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Effect of Acetylcysteine on Experimental Corneal Wounds in Dogs

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Key Words
Acetylcysteine · Corneal ulcers · Wound healing

Abstract
The effects of 3, 10 and 20% concentrations of acetylcysteine on experimental corneal wound healing in dogs were evaluated. Experimental corneal wounds were induced surgically, up to the depth of the anterior third of the stroma, in both eyes of 18 dogs. One of the eyes was treated topically with 0.9% NaCl solution three times a day. The contralateral eye was treated topically with acetylcysteine (3, 10 and 20% concentrations) in each of 6 cases separately. Corneal wounds were measured by fluorescein staining every day. The mean time of healing in the 3% group was significantly different from control eyes (6.17 ± 1.94 days). It was 7.19 ± 0.75 days in the 20% group and 7 ± 2 days in the 10% group. The last two groups were not significantly different from the controls (9.67 ± 3.01 days and 8.17 ± 3.60 days, respectively).

Introduction

N-acetylcysteine (NAC), an acetylated variant of the amino acid *L*-cysteine, is an excellent source of the sulfhydryl groups. Its chemical formula is $C_5H_9NO_3S$ and it has a molecular weight of 163.2 [1].

At first, Webb [2] used NAC for liquefaction of tenacious bronchial secretion. Its efficiency was confirmed in a study that found NAC to be the least irritant and the most stable one among several sulfhydryl agents [3]. Because of its mucolytic and anticollagenolytic properties, NAC has been used in ophthalmology to treat corneal diseases, such as keratoconjunctivitis sicca, filamentary keratitis, corneal mucous plaques and alkali-burned corneas [4–13]. Corneal toxicity of NAC was observed after intracorneal injection [14]. Evaluation of those effects on the cornea and the mucus was performed using light microscopy [15, 16] and histochemistry [17]. Although different treatments with NAC were described, the effective dose without undue toxicity has not been established.

The purposes of this present study were: (1) determination of effects of acetylcysteine on experimentally induced corneal ulcers; (2) compare the 3 different concentrations of the drug on the injured cornea, and (3) to examine microscopically the healing process of the cornea and the correlation between different stages of ulcers pathologically and clinically.

Materials and Methods

Dogs

Eighteen dogs of mixed breed of both sexes (8 months to 5 years old) with normal eye examinations were selected. They were housed under lighting controlled at a dim level for comfort.

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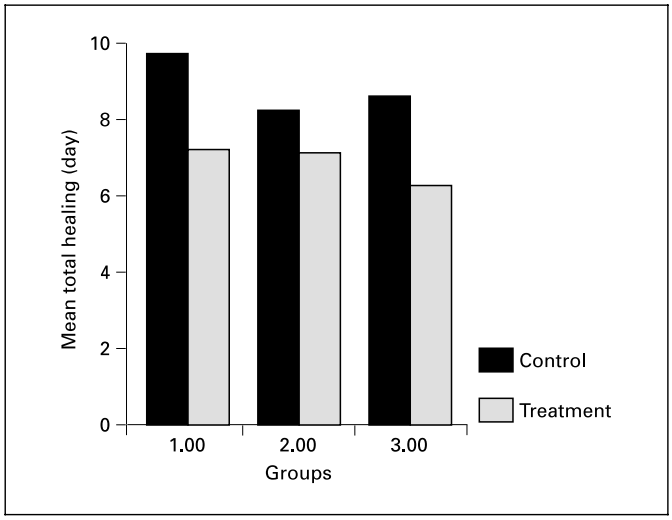

Fig. 1. Mean total healing in different groups.

