animals and humans. Therefore, improving the quality of tears through the addition of electrolytes is important.

Objectives. To assess the effect of adding various electrolyte solutions on tear ferning (TF) patterns collected from goats and camels.

Materials and methods. Tear samples (20 μL) were collected from 5 goats (2 males and 3 females; 3.4 ±1.6 years) and 5 camels (2 males and 3 females; 4.0 ±1.1 years) using microcapillary tubes. A tear sample (0.5 μL) from each animal was mixed with various volumes (0.5–5 μL) of each electrolyte solution to produce homogenous mixtures. A sample (1 μL) of each mixture was dried on a microscopic glass at 22°C with a humidity ≤40%. The obtained TF pattern was observed, graded and compared with those obtained for the corresponding pure tear samples. The effect of dilution using purified water on the TF patterns of animals was also tested.

Results. The TF grades of animals were generally enhanced when mixed with electrolyte solutions. Specifically, the TF grade for tears collected from a goat was improved from 1.4 to 0.7 and to 0.8 when magnesium chloride hexahydrate and calcium chloride were added, respectively. Similarly, the TF grade for tears collected from a camel was improved from 1.8 to 0.9 and to 1.1, when calcium chloride and sodium dihydrogen phosphate solutions were added, respectively.

Conclusions. The TF grades of tears collected from both goats and camels were improved after adding electrolyte solutions, and they were most remarkably improved when divalent electrolyte solutions were added, followed by the hydrogenated electrolyte.

Key words: animals, electrolytes, tears, tear film, tear ferning

Background

The stability of the tear film is critical for a healthy ocular system. Tear film lubricates the eye surface, transports oxygen and nutrients to the cornea, and washes away foreign bodies and waste.[1] It protects the ocular surface against infections, high temperature and chemicals, facilitates eyelid movement, and makes the reflection of the corneal surface smooth.[1] The tear film structure is very complex, but can be simplified as a three-layer model.[2] However, a different model structure has been suggested for the tear film. It includes a mixture of inner aqueous phase containing hydrophilic mucins, electrolytes and large molecules, such as enzymes and lipids as the outermost layer.[3,4] Mucins mix well with aqueous contents and spread over the ocular surface. Both the main and accessory lacrimal glands are responsible for producing aqueous contents of tear film.[4] Notably, meibomian glands produce lipids.[5] In animals, the conjunctiva accessory lacrimal glands produce tears.[6] The disturbance in lacrimal and meibomian glands functions induces tear film abnormality and dry eye disorder.[7]

The ocular tear film can be examined using several tests, in which each test determines a specific parameter. Tear volume can be measured using the phenol red thread test, while the Schirmer's test measures tear production.[8] When the tear evaporates, the breakup and thinning of tear film occur.[9] Therefore, tear breakup time measurement provides valuable information about tear film stability. The tear osmolarity test detects the equilibrium between tear evaporation, absorption, drainage, and production.[10] Osmolality is mainly controlled by the electrolyte concentration in the aqueous contents of tear film.[11] Tear lipid stability can be assessed by measuring the tear evaporation rate. Dry eye, discomfort and tear thinning are associated with excessive tear evaporation rate.[12] The tear ferning (TF) test has been used as a simple and low-cost tool to detect dry eye.[13,14] Capillary tubes are commonly used to collect tear samples with a low coefficient of variation.[15] Tears produce ferns when dried and their shapes are independent of sex, race and duration of day.[16–18] Dry eye tears have a shortage of ferns with large spaces between the branches.[14] The TF test has good specificity and sensitivity and has been used with other tests to assess the quality of tears in both animals and humans.[19–27]

Goats and camels have big eyes and are considered good animal models for surgical interventions.[28,29] Therefore, this study investigated the effect of adding electrolyte solutions on the TF patterns of tears collected from goats and camels. Recently, it has been reported that TF patterns of artificial tears have been improved when mixed with electrolyte and large molecule solutions.[30]

Objectives

To assess the effect of adding various electrolyte solutions on the TF patterns of tears collected from both goats and camels.

