Effect of dexamethasone in combination with acetylcysteine at different times on corneal wound healing in dogs

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Summary

The purpose of this study was to evaluate the effects of different time combinations of dexamethasone and acetylcysteine on experimentally induced corneal ulcers in dogs. Experimental corneal wounds were created surgically to the anterior one third of the cornea in the center of all eyes of 15 mixed breed dogs. The eyes were divided into five groups according to planned post-operative medications: group 1, one drop of N-acetylcysteine 3% and one drop of dexamethasone 0.1% immediately after surgery; group 2, two drops of N-acetylcysteine 3% from day 1, one drop of N-acetylcysteine 3% and one drop of dexamethasone 0.1% from day 15; group 3, two drops of N-acetylcysteine 3%; group 4, two drops of dexamethasone 0.1%; group 5 (control), two drops of normal saline. When applied immediately after corneal ulceration, dexamethasone 0.1% (group 4) decreased corneal haze significantly and did not delay corneal wound healing. Addition of dexamethasone 0.1% to N-acetylcysteine 3% from day 15 (group 2) significantly suppressed opacity at two months after the beginning of the study, but when dexamethasone 0.1% associated to N-acetylcysteine 3% immediately after corneal ulceration (group 1), significant delay in corneal wound healing was induced. It is concluded that combination of dexamethasone 0.1% and NAC 3% immediately after surgery may delay corneal wound healing, also use of these drugs individually, has no obvious clinical effect on corneal haze. On the other hand, use of these drugs in combination with each other may reduce the corneal haze in later months after discontinuation of drugs. However, further studies using larger groups of animals are needed to demonstrate the effectiveness of these pharmacological modulators following experimentally induced corneal wounds in dogs.

Key words: Acetylcysteine, Corneal haze, Dexamethasone, Dogs, Wound healing

Introduction

Corneal wounds still represent an important problem in clinical ophthalmology because of the loss of transparency of corneal scar tissue (Kubota and Fagerholm, 1991; Suzuki *et al*., 2003). This is probably related to the wound-healing response when activated keratocytes lay down new collagen and proteoglycan matrix (Krueger *et al*., 1995). The creation of randomly orientated collagen fibrils with differing dimensions and irregular spacing results in the reflection and diffusion of light rather than transmission (Jester *et al*., 1999). Clinically, this is identified as scarring. On the other hand, in corneal ulcers the combination of overexpression of certain destructive proteinases and reduction in antiprotease activity can lead to rapid degradation of collagen and other components of the corneal extracellular matrix (ECM) (Brown, 1971; Berman, 1980; Ye and Azar, 1998; Strubbe *et al*., 2000). Matrix metalloproteinases (MMPs) and serine proteinases seem to be the predominant proteinases in the corneal wound healing process in dogs (Chandler *et al*., 2003). MMP-2 and MMP-9 are increased in the corneal epithelium of dogs with refractory

superficial ulcers (Chandler *et al*., 2003). MMP-9, produced by corneal epithelial and stromal cells, destroys the adhesive structure of the epithelial cell basement membrane before overt stromal ulceration, and delays the re-epithelialization of the ulcerated cornea (Fini and Girard, 1990; Matsubara *et al*., 1991; Ye and Azar, 1998; Wong *et al*., 2002). MMPs derived from fibroblasts cause corneal stromal remodeling and increased clarity (Wong *et al*., 2002). Furthermore, several researches have been done to find a way to improve the corneal wound healing and to decrease the corneal haze after photorefractive keratectomy (PRK) (Petroutsos *et al*., 1982; Corbett *et al*., 2001; Stiles *et al*., 2003; Esquenazi *et al*., 2005; Kim *et al*., 2006). Attention has been focused on modulating the process of post-procedural wound healing (Alio *et al*., 1998; Brilakis and Deutsch, 2000). MMP inhibitors have been recommended for the treatment of ulcerative keratitis and progressive keratomalacia to reduce the progression of stromal ulceration, speed epithelial healing, and minimize corneal scarring (Brown and Weller, 1970; Brown *et al*., 1970; Brown and Hook, 1971; Berman, 1975; Berman *et al*., 1975; Schultz *et al*., 1992; Barletta *et al*., 1996; Clark, 1998; Brooks, 1999; Ward, 1999; Strubbe *et al*., 2000), and steroids have been used to reduce the corneal haze (Donshik *et al*., 1978; Morlet *et al*., 1993; Park and Kim, 1996; Vetrugno *et al*., 2001; Tani *et al*., 2002; Yulek *et al*., 2006). It has been shown that the use of a collagenase inhibitor (N-acetylcysteine) in combination with a steroid (dexamethasone) immediately after corneal ulceration in rabbits may delay wound healing and is not able to decrease the corneal haze compared to controls (Sarchahi *et al*., 2008). We hypothesized that the use of this combination at different times may lead to different results. To test this hypothesis, we evaluated the effects of dexamethasone in combination with acetylcysteine at different times on experimentally induced corneal wounds in dogs.

