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Short communication

EFFECT OF AMIDOTRIZOATE (UROGRAPHIN 76%) ON TEAR FLUID TOTAL PROTEIN, LYSOZYME, SODIUM AND POTASSIUM CONCENTRATIONS AND TEAR ELECTROPHORESIS AFTER DACRYOCYSTOGRAPHY IN DONKEYS

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Summary

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Dacryocystography has been widely used in the assessment of the nasolacrimal duct system, particularly in patients with epiphora. In most instances, Lipiodol (oil-based contrast media) is the contrast agent of choice among the agents compared. In equids, the effects of Urographin 76%, an oil-based contrast medium on the tear parameters have not been determined in details yet. The aim of this study was to investigate the effects of amidotrizoate (Urographin 76%) on the total protein, lysozyme, sodium and potassium concentrations, and electrophoresis of tears in clinically normal donkeys. Ten normal donkeys from both sexes were submitted to dacryocystography with Urographin 76%. Tear samples were collected before and 2 hours and 2 weeks post dacryocystography. All tested parameters changed significantly, but the electrophoretic diagram of all samples have not shown any quantitative changes. It is concluded that Urographin 76% was not the contrast agent of choice despite providing the greatest conventional radiographic image quality among contrast agents.

Key words: dacryocystography, donkey, electrophoresis, lysozyme, potassium, protein, sodium, tear, Urographin

Dacryocystography, or the visualization of the nasolacrimal system by means of contrast media, has been used clinically in man since 1909 when Dr J. Ewing obtained the first dacryocystogram by using iodized oil (Gelatt *et al.,* 1972; Gammal & Brooks, 1981). This is the traditional radiological investigation for epiphora. The procedure facilitates the delineation of fistulas, tumours, diverticula or calculi, so that therapy can be accurately planned (Munk *et al.*, 1989). Since the introduction of dacryocystography, a variety of contrast agents have been used. They are

categorized in one of two types: water- and oil-based. Having compared water- and oil-based contrast media in dacryocystography, Oil-based contrast media are preferred as they provide higher quality images and highest level of patient comfort (Munk *et al.*, 1989). Water-based preparations however, present several advantages over oil-based products – a viscosity similar to that of normal tears, fewer radiographic artifacts, but at the same time they empty very rapidly (Hogan & Skorin, 2003).

In veterinary medicine, diagnostic dacryocystography have been employed in a case of maxillary bone cyst in a dog (Featherstone & Llabres Diaz, 2003), chronic dacryocystitis in a dog (Giuliano *et al.*, 2006), nasolacrimal duct atresia in an alpaca (Mangan *et al.*, 2008). Experimental studies have been performed for evaluation of nasolacrimal duct stents in rabbits (Wilhelm *et al.*, 2006) and in donkeys to assess the effect of iohexol on tear production (Tabatabaei Naeini & Ziaei Darounkolaei, 2008;) and tear biochemistry (Tabatabaei Naeini *et al.,* 2008). The aim of the former study was to investigate the effects of iohexol on tear production and normal fluctuations of Schirmer tear test (STT) values in donkeys. The STT of ten normal donkeys were determined, which were done pre- and post-treatment of iohexol during dacryocystography; and in the latter we examined 10 normal donkeys that received iohexol once a day. Different tear parameters (total protein, lysozyme, sodium, and potassium and protein electrophoresis of tear) were measured at 0, 2 hours and 2 weeks after dacryocystography with iohexol.

The aim of this study was to investigate the effects of a water-based contrast medium: amidotrizoate (Urographin 76%) used for dacryocystography, on the total protein, lysozyme, sodium and potassium concentrations, and electrophoresis of tears in clinically normal donkeys.

Ten adult donkeys (2 females and 8 males) weighing 240−320 kg, aged from 3 to 6 years were included in this study. Prior to the study, complete physical examination, complete blood counts and ophthalmic examination were performed in all animals and they were all determined to be healthy and to have normal lacrimation. The donkeys had not received any medications before the study. All donkeys were housed together in outdoor paddocks and received hay and water *ad libitum*.

Complete ophthalmic examinations were performed on both eyes on the day of treatment prior to drug administration. Dacryocystography was performed in all donkeys using Urographin 76% (1 mL Urographin 76% contains sodium amidotrizoate 0.10 g and meglumine amidotrizoate 0.66 g; 370 mg I/mL) as a contrast medium at a dose of 3 mL/eye at approximately the same time.

Tear samples were obtained from all donkeys at 10 AM, with the donkeys unsedated. No finger pressure was exerted on the medial commissure to block the lacrimal puncta. Samples were obtained at time 0 (prior to dacryocystography), 2 hours and 2 weeks post dacryocystography, between 10:00 to 14:00 h.