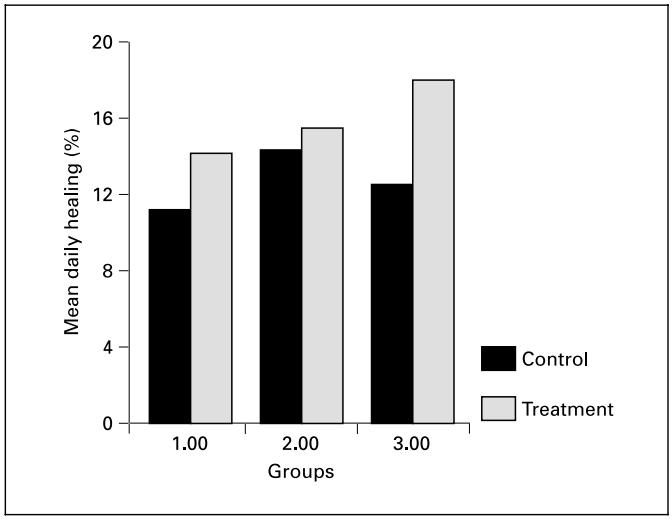

Fig. 2. Mean daily healing.

Table 1. Mean healing time and mean daily healing in different groups

	20% acetylcysteine		10% acetylcysteine		20% acetylcysteine	
	control	treatment	control	treatment	control	treatment
Mean healing time, day	8.5 ± 1.97	6.17 ± 1.94*	8.17 ± 3.6	7 ± 2	9.67 ± 3.01	7.17 ± 0.75
Mean daily healing, %	12.36 ± 3.98	17.73 ± 5.90	14.52 ± 6.68	15.31 ± 5.37	11.10 ± 2.95	14.09 ± 1.54

* $p < 0.05$. Results are means ± SD.

Surgical Operation

The dogs were anesthetized with a combination of 10 mg ketamine HCl/kg +1 mg xylazine HCl/kg +0.11 mg acepromazine/kg. The dogs were placed in lateral recumbency. A pediatric eyelid speculum (lid retractor) was placed for exposure of the cornea. Then a muscle hook was placed under the ventral rectus muscle to control ocular movements during the trephination. A 6-mm calibrated corneal trephine was placed in the center of the cornea. The trephine depth was determined previously to be at a specific setting that would expose the anterior third of the corneal stroma. Trephination was performed. A crescent bevel-up blade was used to perform the keratectomy of the trephinated cornea. The corneal tissue buttons that had been removed were then placed in formalin for histopathologic examination. This procedure was done on both eyes of all dogs.

Experimental Groups

The 18 dogs were divided into three groups of 6 dogs. The left eyes of all dogs were treated topically with 0.9% NaCl drops and used as controls. The right eyes of the 6 dogs in group 1 were treated with 20% acetylcysteine, the 6 dogs in group 2 with 10% acetylcysteine and the 6 dogs in group 3 with 3% acetylcysteine drops. It was given three times a day, at 6 a.m., 1 p.m. and 9 p.m. for 2 months.

Corneal healing was measured by fluorescein staining every day at 4 p.m. Photographs were taken every week, when the wounds changed. Healing was determined to be complete when no fluorescein stain uptake was visible with a cobalt light source. But drops were continued for 2 months. At the end of the experiment, 2 months after the initial surgical operation, 3 eyes from each group were enucleated, stained with hematoxylin and eosin and prepared for histopathologic examination.

Statistical Methods

The data for reepithelialization 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 control and treatment groups, the data were analyzed by Student's t test.

Results

Data of mean healing time and mean daily healing are shown in table 1, and figures 1 and 2. p values obtained by the Student's t test reveal the difference of the mean heal-

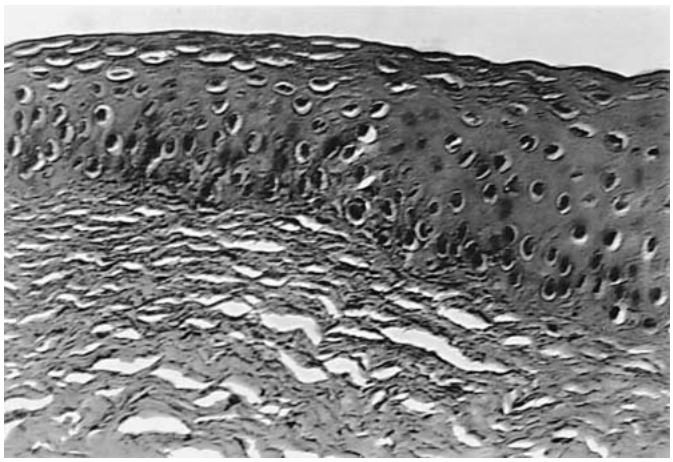

Fig. 3. Hyperplasia and hypertrophy in the corneal epithelial wound repairing area.