Materials and methods

Animals

The animals were randomly selected from a farm 120 km east of Riyadh, Saudi Arabia. The animals were healthy, without ocular disorders or diseases. The tear samples were collected from the lower meniscus of the right eye of 5 goats (2 males and 3 females; 3.4 ±1.6 years) and 5 camels (2 males and 3 females; 4.0 ±1.1 years) in the same environment by the same examiner. During the tear collection, no lacrimation instruments or anesthetics were used. The tears were stored in Eppendorf tubes in a cooled container and were transported immediately to the clinic. The study was ethically approved before tear collection. The study was approved by the Institutional Review Board of King Saud University Riyadh, Saudi Arabia (approval No. 131-3637).

Electrolyte solutions

The salts were obtained from Avonchem Limited (Macclesfield, UK). Solutions of sodium chloride (NaCl; 680 mg/mL), potassium chloride (KCl; 140 mg/100 mL), calcium chloride (CaCl$_2$; 5 mg/100 mL), magnesium chloride hexahydrate (MgCl.6H$_2$O; 12 mg/100 mL), and sodium hydrogen phosphate (NaH$_2$PO$_4$; 9.4 mg/100 mL) were prepared in double-distilled water (100 mL). The mixtures were stirred for 5 min using a Stuart magnetic stirrer (Cole-Parmer, St Neots, UK) to produce a solution of each electrolyte. The concentration of electrolytes was the same as in the basic tear solution.[31]

TF test

Microcapillary tubes (50 μL) obtained from Merck (Darmstadt, Germany) were used to collect the tear samples (20 μL) from the right eyes of the animals. A tear sample (1 L) of each animal was transferred over a microscopic slide to dry at normal environmental conditions (22°C with humidity ≤40%). The formed TF patterns were observed using an Olympus DP72 digital microscope (Olympus Key Med Limited, Southend-On-Sea, UK) at ×20 magnification. The produced ferns were graded based on the 5-point grading scale in 0.1 increments.[19] A tear sample (0.5 μL) from each animal was thoroughly mixed with different volumes of each electrolyte (0.5–5 μL). A tear sample (1 μL) of each mixture was dried over a glass slide and the produced ferns were graded and compared with those of the corresponding pure tears collected from each animal.

For comparison, a tear sample from each sheep (0.5 μL) was diluted with double-distilled water (0.5 μL) and the TF patterns were recorded for each mixture. The TF grades of diluted tears were the same as those of pure animal tears before the dilution. Three independent examiners graded the TF patterns. The 2nd and 3rd examiner were blinded to avoid bias. In many cases, the scores from the examiners were exactly the same and in other cases, the variation was less than ±0.05. An average TF grade was recorded and rounded to the nearest decimal place. The standard deviation (SD) has not been recorded since it was negligible in all cases (less than ±0.05). The TF patterns were graded in 0.1 increments.[19] No statistical tests were used to test the significance of the differences between the tear ferning grades (TFG) before and after the addition of the electrolyte solutions, mainly due to the small sample size.

Results

The age and sex of goats (n = 5; 2 males and 3 females; 3.4 ±1.6 years) and camels (n = 5; 2 males and 3 females; 4.0 ±1.1 years) from whom the tear samples (20 μL) were collected are shown in Table 1.

Table 2 shows the TF grades for tears collected from goats and their homogenous mixtures with different

Table 1. The age and sex of goats and camels from whom tear samples were collected

Tear sample	Age [years]	Sex
G1	6	female
G2	3	male
G3	4	female
G4	1	female
G5	3	male
C1	3	female
C2	4	female
C3	6	female
C4	3	male
C5	4	male

G1 – 1st goat tear sample; G2 – 2nd goat tear sample; G3 – 3rd goat tear sample; G4 – 4th goat tear sample; G5 – 5th goat tear sample; C1 – 1st camel tear sample; C2 – 2nd camel tear sample; C3 – 3rd camel tear sample; C4 – 4th camel tear sample; C5 – 5th camel tear sample.

