Refractive operations such as photorefractive keratectomy (PRK) and laser in situ keratomileusis (LASIK) are popular surgical procedures for the correction of refractive errors. Both operations cause a large-area wound in the corneal stroma. After the operation, compensative keratocyte proliferation always occurs in PRK and LASIK-treated eyes [1]. Overproliferation of corneal keratocytes after injury may cause corneal haze [2] and result in the regeneration of myofibroblasts in the corneal stroma [3,4]. However, poor proliferation of corneal keratocytes may also affect extracellular matrix reorganization, stromal remodeling, and wound healing in the cornea after surgery [5].

For prevention of inflammation and infection after surgery, many antibiotics and corticosteroids are always applied locally on the corneal wound area. Although many antibiotics and corticosteroids have been used to prevent ocular bacterial infection and inflammation [6,7], few studies have reported the effects of antibiotic and corticosteroid eyedrops on human corneal keratocyte proliferation. Thus, the
The purpose of this study was to assess the effects of commonly used antibiotic and corticosteroid eyedrops, such as gentamicin, sulfisomezole, fluorometholone, dexamethasone, and betamethasone, on cultured human corneal keratocytes.

**Materials and Methods**

**Materials**

Culture materials including trypsin, minimal essential medium (MEM), glutamine, gentamicin, and fetal bovine serum were obtained from GIBCO (Grand Island, NY, USA). [3H]-thymidine (specific activity 15.0 Ci/mmol) was purchased from New England Nuclear (Du Pont, Boston, MA, USA). Commercially available preparations of drugs, e.g., 0.3% gentamicin (Gentamycin, Shionogi & Co. Ltd., Osaka, Japan), 4% sulfisomezole (Sinomin, Shionogi & Co. Ltd.), 0.1% fluorometholone (FML, Allergan Inc., Irvine, CA, USA), 0.1% dexamethasone (Maxidex, Alcon Laboratories Inc., Fort Worth, TX, USA), and 0.1% betamethasone (Rinderon-A, Shionogi & Co. Ltd.) were prepared with a serum-free medium without gentamicin to make three dilutions (1/10, 1/100, and 1/1,000) of these drugs for 100 minutes of incubation in all experiments. All other chemicals were obtained from Merck (Darmstadt, Germany).

**Culture of human corneal keratocytes**

Human corneal keratocyte primary cultures were obtained and cultured using human donor corneas that were discarded after transplantation, as described in earlier publications [8,9]. The endothelial and epithelial layers were removed and the corneal stroma minced into about 1 mm cubes before being plated in a culture flask for culture of keratocytes. The culture medium for cells contained RPMI-1640, 10% fetal bovine serum, 3.8 mM L-glutamine and 50 μg/mL gentamicin. The culture medium was kept in a humidified chamber with 5% CO₂ at 37°C, and changed every 2–3 days. Cells usually appeared within 3–7 days. The cells were used in this experiment with passage three to five.

**[3H]-thymidine uptake assay**

Corneal keratocytes were plated into 12-well plates for at least 24 hours for attachment. Cultured cells were incubated for 100 minutes with various concentrations of drugs that were diluted with serum-free medium. Fresh culture medium was then added to the cells. [3H]-thymidine 1 μCi/mL was added into each well for 4 hours’ incubation, then cells were washed with phosphate-buffered solution and 2% sodium dodecyl sulfate for liquid scintillation count.

**Statistical analysis**

Values are presented as mean ± SEM from three experiments with triplicate determinations. All data were analyzed with ANOVA followed by comparison with Dunnette test. Values were considered to be significantly different from corresponding controls at p < 0.05.

**Results**

To estimate the effects of antibiotic eyedrops such as gentamicin and sulfisomezole on cellular proliferation, [3H]-thymidine uptake was performed after treating the cells with three dilutions of these drugs at 1/10, 1/100, and 1/1,000 for 100 minutes’ incubation. We found that [3H]-thymidine uptake in cultured human corneal keratocytes was not affected by gentamicin at 1/10, 1/100, and 1/1,000 dilutions (data not shown). However, [3H]-thymidine uptake in cells was significantly inhibited by 1/10 (0.4%) and 1/100 (0.04%) rather than by 1/1,000 of sulfisomezole to 82±3% and 90±3% when compared with the control group (which was defined as 100% response) (Figure 1).
In the presence of the corticosteroid fluorometholone at a concentration of 0.01% rather than 0.001% and 0.0001%, cellular $[^{3}H]$-thymidine uptake was decreased to $75\pm 3\%$ of control (Figure 2). Incubation with the corticosteroid dexamethasone 0.01% (1/10), 0.001% (1/100), and 0.0001% (1/1,000) for 100 minutes resulted in cellular $[^{3}H]$-thymidine uptake being significantly inhibited to $82\pm 4\%$, $86\pm 3\%$, and $90\pm 3\%$ compared with the control (Figure 3). On exposure of the cells to the corticosteroid betamethasone 0.01% (1/10), 0.001% (1/100), and 0.0001% (1/1,000) for 100 minutes, $[^{3}H]$-thymidine uptake was significantly inhibited only by 0.01% and 0.001% and not by 0.0001% to $84\pm 4\%$ and $86\pm 3\%$ of control (Figure 4).