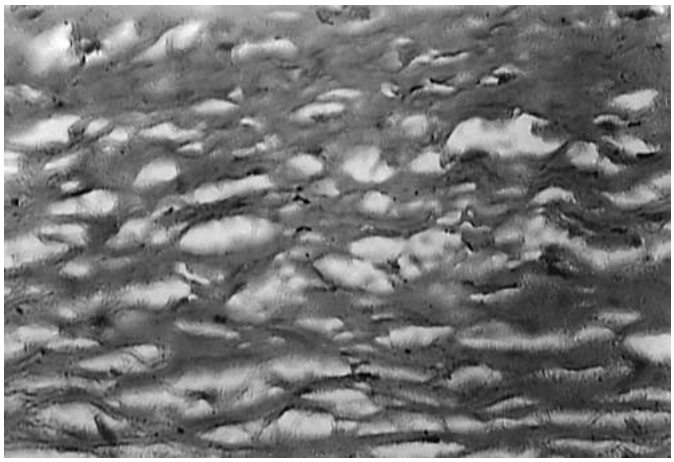

Fig. 4. Edema within the collagen fibers in the wound healing area.

ing time between the controls and acetylcysteine-treated eyes (20 and 10% concentrations). Although there was no statistically significant difference at the $p < 0.05$ level, the 3% concentration of acetylcysteine had significantly decreased the mean healing time compared with the control group ($p < 0.05$). On the other hand, mean daily healing in all groups was no significantly different between treatment and control groups.

Histopathological examination of the formalin-fixed corneal buttons revealed them to be of slightly variable thickness, but all of them were taken in the anterior third of the corneal stroma. The trephinated areas of the healed corneal epithelium in only some eyes demonstrated hypertrophy and hyperplasia and no difference was detected between control and study groups (fig. 3). In most eyes, the epithelial basement membrane of the healed corneas was not distinct from the adjacent normal basement membrane. The subepithelial stroma seemed variably edematous (fig. 4). The degree of stromal edema was independent of the treatment groups. Neovascularization was not seen in all healing corneas. The Descemet membrane and endothelium seemed normal in all eyes.

Discussion

In general, corneal ulcers healed without any complications. Corneal transparency is dependent upon epithelial and stromal regularity. Stromal fibers must be of uniform diameter, and the distance between the fibers must be less than half the wavelength of visible light to prevent the scattering of light (?). Clinically the healed corneas in the present study seemed variably opaque in the trephinated areas (in ophthalmoscopy and retinoscopy). The opacity around the wound was severer than that in the center. In manual trephining, a pressure of more than 40 lb on the globe is necessary, which deforms the tissue and leads to irregular margins [18]. The combination of the corneal epithelial hyperplasia and stromal edema and more importantly the disarrangement of collagen fibers account for this appearance at this stage. This opacity may have resolved with time, as the histopathologic examination revealed no evidence of fibroplasia or neovascularization.

The corneal collagenases in the wounded cornea arise from several cell sources: the injured epithelium, the activated keratocytes, and invading inflammatory cells such as macrophages and polymorphonucleocytes. Whereas collagenolytic and gelatinolytic activity is essential for tissue remodeling and wound healing, downregulation of the response is essential to maintain tissue stability after initial wound healing [19].

It has been shown that cysteine and acetylcysteine are effective in the inhibition of ulceration of the cornea due to alkali burns in rabbits. Both substances probably act by mechanisms of binding to the Zn^{2+} site of an enzyme [20–22].