Materials and Methods

Thirty eyes of 15 mixed breed dogs of both sexes (10 months to 30 months) weighing 8.55 to 24 kg (mean ± SD, 16.54 ± 4.23 kg), with normal eye examination, were used. The study was approved by the Research Animal Care and Use Committee of the School of Veterinary Medicine, Shiraz University. Dogs were anesthetized with an intravenous injection of a mixture of ketamine hydrochloride (10 mg/kg), xylazine hydrochloride (1 mg/kg) and acepromazine maleate (0.11 mg/kg). A pediatric eyelid speculum (Lid retractor) was used to open the eyelids and expose the cornea; a muscle hook was then placed under the inferior rectus muscle to control ocular movements during the trephination. A 6.5-mm calibrated corneal trephine was placed in the center of the cornea and trephination was performed. The trephine depth was determined previously to be at a specific setting that would expose about the anterior one third of the cornea (200 microns). A crescent bevel-up blade was used to perform the keratectomy of the trephinated area. This procedure was performed on both eyes of all dogs.

All eyes were administered one drop of gentamicin ophthalmic solution topically at least 5 min before treatment for 5 days in order to prevent infection. The eyes of the animals were randomly divided into five groups according to planned post-operative medications: eyes in group 1 were treated topically with one drop of N-acetylcysteine (NAC) 3% (Rotexmedica, Germany) and one drop of dexamethasone 0.1% (Sinadarou, Tehran, Iran) immediately after surgery; eyes in group 2 were treated with two drops of NAC 3% from day 1 and with one drop of NAC 3% and one drop of dexamethasone 0.1% from day 15; eyes in group 3 were treated with two drops of NAC 3% from day 1; eyes in group 4 were treated with two drops of dexamethasone 0.1% from day 1; and eyes in group 5 (control) were treated with two drops of normal saline from day 1. All eyes were treated topically 6 times a day (once every two h from 8 a.m. to 6 p.m.) for 25 days. After the staining of wounds by fluorescein every day at 8 a.m. the epithelial defects were photographed daily and the pictures transferred to computer. The area of defects was then calculated in mm^2 by Autocad

program (2005). Healing was determined to be complete when no fluorescein stain uptake was visible with a cobalt light source, however, drops were continued to day 25 in all eyes. All eyes were thoroughly examined to assess corneal haze monthly to 3 months after the beginning of study. The corneal haze was determined objectively by the same examiner at all times and the rate of corneal haze scored in each eye (0 = clear cornea without opacity, 1 = mild opacity, 2 = moderate opacity, 3 = sever opacity) on the basis of light transmission and visibility of the fundus. Each time after examination, two dogs from each group were killed humanly and both eyes were enucleated, fixed in buffered formalin 10%, processed and 5 µm thick sections prepared. Finally, specimens were stained with hematoxylin and eosin and examined by light microscopy searching for changes in epithelium, stroma and endothelium.

Statistical analysis

Data for re-epithelialization and daily healing of each eye were analyzed by linear regression and then slopes were analyzed by Student's t-test. For the comparison of the total healing time in the control and treatment groups, the data were analyzed by Student's t-test and scores from the corneal haze were analyzed by the non parametric Mann-Whitney U test. A p-value less than 0.05 was considered statistically significant. All results were expressed as mean ± SD.

Results

Clinical evaluation results

Thirty healing curves were obtained and their slopes evaluated from the areas of photographed defects. Data of mean healing time and mean daily healing are shown in Table and Fig. 1. Results of the statistical analysis revealed that the difference of the mean healing time and mean daily healing between the control and treatment eyes in group 1 (NAC + Dexa1) were statistically significant and drug combinations increased the mean healing time (P = 0.009) and decreased the mean daily healing (P = 0.018) compared to the control group; On the other hand, differences of the mean healing time and mean daily healing between the control and treatment eyes in group 2 (NAC + Dexa15), group 3 (NAC) and group 4 (Dexa) were not significant.