Total protein in tears was analyzed by Lowry's method, based on reaction of Cu (II) ion in alkaline solution with protein to form complexes, reacting with the Folin-phenol reagent, and forming product quantitated at 750 nm (Lowry *et al.*, 1951).

Lysozyme content of tears was assessed by enzymatic assay (Shugar, 1952). During the procedure, *Micrococcus lysodeikticus* cell wall (9 mg) was added to 25 mL 0.9 M potassium phosphate buffer (pH 7.0), and then the final

Table 1. Tear samples dilutions for atomic absorption spectrometry

Elements	Before administration of Urographin 76%	After 2 hours	After 2 weeks
K	1/450	1/450	1/450
Na	1/3500	1/3500	1/4500 and 1/7500

Table 2. Total protein, lysozyme, sodium and potassium concentrations in tear fluid of donkeys before and after administration of Urographin 76% at a dose of 3 mL/eye (mean ± SEM, n=10)

Tear fluid parameters	Before dacryocystography (0 hour)	Time after dacryocystography	
		2 hours	2 weeks
Total protein (mg/mL)	5.88 ± 0.96	3.66 ± 0.19	6.44 ± 2.32#
Lysozyme (U/mg protein)	858 ± 76	1079 ± 68	552 ± 136#
Sodium (ppm)	3049 ± 324	2738 ± 218	6777 ± 1639*#
Potassium (ppm)	863 ± 23	662 ± 32*	1648 ± 87*#

* $p<0.05$ vs hour 0, # $p<0.05$ vs hour 2.

mixture volume was adjusted to 30 mL with the same buffer. Tear lysozyme was quantitated by adding 100 µL tear to the 2.9 mL mixture in a cuvette at 450 nm and recorded for 1 min. Free Na and K concentrations were quantitated by a Shimadzu (AA-670) atomic absorption unit with air-C_2H_2 burner. Aldrich™ standards of Na and K were applied to setting up their standard curves at 589.0 and 766.5 nm respectively (Cantle, 1982). Prior to the analysis, tear samples were diluted as shown in Table 1.

SDS-PAGE was performed on gradient (5−20%) gel casts. Tear samples were preincubated in sample buffer at 100 ºC water for one minute before electrophoresis. Standard molecular weight marker (Fluka, Co. Ltd.) was performed in the gels. From the samples, up to 20 µL was applied with Hamilton sample applicator. Total tear protein applied on each pit was estimated up to 1 µg/µL sample, because of inadequate tears remaining in part of samples. Electrophoresis was performed under the manufacturer's instructions (approximately 3 hours, 60 mA), using casted 5−20% gradient gels. After electrophoresis, gels were Coomassie Brilliant Blue stained.

Data were analyzed using a one-way analysis of variance (ANOVA) for repeated measures. An alpha level of 0.05 was set as statistically significant.

Two hours after administration of Urographin 76%, the tear potassium level was the only parameter that changed. All other parameters changed significantly 2 weeks after the procedure compared to hour 2 (Table 2).

Results of SDS-PAGE confirmed that numerous protein bands were present in all donkeys (Table 3). At least 11 electrophoretic bands were demonstrated on SDS-PAGE with Coomassie Brilliant Blue staining between 10−150 kDa (Fig. 1). No variation among samples was detected in numbers and molecular weights of protein bands obtained by gradient gel electrophoresis. By the methods used, all samples have not shown any changes in number of bands electrophoretically.

Image quality and level of patient discomfort during examinations are parameters of choice for contrast media in dacryocystography (Munk *et al.*, 1989); but safety of used contrast media still remains unknown. Munk *et al.* (1989) have re-

Table 3. Molecular weights of the electrophoretic bands, detected on tears SDS-PAGE 5-20%*

№	Molecular weights of electrophoretic bands, KDa
1	10
2	~ 15
3	~ 18
4	~ 20
5	25
6	~ 30–35
7	40
8	50
9	~ 60–70
10	~ 100–120
11	150

* Table 3 as well as Fig. 1. are revised by Prof. M. Aminlari, Professor in Biochemistry, Department of Basic Sciences, Shiraz Faculty of Veterinary Medicine, Shiraz University, Iran.

ported Lipiodol (oil-based contrast media) to be the contrast agent of choice because it was well tolerated by the patients and provided highest conventional radiographic image quality.