**DISCUSSION**

Corneal keratocytes are fibroblasts and are found throughout the stroma between the stroma lamellae. They play a crucial role in producing ground substance and collagen fibrils during embryogenesis and after corneal injury [10]. The change in keratocytes after photorefractive surgery is a complex process. Apoptosis and subsequent proliferation of keratocytes are both observed in corneal stroma [11]. Keratocytes in the posterior and peripheral cornea are reported to begin mitosis about 12–24 hours after PRK and LASIK [12]. Many factors such as the kinetics of keratocytes undergoing apoptosis and proliferation or drugs may affect visual acuity and stromal haze [11,13]. There is evidence to show that betamethasone decreases the formation of subepithelial haze by inhibiting keratocyte proliferation and synthesis of extracellular matrix in the corneal stroma [14].

There is also evidence that topically applied corticosteroids can modulate stromal wound healing by changing collagen thickness and keratocyte density.
after excimer laser keratectomy [7]. Dexamethasone is reported to increase thymidine incorporation in cultured human retinal pigment epithelial cells [15], but induces apoptosis in cultured human corneal keratocytes [16] and epithelial cells [17]. We also found that dexamethasone eyedrops inhibited cellular proliferation at dilutions of 1/10, 1/100, and 1/1,000. Nevertheless, inhibition of keratocyte proliferation by betamethasone may decrease the formation of subepithelial haze after corneal injury [14].

Gentamicin is a popular antibiotic for ocular use to prevent bacterial infection. We found that gentamicin did not inhibit cultured human keratocyte proliferation at 0.03%, 0.003%, and 0.0003% after 100 minutes of incubation. It was reported that even after 24-hour incubation with gentamicin at concentrations ranging from 0.024% to 0.009%, cellular proliferation was not affected in cultured rabbit keratocytes [6]. In contrast, 0.01% and 0.001% sulfisoxazole was found to inhibit cellular proliferation of corneal keratocytes in this study. Up to now, the effect of sulfisoxazole on corneal keratocytes has been poorly reported even though the topical use of this drug is very popular for the prevention of ocular infection [18].

In summary, cellular proliferation of corneal keratocytes was significantly inhibited by many commercial antibiotics and corticosteroids, especially at high drug concentrations. Thus, the application of these commercial eyedrops in patients with corneal damage from refractive surgery needs to be monitored carefully during corneal stroma change.

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REFERENCES

抗生素及類固醇眼藥水對人類角膜縫維母細胞增生之影響

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本實驗之主要目的是研究不同的抗生素包括 gentamicin，sulfisomezole 及類固醇 fluorometholone，dexamethasone 及 betamethasone 眼藥水對人類角膜縫維母細胞增生的影響。人類角膜細胞細胞經 RPMI－1640 及 10% 胎牛血清培養後加入經 1/10，1/100 及 1/1,000 稀釋的上述眼藥水，經過 100 分鐘培養後測試細胞對 [3H]-thymidine 的攝取量。結果發現三種稀釋濃度的 gentamicin 都不影響細胞的增生，但是細胞對 [3H]-thymidine 的攝取量會被 1/10，1/100 稀釋的 sulfisomezole 抑制到只有控制組的 82% 及 90%，也被 1/10 稀釋的 fluorometholone 及 1/10，1/100 稀釋的 betamethasone 抑制到只有控制組的 75%、84% 及 86%，而且 1/10，1/100 及 1/1,000 稀釋的 dexamethasone 皆會明顯抑制細胞增生速率到只有控制組的 82%，86% 及 90%。因此本實驗顯示類固醇眼藥水會抑制人類角膜縫維母細胞的增生而可能會改變角膜基質組織傷口愈合的過程。

關鍵詞：betamethasone，細胞增生，角膜縫維母細胞，dexamethasone，fluorometholone，sulfisomezole

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