volumes of electrolyte solutions (goat tear samples to electrolyte solutions = 1:1, 1:2, 1:4, 1:6, 1:8, and 1:10), based on the 5-point grading scale in 0.1 increments. Examples of the TF images of tears collected from the 3rd and 4th goat, and those obtained from their homogenous mixtures with some electrolyte solutions, which lead to the most improvements, are shown in Fig. 1 and Fig. 2, respectively.
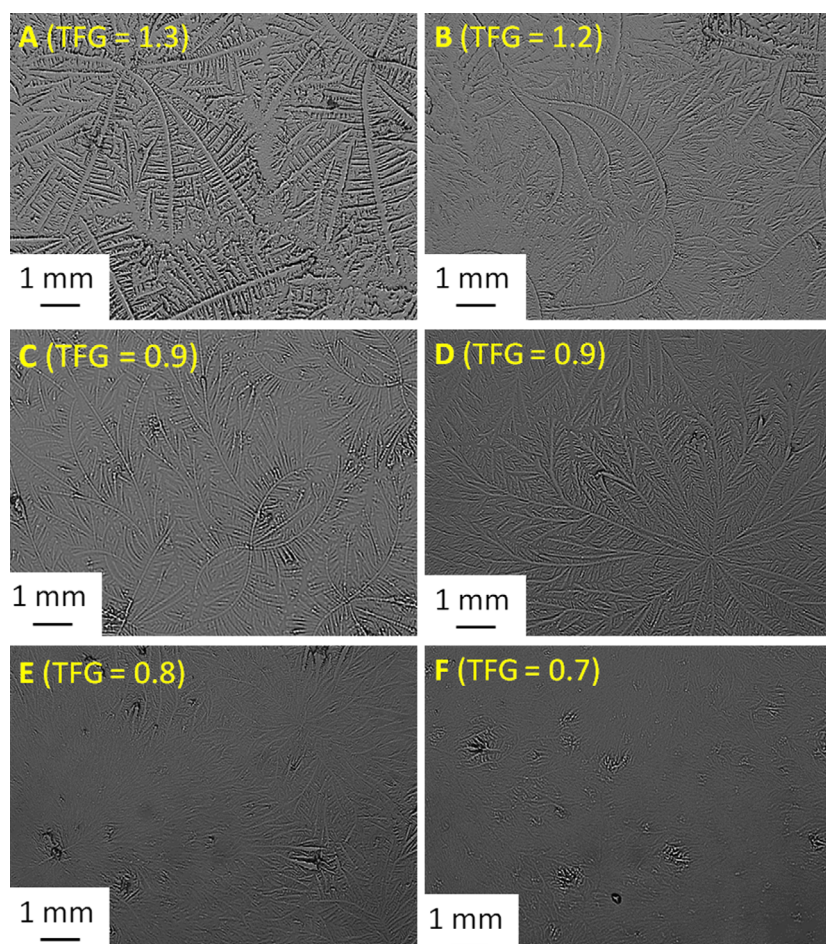

Fig. 1. The tear ferning (TF) patterns of A. pure G3; B. G3:NaCl (1:1); C. G3:KCl (1:4); D. G3:CaCl$_2$ (1:1); E. G3:MgCl$_2$.6H$_2$O (1:8); and F. G3:NaH$_2$PO$_4$ (1:8)

G3 – 3rd goat tear sample; TFG – tear ferning grade.

Table 2. Tear ferning (TF) grades of goat tears and those obtained from their corresponding mixtures with electrolyte solutions

Electrolyte	Ratio[a]	Tear sample				
–	–	0.8	1.5	1.3	1.2	1.4
NaCl	1:1	1.5	1.6	1.2	1.4	1.6
	1:2	1.4	1.6	1.3	1.2	1.3
	1:4	1.3	1.6	1.2	1.1	1.4
	1:6	1.4	1.6	1.2	1.2	1.5
	1:8	1.4	1.7	1.3	1.2	1.5
	1:10	1.4	1.7	1.3	1.2	1.3
KCl	1:1	1.5	1.5	1.2	14	1.4
	1:2	1.5	1.5	1.2	0.9	1.1
	1:4	1.5	1.4	0.9	0.9	1.1
	1:6	1.4	1.4	1.1	0.8	1.3
	1:8	1.4	1.4	1.1	0.5	1.2
	1:10	1.4	1.4	1.1	0.9	1.1
$CaCl_2$	1:1	1.3	1.3	0.9	0.8	1.3
	1:2	1.2	1.3	0.9	1.1	1.2
	1:4	1.2	1.3	0.9	0.4	0.9
	1:6	1.2	1.3	0.9	0.9	0.8
	1:8	1.2	1.3	1.0	0.7	0.8
	1:10	1.2	0.8	1.0	0.8	0.8
$MgCl_2.6H_2O$	1:1	1.3	1.2	1.1	0.6	1.3
	1:2	1.3	1.2	1.2	1.2	1.1
	1:4	1.2	1.2	0.9	1.1	1.1
	1:6	1.1	1.1	1.1	1.1	1.1
	1:8	1.1	1.2	0.8	1.1	0.7
	1:10	1.1	1.2	1.2	1.1	0.7
NaH_2PO4	1:1	1.4	1.3	1.1	1.1	1.0
	1:2	1.3	1.3	0.8	1.1	1.1
	1:4	1.2	1.3	0.8	1.1	1.1
	1:6	1.1	1.2	0.8	0.7	1.1
	1:8	1.1	1.2	0.7	0.6	0.9
	1:10	0.6	1.2	0.8	0.9	0.9