All dogs showed blepharospasm and photophobia during the first two days after operations. On the first day after operation all dogs showed conjunctival hyperemia.

One month after surgery, ophthalmologic examinations revealed that, corneal haze in groups 1, 2 and 3 was just the same as the control group, while there was significantly less corneal haze in group 4. Two months after surgery, there was significantly less corneal haze in groups 1

Table 1: Mean healing time and mean daily healing in the treatment and control groups (in each group, n = 6)

Parameters	Groups								
	Control (Saline)	Group 1 (NAC+Dexa1)		Group 2 (NAC+Dexa15)		Group 3 (NAC)		Group 4 (Dexa)	
	Mean±SD	Mean±SD	P-values[a]	Mean±SD	P-values[a]	Mean±SD	P-values[a]	Mean±SD	P-values[a]
Mean healing time (Days)	4.67 ±1.03	9.00 ±2.68	0.009	7.00 ±2.83	0.104	6.67 ±3.33	0.190	6.67 ±2.94	0.147
Mean daily healing (%)	22.50 ±5.94	12.59 ±6.16	0.018	16.13 ±5.85	0.091	18.52 ±9.24	0.399	18.22 ±9.24	0.366
Mean slopes of regression	-0.1200 ±0.0249	-0.2383 ±0.0791	0.013	-0.1675 ±0.0571	0.105	-0.1725 ±0.0951	0.241	-0.1657 ±0.0586	0.124
Clinical scar score 1st month after surgery	2.25 ±0.27	2.58 ±0.49	0.201	2.58 ±0.49	0.201	2.25 ±0.27	1.0	1.75 ±0.42	0.031
Clinical scar score 2nd month after surgery	1.63 ±0.48	1 ±0	0.046	1 ±0	0.046	1.5 ±0.87	1.0	1 ±0.50	0.146
Clinical scar score 3rd month after surgery	1.25 ±0.35	1 ±0	0.317	1 ±0	0.317	0.75 ±0.35	0.221	0.75 ±0.35	0.221

[a]: Compared with control group, NAC: N-acetylcysteine 3%, Dexa: dexamethasone 0.1%, Dexa1: dexamethasone 0.1% used immediately after operation, Dexa15: dexamethasone 0.1% used from day 15, and Clinical scar scores: 1, mild haze; 2, moderate haze; 3, severe haze

