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Effect of mitomycin C on the tensile properties of the upper lacrimal canaliculi in a rabbit model

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ABSTRACT

The upper lacrimal canaliculus consists of a tubular structure, and the tensile properties which in lacrimal tissues might contribute to structural integrity and tear drainage. We evaluated the characteristics of the tensile properties of the upper lacrimal canaliculi and the clinical implications of using a mitomycin C (MMC)-treated rabbit model. Mitomycin C (0.04%) was applied to the punctum of rabbits for 5 min, and the upper lacrimal tissues including the punctum were excised and attached to a forced transducer to record the tensile properties in a resting state 1 month later. The recording showed continuing decrement of basal tension with time in the lacrimal tissues treated with MMC in contrast with normal controls which maintained initial tension throughout the experiment. The rabbits were then randomly divided into the following 3 groups: vertical punctal incision with the MMC application group; vertical punctal incision with a balanced salt solution application group; and a balanced salt solution application only group. Four weeks after surgery, the puncta of rabbits treated with an incision and MMC application were more dilated clinically and showed less elasticity as compared with the other groups. Histological staining revealed that MMC treatment combined with incision decreased the amount of collagen and elastin fibers in the canaliculi. These results suggest that lacrimal canaliculi of rabbits have rheological basal tension and elasticity, which can be decreased by the use of MMC treatment.

1. Introduction

Tear drainage is performed by a complex pumping action, including an interaction of muscles and periorbita around the medial canthal area, as was suggested by Jones (1961) and Doane (1981). Gravity, capillary and reservoir drainage, microcilliation of the nasolacrimal duct, tear vaporization on the ocular surface, and tear absorption by lacrimal sac mucosa have also been reported to support tear drainage (Ahl and Hill, 1982; Becker, 1992).

The lacrimal passage consists of a tubular structure with passive tensile strength, similar to vascular and digestive tubular organs. The passive tensile force existing in the lacrimal pathway may contribute to tear drainage, and might be used as a new treatment modality or effective method to increase the efficiency of established therapy for disease of the tear drainage system. Previous reports involving the tear drainage system have focused on the interaction between tissues surrounding the lacrimal pathway, but studies involving the mechanics and physiology of the lacrimal pathway have been limited.

Mitomycin C (MMC) is an anti-neoplastic agent isolated from *Streptomyces caespitosus*, which inhibits fibrosis and neovascularization by interruption of protein synthesis (Reddy and Randerath, 1987; Verweij and Pinedo, 1990). Mitomycin C is now widely used in the field of ophthalmology, including ocular surface, refractive surgery, and glaucoma, to increase the success rate by modulating the wound healing process (Abraham et al., 2006; Forseto Ados et al., 2010). In lacrimal surgery, previous studies showed that direct application of the MMC to the lacrimal sac and nasal mucosa during a dacryocystorhinostomy improved the patency of the ostium without significant complication (Apuhan et al., 2011; Deka et al., 2006; Kao et al., 1997; You and Fang, 2001). Nevertheless, wound healing after an MMC raises concerns about residual tissue damage causing a problem of predictability and stability of the healing process. Besides, the possible mechanical and physiological effect of MMC on the punctum and canaliculus has not been revealed yet. Though good surgical results of canalicular irrigation by MMC in the lacrimal system have been reported by several authors (Choontanom, 2010; Kim et al., 2007;

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Tabatabaie et al., 2007), the reports of punctual and canalicular stenosis after topical MMC treatments also caused uncertainty in the actual effect of MMC on the punctum and canaliculus (Billing et al., 2003; Khong and Muecke, 2006; Kopp and Seregard, 2004).

Based on the above findings, we first attempted to demonstrate the mechanical properties of tubular lacrimal canaliculi using a rabbit model, and then investigated the possible effects of MMC on the tensile properties of the canaliculi. We also simulated surgery by punctal incision combined with MMC application and evaluated the functional and morphological changes of the puncta and canaliculi to explore the clinical implications of MMC on lacrimal canaliculus.

2. Materials and methods

The animals in this study were treated according to the ARVO Statement for the Use of Animals in Ophthalmology and Vision Research. All protocols described herein were approved by the Ethics Committee for the Protection of Persons and Animals in Biochemical Research in the Institute of Medical Science of Chung-Ang University (Seoul, Korea).

2.1. Animals and surgical procedures

Twenty-four New Zealand white rabbits of either sex, weighing 2–3 kg, were used for this study. Before the surgical procedure, the rabbits were anesthetized with an intramuscular injection of a Tiletamine and Zolazepam mixture (Zolatil®, 12.5 mg/kg; Virbac Lab, Carros Cedex, France) and xylazine (Rompun®, 12.5 mg/kg, Bayer Korea, Ansan, Korea), and the eyes were prepared using 0.5% proparacaine hydrochloride (Alcaine®; Alcon, Fort Worth, TX, USA). For 6 rabbits, 0.04% MMC was applied to the punctum for 5 min using a cotton applicator and washed with excess PBS. A balanced salt solution (BSS) was applied to the contralateral eyes for 5 min as controls. After 4 weeks, at least 6-mm lengths of lacrimal canaliculi, including puncta were excised and sent to measure basal tension.