[a] – the volume ratio between tear sample and electrolyte solution. The TF grade was rounded to the nearest one decimal place.

The TF grades for the diluted animal tear samples using double-distilled water were the same as those recorded before the dilution. Such result eliminates the effect of water used to prepare the salt solution on the TF patterns of animal tear samples.

The TF grades of tears collected from goats (1.5−0.8) were improved after adding electrolyte solutions, and were mostly improved when the $CaCl_2$ solution was added. For example, the TF grade of tears collected from the 4th goat (G4) was improved from 1.2 to 0.4 when $CaCl_2$ solution (G4 tears to $CaCl_2$ ratio = 1:4) was added. Similarly, adding KCl solution (G4 tears to KCl ratio = 1:8) improved the TF grade of G4 to 0.5. Additionally, the TF grade of G4 was improved to 0.6 after either $MgCl_2.6H_2O$ (G4 tears to $MgCl_2.6H_2O$ ratio = 1:1) or NaH_2PO_4 solution (G4 tears to NaH_2PO_4 ratio = 1:8) was added. Limited

or no improvement was observed when the NaCl solution was used, regardless of its proportion within the mixtures. Adding divalent ($CaCl_2$ and $MgCl_2.6H_2O$) and hydrogenated (NaH_2PO_4) electrolyte solutions provided a better improvement in TF grades of goat tear samples compared with monovalent electrolytes (NaCl and KCl).