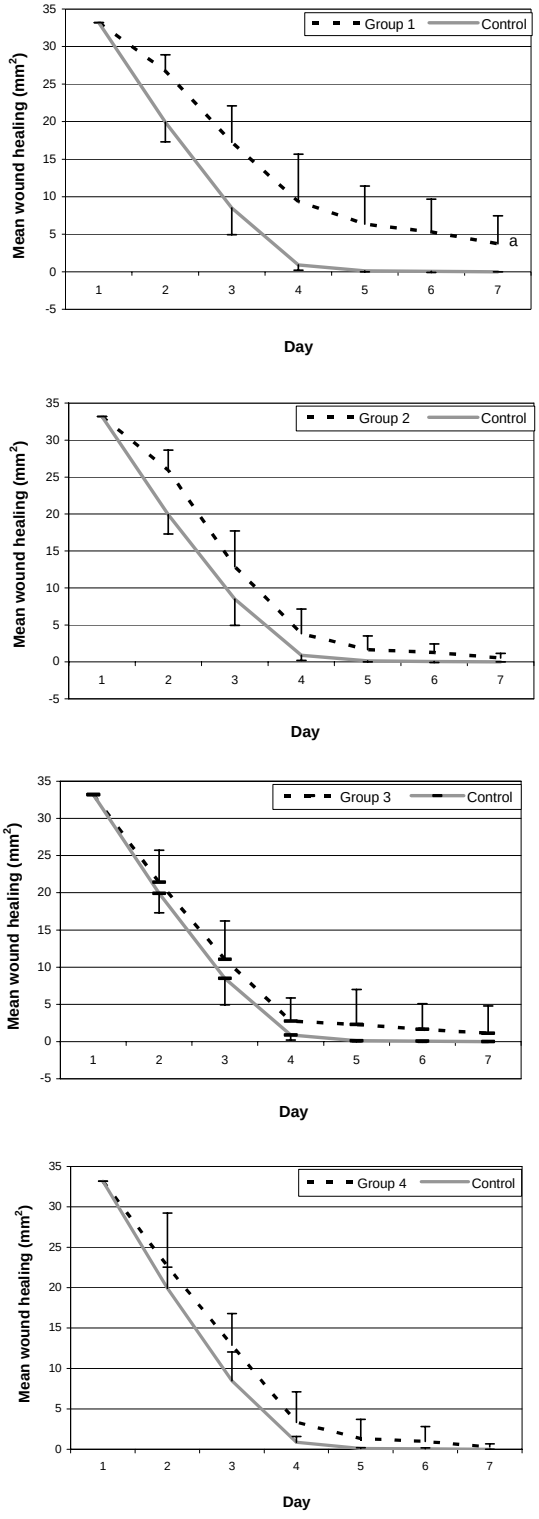

Fig. 1: Comparisons of mean daily corneal wound healing in dogs of groups 1, 2, 3 and 4 and control (n = 6). [a] is significantly different compared to control

and 2, but the decrease of corneal haze in groups 3 and 4 was not statistically significant. Three months after surgery, there was less corneal haze in all treatment groups than the control, but these differences were not statistically significant (Table 1).

Histopathologic examination results

Histopathologic examination of the formalin-fixed corneas revealed more epithelial hyperplasia in some eyes of the groups receiving NAC (group 1, 2, 3) (Figs. 2 and 3). Stromal lamellae irregularity and scarring was similar in all groups, and Descemet's membrane and endothelium seemed normal in all eyes.

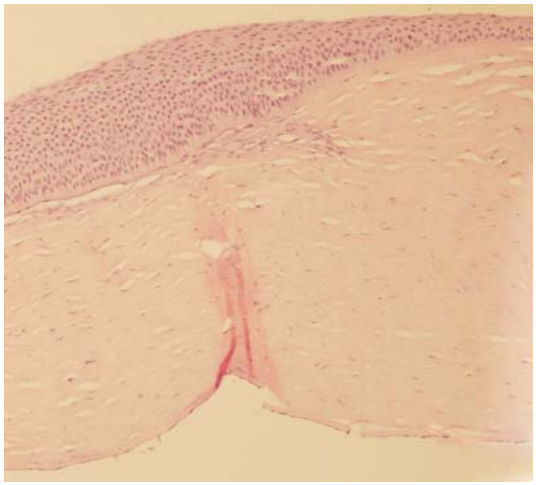

Fig. 2: Epithelial hyperplasia in the cornea of a dog in group 2. Note the normal epithelial layers on right compared to more than three fold increased cellular layers on left, (H&E, ×100)

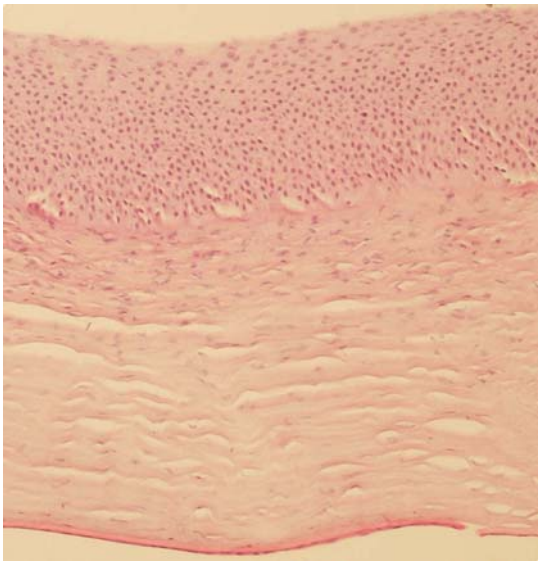

Fig. 3: Epithelial hyperplasia overlying stromal scar in cornea of a dog in group 3 (H&E, ×100)