The fluid secreted by the lacrimal glands is a complex solution of ions and proteins produced by two resident secretory cell populations: the plasma cells of the immune system and the acinar and duct cells of the secretory epithelium of the gland (Walcott, 1998); by the way fibronectin in the tear fluid was derived from plasma and that the increase in concentration in closed eye and reflex tear fluid was caused by leakage from dilated conjunctival blood vessels (Salvatore *et al.*, 1999). The quantity of overall protein concentration, is influenced by the sensory and/or autonomic innervation to the lacrimal gland (Salvatore *et al.*, 1999). Tear protein complex changes during eye disease (Kuizenga *et al.*, 1991) and is shown to be elevated during dry eye (Grus *et al.*, 2002). In our study, tear total protein concentration was elevated sig-

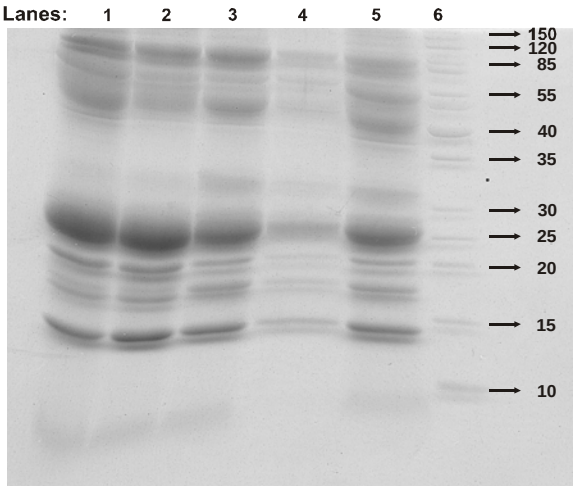

Fig. 1. Molecular weights (kDa) of the electrophoretic bands, detected on tears SDS-PAGE 5-20%. Lanes 1, 2 and 3: distribution of tear proteins before, 2 hours and 2 weeks post dacryocystography of one of donkeys; lanes 4 and 5: distribution of tear proteins before and 2 hours after dacryocystography of another donkey; lane 6: molecular weight markers between 10–150 KDa.

nificantly 2 weeks post dacryocystography.

The lacrimal gland-specific proteins found at highest concentrations in the tears are lactoferrin, tear-specific prealbumin (TSP or lipocalin), and lysozyme (Walcott, 1998). Tear lysozyme content decreases with age. There are no gender-related statistical differences between subjects (Moss *et al.*, 2004; Hartley *et al.*, 2006). Tear lysozyme is in normal range in conjunctivitis and keratitis, although decreases in herpes keratitis (Chao *et al.*, 1990). Tear lysozyme increases in patients with complete or partial obstruction of nasolacrimal duct (Lew *et al.*, 2005). Some species as llamas, goats and sheep have high levels of tear lysozyme, but cows exhibit lower concentration (Gionfriddo *et al.*, 2000a; 2000b). Increased level of lysozyme with inflammation (Gionfriddo *et al.*, 2000a) and infection (Pinard *et al.*, 2003) makes it a fitness indicator of eye. Gionfriddo *et al.* (2000a) demonstrated high tear lysozyme levels in llamas make them resistant to pink eye. Results of our study showed that tear lysozyme content of donkeys was greatly higher than that in llamas, sheep and cows. In the other hand, the significant decrease in lysozyme in the current study probably documented the lack of any bacterial infections. Together with the significant increase in total protein, decreased lysozyme content indicated presence of enhanced levels of other protein-based molecules such as lactoferrin, tear-specific prealbumin (TSP or lipocalin), immunoglobulins etc., which have important roles against pathogens and/or pathological conditions.

One of the major secretory "products" of the lacrimal gland is water. It is moved from the interstitial spaces of the gland into the lumen of the gland where it is mixed with the other secretory products. This water movement is accomplished by osmosis, which depends on the movement of particles (ions) from the acinar cells into the lumen. Therefore, most studies have examined the process of water movement indirectly by characterizing the membrane channels through which ions move in and out of the acinar cells (Walcott, 1998). The exchange of sodium and potassium ions in the current research showed statistically significant increase in both levels of sodium and potassium ions 2 weeks after the dacryocystography.

Gionfriddo *et al.* (2000b) have reported 10 individual proteins in llama and cattle tears; however several bands differed between the 2 species. Remarkably we have observed the same number of protein bands in donkeys too. Because of different markers used in both researches, we couldn't make a consistent comparison of them. On SDS-PAGE, despite molecular weights of tear protein bands had not shown changes (in the number of band points) at any time, it could not be said categorically that nothing happened because we couldn't compare the density of protein bands, due to the little volume of collected tear samples. In these instances a little protein were loaded in gel. Altogether the SDS-PAGE couldn't demonstrate the safety of Urographin 76%.

The obtained results allowed us to conclude that in this study, Urographin 76% caused significant changes in tear components in donkeys and that this contrast agent should be used cautiously for dacryocystography.

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