Table 3 shows the TF grades for pure tears collected from 5 camels and those obtained from the corresponding homogenous mixtures with different volumes of electrolyte solutions (camel tear samples to electrolyte solutions = 1:1, 1:2; 1:4, 1:6, 1:8, and 1:10) in 0.1 increments. Figure 3 shows TF images of tears collected from one of the camels (C3), and those obtained from their homogenous mixtures with some electrolyte solutions. The mean ±SD for the TF grades for both goats and camels after the addition of electrolytes are shown in Table 2,3, respectively.
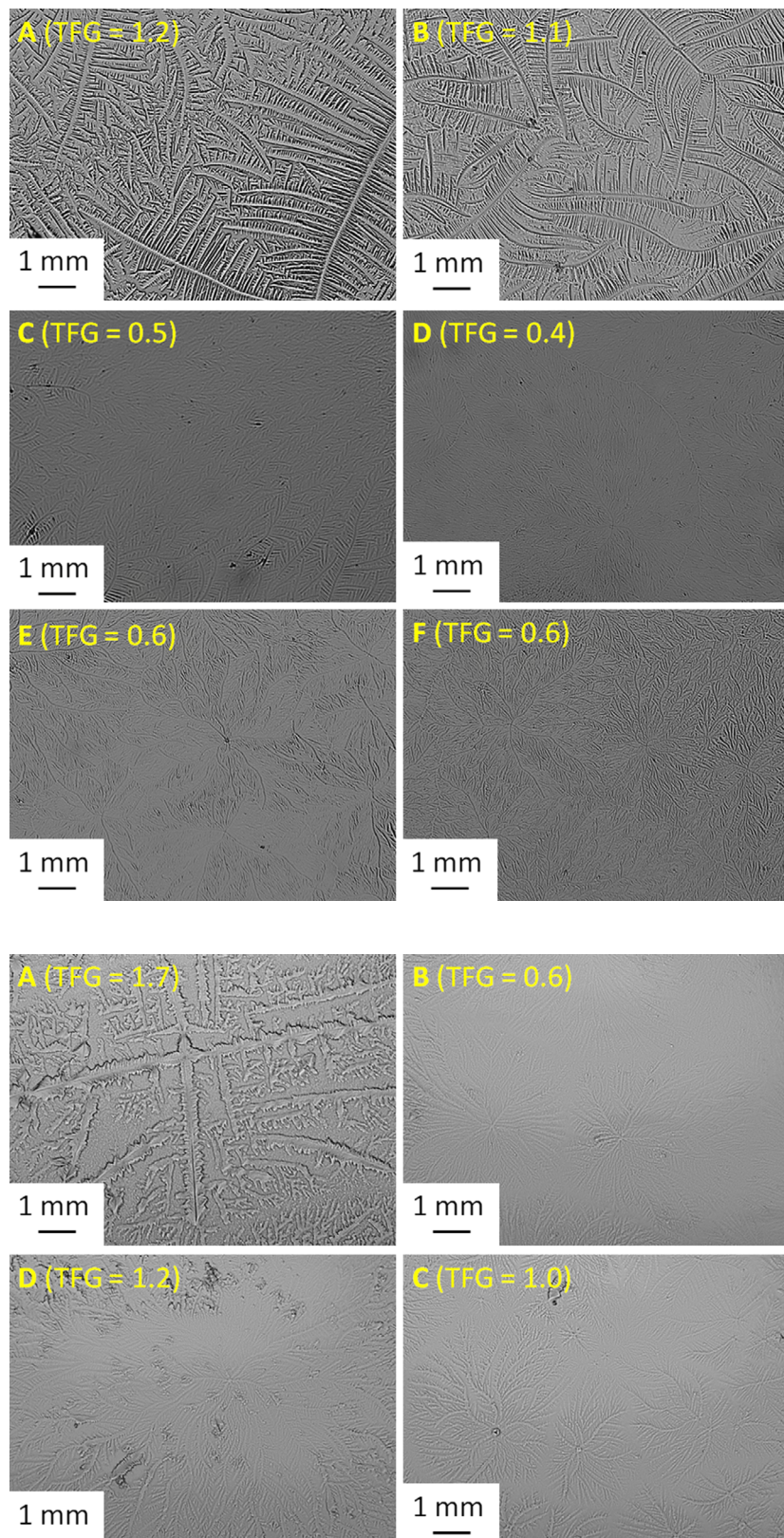
Fig. 2. The tear ferning (TF) patterns of A. pure G4; B. G4:NaCl (1:4); C. G4:KCl (1:8); D. G4:CaCl$_2$ (1:4); E. G4:MgCl$_2$.6H$_2$O (1:1); and F. G4:NaH$_2$PO$_4$ (1:8)

G4 – 4th goat tear sample; TFG – tear ferning grade.

Fig. 3. The tear ferning (TF) patterns of A. pure C3; B. C3:CaCl$_2$ (1:4); C. C3:MgCl$_2$.6H$_2$O (1:2); and D. C3:NaH$_2$PO$_4$ (1:2)

C3 – 3rd camel tear sample; TFG – tear ferning grade.

The TF grade improvements of tears collected from camels when mixed with electrolyte solutions were limited in all cases. However, the TF grade of tears collected from one of the camels (C3) was improved from 1.7 to 0.6–0.7 when CaCl$_2$ was added, regardless of its proportion in the mixtures. Similarly, the TF grade of the same camel was improved to 0.9 when NaH$_2$PO$_4$ (tears to electrolyte ratio = 1:1) was added to camel tears. The effect of the addition of different prorations of various electrolyte solutions on the TFG of the tears collected from the 3rd goat

Table 3. Tear ferning (TF) grades of camel tears and those obtained from their corresponding mixture with electrolyte solutions