Discussion

NAC is an MMP inhibitor commonly used in human as well as veterinary ophthalmology (Slansky *et al*., 1969; Berman, 1980; Petroutsos *et al*., 1982; Kanao *et al*., 1993; Ward, 1999). The beneficial effects of NAC on corneal wound healing have been reported by several authors (Absolon and Brown, 1968; Brown *et al*., 1969; Slansky *et al*., 1969; Brown and Weller, 1970; Brown *et al*., 1972; Berman and Manable, 1973; Sugar and Waltman, 1973; Williamson *et al*., 1974; Berman, 1975; Berman and Dohlman, 1975; Fraunfelder *et al*., 1977; Haut *et al*., 1977; Petroutsos *et al*., 1982; Burns *et al*., 1989; Kanao *et al*., 1993; Corbett *et al*., 2001; Aldavood *et al*., 2003). Most of these studies have shown that low concentrations of NAC, if used topically, have no toxic effects on the cornea, yet accelerate the corneal wound healing (Brown *et al*., 1969; Slansky *et al*., 1969; Brown and Weller, 1970; Sugar and Waltman, 1973; Berman, 1975; Petroutsos *et al*., 1982; Aldavood *et al*., 2003).

The use of topical corticosteroids in the management of corneal wounds is controversial. The beneficial effects of dexamethasone on corneal wound healing have been reported by some investigators (Donshik *et al*., 1978; Morlet *et al*., 1993; Park and Kim, 1996; Vetrugno *et al*., 2001; Tani *et al*., 2002; Yulek *et al*., 2006). Conversely, others have reported that corticosteroids have no long lasting effect on either haze or regression after refractive surgery, and were associated with an unacceptably high incidence of unwanted effects (Brown *et al*., 1970; Francois and Feher, 1973; Puelhorn *et al*., 1978; Gartry *et al*., 1992; Corbett *et al*., 1995; Chung *et al*., 1998).

It has been shown that co-administration of 3% concentration of NAC and 0.1% concentration of dexamethasone immediately after surgery may delay wound healing and is not useful to inhibit corneal haze formation compared to controls (Sarchahi *et al*., 2008).

In the present study, the combination of NAC 3% and dexamethasone 0.1%, when used immediately after ulcerations, significantly delayed the corneal wound healing. It seems that in this state, dexamethasone-induced collagen degradation by corneal fibroblasts (Lu *et al*., 2004) and lysis of disulfide linkages between mucopolysacharides and tissue proteins by acetylcysteine is responsible for the tissue destruction (Obenberger and Cejkova, 1972; Sugar and Waltman, 1973), although the exact mechanism of this alteration remains uncertain.

Ophthalmologic examinations in 5 groups showed that one month after operations, the corneal haze in group 4 was significantly less than the control group. Corneal haze in group 1, 2 and 3 was the same as the control group at this time. Two months after surgery there was significantly less corneal haze in group 1 and 2, there was also less corneal haze in group 3 and 4, but these differences were not statistically significant. At three months after surgery, there was non-significant less corneal haze in all treatment groups. These results show that when dexamethasone is prescribed in combination with NAC immediately after corneal ulceration and before completion of epithelial defects (group 1), wound healing may be delayed, and this caused the corneal haze not to decrease one month after operation, but, dexamethasone decreased the corneal haze after two and three months in this group. Lack of delay in wound healing and the significant decreased corneal haze at two and three months after ulceration in group 2 support these conclusions. In group 3, although NAC did not cause any toxic effects on corneas, it did not decrease corneal haze significantly and did not accelerate wound healing. On the other hand, in group 4 dexamethasone decreased the corneal haze significantly at the first month after surgery compared to the control group, but reduction of corneal haze was not significant two and three months after surgery in this group. This may explain why dexamethasone initially caused decreased keratocytes activity, and therefore, decreased collagen synthesis (Polack and Rosen, 1967; Gassett *et al*., 1969; McDonald *et al*., 1970); However after discontinuation of dexamethasone, the collagen synthesis probably increased. These results are in agreement with the results noted in rabbit eyes in which the short term use of

dexamethasone decreased the corneal haze (Tuft *et al*., 1989). Although the corneal haze in all treatment groups decreased at three months after surgery, however, it was not statistically significant, when compared with the control group.

Finally, the results of this study showed that combination of dexamethasone 0.1% and NAC 3% immediately after surgery may delay corneal wound healing. Also, the use of these drugs individually, has no obvious clinical effect on corneal haze. On the other hand, use of these drugs in combination with each other may reduce the corneal haze in later months after discontinuation of drugs. However, further studies using larger groups may provide more evidence of the effectiveness of these pharmacological modulators following experimentally induced corneal wounds in dogs.

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