The remaining 18 rabbits were randomly divided into 3 groups, two which had punctual incision ± MMC, and negative controls which were treated with BSS only without incision. A 3-mm vertical incision was made using Westcott scissors on the punctum of the left eye, then 0.04% MMC or BSS was applied to the incision site for 5 min. After surgery, the rabbits received topical neomycin-polymyxin B sulfate-dexamethasone eye drops (Maxitrol®; Alcon) four times daily until the end of the study. After 4 weeks, the puncta of rabbits were examined and photographed under a slit lamp biomicroscopy. Then, at least a 6-mm length of lacrimal canaliculi were excised and sent to measure elasticity. Among them, two circular pieces from each group were randomly selected and used for histological analysis.

2.2. Preparation of strips and tension recordings

The excised lacrimal canaliculus was promptly placed in a Petri dish containing HEPES-buffered physiologic salt solution (PSS) (composition in mM: NaCl, 140; KCl, 4.7; $CaCl_2$, 2.5; NaH_2PO_4, 1.2; L-glucose, 11; and HEPES, 5; [pH 7.4]) with 100% O_2 saturation and trimmed into circular strips (3 × 3 mm), including the puncta and longitudinal strips (3 × 3 mm) below the circular strip. The strips were mounted in a 20-ml organ bath filled with bicarbonate-buffered PSS (composition in mM: NaCl, 114; $NaHCO_3$, 26; KCl, 4.7; $CaCl_2$, 2.5; NaH_2PO_4, 1.2; and L-glucose, 11). The solution in the bath was bubbled with mixed gas (95% O_2/5% CO_2) and maintained at 37 °C and pH 7.4, and exchanged at 30-min intervals. The strips were connected to a force transducer (52–9545; Harvard Instruments, London, UK) and the basal tension was recorded on a MacLab4e recording system (AD Instruments, NSW, Australia; Myung et al., 2006). The basal tension for each strip was adjusted to 0.02 N over a 60-min equilibration period, and the developed tension was recorded.

2.3. Assessment of elasticity

The circular and longitudinal strips from 3 different groups were placed in a 20-ml organ chamber containing bicarbonate-buffered PSS, and attached to a force transducer (52–9545; Harvard Instruments). The strips were stretched for 1 mm using a puller system, after which the force needed to stretch the lacrimal strip was measured. Then, the time needed for the strips to restore to 70% of the original length was recorded.

2.4. Histology

The lacrimal canaliculi were first fixed with 10% formalin for 24 h, then stained with hematoxylin-eosin. Lacrimal canaliculi were additionally stained with Masson trichrome and Victoria blue to assess the collagen contents and degree of fibrosis occurring inside the lacrimal pathway, as well as the degree of destruction of the elastic lamina on the lacrimal tissue wall.

Immunohistochemistry was performed to identify the expression of fibroblasts in the lacrimal tissue by localizing fibroblast marker S100A4. Formalin-fixed, paraffin-embedded, 5-μm-thick sections were dewaxed in xylene, rehydrated through graded alcohol, and placed in an endogenous peroxide-block for 15 min. Sections were placed in a citrate buffer (10% citrate buffer stock in distilled water, pH6.0). Nonreactive blocking staining was blocked by 1% horse serum in Tris-buffered saline (pH6.0) and applied for 3 min. The primary antibody (S100A4: Thermo Scientific, USA, 1:100) was left on the sections overnight, respectively. Antibody-binding was detected using a standard labeled streptavidin-biotin system (Life science division, Mukilteo, USA). Tonsil was used for external positive controls and for negative control and the primary antibodies were omitted.

Each of the sections were examined under a microscope (Carl Zeiss, Germany) and photographed. The number of fibroblasts per high power (40× objective) field (HFP) was counted from five different regions of each sample.

2.5. Statistical analysis

Data are expressed as the mean ± SD. Statistical analysis was performed using SPSS statistical software (version 18.0; SPSS, Inc., Chicago, IL, USA). Statistical significance was determined by a Kruscal–Wallis test, and multiple comparison post tests were made using the Mann–Whitney test. A $p < 0.05$ was considered statically significant.

3. Results

3.1. Basal tension of lacrimal canaliculi

For six rabbits, lacrimal canaliculi treated with MMC were compared with contralateral lacrimal canaliculi. During the recording period, basal tension of the lacrimal canaliculus was kept at a constant level for more than 3 h. In comparison, the lacrimal tissues treated with MMC showed a pattern of decrement of basal tension with time (Fig. 1).

3.2. Clinical findings after MMC treatment combined with surgical incision over the punctum

Before treatment, the normal puncta of rabbits showed <0.1-mm contracted orifices. All of the puncta healed well without