Electrolyte	Ratio[a]	Tear sample				
–	–	1.8	1.9	1.7	1.8	1.9
NaCl	1:1	1.6	1.4	1.6	1.6	1.4
	1:2	1.7	1.4	1.5	1.4	1.4
	1:4	1.7	1.3	1.6	1.4	1.4
	1:6	1.6	1.3	1.7	1.5	1.5
	1:8	1.6	1.5	1.7	1.5	1.5
	1:10	1.7	1.5	1.7	1.5	1.6
KCl	1:1	1.4	1.4	1.3	1.3	1.3
	1:2	1.5	1.3	1.4	1.4	1.3
	1:4	1.5	1.5	1.4	1.4	1.4
	1:6	1.6	1.6	1.5	1.5	1.4
	1:8	1.6	1.6	1.5	1.6	1.5
	1:10	1.6	1.7	1.5	1.6	1.6
$CaCl_2$	1:1	1.7	1.3	0.7	1.2	1.2
	1:2	1.4	1.2	0.6	1.5	1.1
	1:4	1.5	1.4	0.6	1.2	1.4
	1:6	1.3	1.2	0.7	1.1	1.3
	1:8	1.3	1.2	0.7	1.1	1.4
	1:10	1.4	1.2	0.7	1.1	1.4
$MgCl_2.6H_2O$	1:1	1.4	1.6	1.2	1.5	1.3
	1:2	1.5	1.7	1.2	1.5	1.5
	1:4	1.4	1.6	1.2	1.4	1.5
	1:6	1.8	1.6	1.6	1.6	1.6
	1:8	1.6	1.6	1.5	1.4	1.5
	1:10	1.7	1.7	1.5	1.4	1.4
NaH_2PO4	1:1	1.4	1.5	0.9	1.5	1.2
	1:2	1.6	1.6	1.0	1.5	1.3
	1:4	1.4	1.5	1.4	1.3	1.4
	1:6	1.3	1.4	1.3	1.2	1.3
	1:8	1.4	1.3	1.2	1.2	1.4
	1:10	1.4	1.3	1.2	1.2	1.4

[a] – the volume ratio between tear sample and electrolyte solution. The TF grade was rounded to the nearest one decimal place.
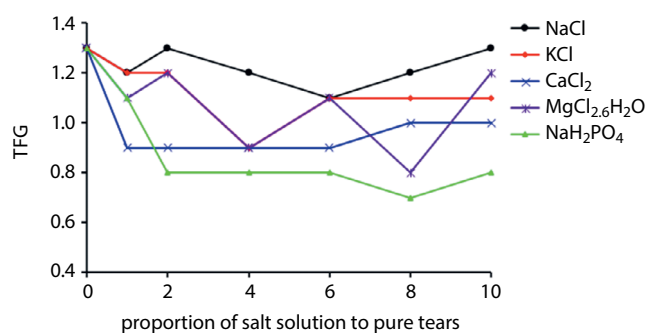

Fig. 4. Effect of various electrolyte solutions on the tear ferning grade (TFG) of the tears collected from the 3rd goat (G3)
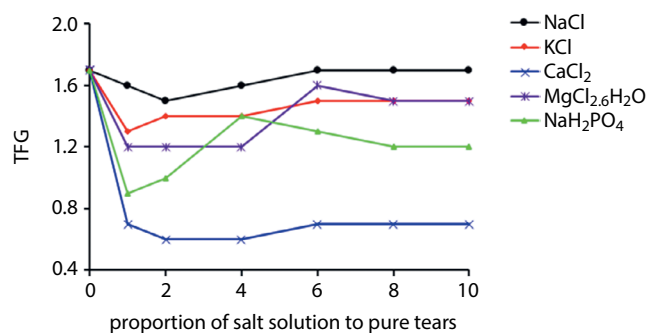

Fig. 5. Effect of various electrolyte solutions on the tear ferning grade (TFG) of the tears collected from the 3rd camel (C3)

(G3) and the 3rd camel (C3) are shown in Fig. 4 and Fig. 5, respectively. Clearly, NaH_2PO_4 solution led to the highest improvement in TF patterns of tears collected from the 3rd goat. At the same time, the addition of $CaCl_2$ solution led to the most noticeable improvement in TF patterns of the tears collected from the 3rd camel.

Discussion

This study showed that adding different volumes of several electrolyte solutions to tear samples collected from goats and camels improved their TF grades. The TF grades most remarkably improved when a solution of $CaCl_2$, NaH_2PO_4 or $MgCl_2.6H_2O$ was used. While relatively low proportions of $CaCl_2$ solution improved TF grades of goats, large volumes of NaH_2PO_4 solution were required to improve these grades. The NaCl and KCl solutions did not improve TF grades of tears collected from camels, since the high levels of ions (Na^+, K^+ and Cl^-) could upset the balance between electrolytes and large molecule concentrations in the camel tears.