Our results show that 3% acetylcysteine is effective in decreasing the healing time of corneal wounds in dogs, and although it was not significant, the 20 and 10% concentrations of this drug decreased the healing time of corneal wounds. On the other hand, no drug toxicity was

found in the light microscopic study. The findings are the same as the results of Kanao et al. [23]. They report that acetylcysteine at the 3% concentration improves corneal diseases in 71 dogs, and that there were no adverse effects on these dogs or on 82 healthy controls.

Absolon and Brown [4] demonstrated that topical applications of 20% acetylcysteine produce better results on objective signs than artificial tears.

Brown et al. [8] reported that cysteine is very effective in the treatment of human corneal wounds.

Berman [11] reported that both 20% mucomyst (1.2 *M* acetylcysteine, containing 1 m*M* EDTA) and 20% acetylcysteine (without EDTA) are not toxic, and of 13 patients with long-standing epithelial defects that did not respond to other kinds of therapy, 10 cases healed the defects within 10 days.

Williamson et al. [9] reported that 20 of the 98 patients (suffering from Sjögren's syndrome and keratoconjunctivitis sicca) who had not responded to tear substitute therapy or nasolacrimal canalicular obliteration were treated with a 5% acetylcysteine solution, pH 8.4 for 1 year. 30% of the cases in this group improved in both symptoms and signs.

Frauenfelder et al. [12] demonstrated that 10% acetylcysteine has good effects on corneal mucous plaques and Marsh and Cooper [13] reported the same results.

Burns et al. [24] applied the concentration of acetylcysteine that could inhibit 50% of collagenase purified from culture medium of alkali-burned rabbit corneas showing that the anticollagenase effect of this drug is by far weaker than synthetic peptides (carboxyl peptide and thiol peptide). The results of the present study are somewhat similar to those by Burns et al. [24].

Sugar and Waltman [16] injected intrastromally 0.1 ml of the 20, 10 and 3% concentrations of acetylcysteine to rabbit corneas and showed that within 1 h after intrastromal injection of 20% acetylcysteine, the cornea became edematous with a marked localized protuberance resembling keratoconus with hydrops. The clouding progressed slightly over the following 10 days, while the protuberance decreased somewhat. Usually the anterior stroma sloughed. The toxicity of the 3% concentration of acetylcysteine was essentially negligible with slight haze lasting a few days in only 2 eyes (from 10 eyes). Ten percent acetylcysteine had an intermediate effect with moderate haze and distortion. These investigators, in their second experiment, denuded the epithelium of 10 rabbit corneas with a scalpel under topical proparacaine anesthesia. Scraping was repeated every 2–3 days to maintain a central 4- to 5-mm defect. One drop of 20% acetylcysteine was applied to each of the 10 eyes four times daily for 10 days.

Twenty percent acetylcysteine applied topically four times daily to corneas denuded of epithelium caused a fine superficial stromal haze in 4 of the 10 eyes at 6 and 10 days. By the tenth day of treatment, minimal opacity could be detected in only 2 eyes [16].

Petroutsos et al. [15] showed that 20 and 10% concentrations of acetylcysteine had no toxicity on epithelial wound in rabbits. They reported that the healing of normal superficial epithelial ulcers is not affected by acetylcysteine. The nontoxicity and some improving effects of acetylcysteine in the present study are slightly similar to those of Absolon and Brown [4], Brown and Weller [7], Berman [11], Williamson et al. [9], Frauenfelder et al. [12], Marsh and Cooper [13], Burns et al. [24], Sugar and Waltman [16] and Petroutsos et al. [15].

On the other hand, Obenberger and Cejkova [14], Fitton et al. [19], Therms et al. [25] and Chen et al. [26] showed that acetylcysteine does not have beneficial effects on corneal wound healing and that it additionally causes cell toxicity and cell lysis, necrosis and desquamation of epithelial cells and corneal inflammation, while none of these signs were seen in the present study.

In conclusion, the 3% concentration of acetylcysteine improves the corneal wound healing in dogs, while the 20 and 10% concentrations do not have this effect.

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