Tear ferns are formed due to the interactions between electrolytes, specifically Na^+ and Cl^- ions, with macromolecules (mucins and proteins) within tear film.[32] The ratio between monovalent (e.g., Na^+ and K^+) and divalent (e.g., Ca^{+2} and Mn^{+2}) cations controls fern formation.[33] Notably, the TF patterns of tears collected from camels outperformed the corresponding ones for Refresh Plus eye drops.[27] The scanning electron microscope revealed that the tears collected from camels have perfect tertiary and quaternary divisions.[27] The ions such as K^+ and Cl^- are responsible for fern formation and were more prevalent in tears collected from camels compared with human tears and Refresh Plus eye drops.[27] Presumably, these anions help maintain healthy TF patterns in camels.[27] Also, the balance between Na^+, K^+ and Cl^- affected the fern formation rather than each individual ion concentration.[33] Both proteins and mucins facilitate fern formation, but are uninvolved in the fern structure.[33,34] Adding electrolyte and large molecule solutions improved the TF grades of artificial tears.[30]

Animals and humans have different types of protein in their tears.[35] In animals, season and sex can influence tear production.[36,37] This study showed no difference among TF grades in tears collected from both goats and camels, and those obtained from their corresponding homogeneous mixtures with electrolyte solutions.

Very recently, the improvement of TF patterns of sheep tears has been achieved through the addition of electrolyte solutions. The divalent electrolytes led to a noticeable improvement in TF patterns of sheep tears followed by sodium dihydrogen phosphate. The use of various concentrations of sodium chloride led to no improvement in TF patterns when added to sheep tears.[38] The TF patterns depend on the type and concentration of electrolyte in tears.[39] Salts are essential to adjust the osmolarity of tears, improve the secretion of tears, and suppress tear evaporation.[40] However, it is not clear why divalent electrolytes improve the TF patterns of tears obtained from both goats and camels compared with monovalent salts. The concentration of monovalent electrolytes used in this study was much higher (140–680 mg in 100 mL water) compared with that used for divalent salts (5–12 mg in 100 mL water), as in the basic tear solution.[31] Such high concentrations

of salts (NaCl and KCl) could lead to hyperosmolality and as a result, high TF grades. To confirm this hypothesis, further research is needed to test the effect of a low concentration of monovalent electrolytes on the TF patterns of natural tears (in animals and humans).

The mean difference and 95% limits of agreement for TF grades of normal eye tears for different day durations were 0.1 ±0.4.[17] The TF grade mean difference for tears collected from goats and camels and those obtained from their corresponding mixtures with electrolyte solutions was minute (±0.1). The scores recorded by 2 examiners were exactly the same in many cases and the changes in TF grades were minute (less than ±0.05) in other cases, particularly for camel tears. The correlations between TF grades and the scores from other dry eye tests in healthy and dry eye subjects are generally weak.[17,23,41] Nevertheless, the TF test is repeatable and reliable to detect dry eye.[41–43]

Limitations

The current study has some limitations, which can be addressed in related future research. First, the sample size for both goats (n = 5) and camels (n = 5) was small. Second, the animals involved in the study had healthy eyes. No animal with a dry eye was involved in the study (the TF grades of tears were less than 2). We anticipated that the effect of electrolytes solution will improve the TF patterns of tears collected from dry eyes even better than those recorded for normal eye tears. However, such prediction needs to be investigated. Third, the effect of other electrolytes in different concentrations needs to be tested. Fourth, the animals have been recruited from a single farm that represents a specific location in Saudi Arabia. Fifth, no statistical tests were used to test the significance of the differences between the TFG before and after the addition of the electrolyte solutions, mainly due to the small sample size.

Conclusions

The TF grades of tears collected from both goats and camels were improved after adding the electrolyte solutions, and were most improved when divalent electrolytes (calcium chloride and magnesium chloride hexahydrate) were added, followed by the hydrogenated electrolyte (sodium dihydrogen phosphate).

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