to the alloimmunization of the recipient, and targeting the donor endothelium for cell-mediated immune destruction. The importance of la alloantigen induction to endothelial rejection of human corneal allografts merits further study.

Key words: Class II alloantigens, la antigens, corneal endothelium, immune interferon, rabbit

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5-Fluorouracil Toxicity to the Ocular Surface Epithelium

The antimetabolite, 5-fluorouracil (5-FU), has been used to control proliferation of retinal pigment epithelial cells and fibrocytes, and is currently the subject of a multicenter clinical trial of its value in the control of scarring after glaucoma operations. To evaluate possible ocular surface toxicity, the effect of 5-FU on the mitotic rate and differentiation of the ocular surface epithelium in rabbits was measured. 5-FU was instilled into eyes with 10-mm diameter central epithelial wounds and into nonwounded eyes at a dose of 9 mg per day for 4 days. Saline treated control wounds healed within 4 days (n = 5) while 40% (4 of 10) of the 5-FU treated wounded eyes had defects at 4 days. The normal mitotic rate of the corneal epithelium was 1.0 ± 0.3 (n = 4) tritiated thymidine labeled cells per 100 basal corneal epithelial cells after 2.5 hr incubation. Saline treated control wounds had an increased mitotic rate, 7.1 ± 1.3 (n = 5) labeled cells per 100 basal corneal epithelial cells after 2.5 hr incubation. Topical 5-FU decreased both of those rates to about 1% of normal. The normal conjunctival epithelial mitotic rate was 1.8 ± 0.4 (n = 4) labeled cells per 60 basal cells after per 2.5 hr incubation. This rate was the same in wounded eyes, but was decreased in eyes treated with 5-FU. Thus, 5-FU (9 mg/day topically) has serious toxic effects to ocular surface epithelium which must be carefully considered if this drug is to be used clinically. Invest Ophthalmol Vis Sci 26:580-583, 1985

The antimetabolite, 5-fluorouracil (5-FU), is a pyrimidine analog which has been shown to block mitosis of retinal pigment epithelial cells and of fibrocytes in vitro and in vivo. It has also been used experimentally to prevent fibrocyte proliferation and scarring of the filtering bleb in glaucoma surgery, 1-4 and is now the subject of a multicenter clinical trial to evaluate its effectiveness in controlling scarring of filtration blebs. In some studies, there appeared to be some toxicity of 5-FU to the ocular surface epithelium in the form of persistent epithelial defects.

The purpose of this article is to evaluate these potential toxic effects of 5-FU. To do so, topical 5-FU was applied to normal and wounded rabbit epithelium and the effect of the drug on the appearance and mitotic rate of the epithelium was studied.

Materials and Methods. Animals: All investigations involving animals that are described in this manuscript

References
Fig. 1. Clinical and morphological appearance of wounded corneas at day 4. A, Healed cornea of wounded, saline treated eye at 4 days. B, Corneal epithelium of wounded, saline treated eye at day 4. Note tritiated thymidine labeled cells (arrows) and multilayered epithelium (PAS, ×750). C, Epithelial defect on day 4 in wounded eye treated with 5-FU for 4 days. D, Corneal epithelium of wounded, 5-FU treated eye at 4 days. Note thin epithelium and lack of tritiated thymidine labeled cells (PAS; original magnification, ×750).

conform to the ARVO Resolution on the Use of Animals in Research.

Albino rabbits, weighing 2–3 kg, were anesthetized with intramuscular injection of chlorpromazine hydrochloride (25 mg) and ketamine hydrochloride (200 mg) supplemented with topical proparacaine. Using a #15 Bard Parker blade, a 10-mm diameter corneal epithelial defect was made by scraping the epithelium from an area circumscribed by a shallow 10 mm-diameter trephine mark. After wounding, the epithelial defect was stained with Richardson's stain to confirm complete epithelial removal. Control animals were not wounded. The eyes were divided into four groups, based on the presence or absence of wounds and the topical medication used (see below for description of medications). The four groups were (1) normals: no wound, topical saline, n = 4 eyes; (2) no wound, topical 5-FU, n = 5 eyes; (3) wound, topical 5-FU, n = 10 eyes; and (4) wound, topical saline, n = 5 eyes.

5-Fluorouracil (5-FU), saline preparation, and administration: 5-fluorouracil (5-FU) (Roche Laboratories; Nutley, NJ) at a concentration of 50 mg/ml was diluted with phosphate buffered saline to a concentration of 10 mg/ml with a pH of 8.5. 150 μl was administered topically six times daily at 2-hr intervals to those rabbit eyes receiving 5-FU treatment. This, theoretically, yielded a dose of 9 mg/day, although sometimes a small amount of fluid (less than 10%)
Corneal Epithelial Mitoses

![Figure 2. Corneal epithelial mitoses per 100 basal corneal epithelial cells per 2.5 hr in normal (saline treated, nonwounded), 5-FU treated, wounded-saline treated, and wounded-5-FU treated eyes at 4 days. Values are presented as averages bracketed by the standard errors of the mean with the number of eyes counted in parentheses.](image)

Mitoses per 100 basal cells

- Normal: 1.0±0.25 (4)
- 5 FU: 0.016±0.016 (5)
- Wounded: 7.1±1.3 (5)

Wounded + 5 FU: 0.13±0.03 (10)

Conjunctival Epithelial Mitoses

![Figure 3. Conjunctival epithelial mitoses per 60 basal epithelial cells per 2.5 hr in normal (saline treated, nonwounded), 5-FU treated, wounded-saline treated, and wounded-5-FU treated eyes at 4 days. Values are presented as averages bracketed by the standard errors of the mean with the number of eyes counted in parentheses.](image)

Mitoses/60 basal cells

- Normal: 1.8±0.4 (4)
- 5 FU: 0.03±0.03 (10)
- Wounded: 2.0±0.6 (5)
- Wounded + 5 FU: 0.03±0.03 (10)

o'clock position of the eye for orientation, and the eye was immediately enucleated. All animals were sacrificed between the hours of 8 and 10 am.

Corneal-scleral-conjunctival preparations were incubated in 3 ml SHEM epithelial tissue culture medium containing 20 uCi tritiated thymidine (20 Ci/mmol, New England Nuclear; Boston, MA) for 2.5 hr in an air-CO2 (95%:5%) water-jacketed incubator at 37°C, followed by half an hour incubation in isotope-free medium. Samples were then fixed in 10% buffered formalin and routine 7-micron histological sections were prepared. All sections were cut in the 12 o'clock:6 o'clock meridian and stained with the periodic acid Schiff (PAS) reaction or hematoxylin and eosin (H&E).

Other sections used for analysis of tritiated thymidine uptake were dipped in Kodak NTB 2 emulsion (Kodak; Rochester, NY), stored at −20°C for 2 weeks, and then developed in Kodak D19 developer and stained with PAS and hematoxylin.

Analysis of mitotic rate of ocular surface epithelium: The number of cells which incorporated tritiated thymidine (s-phase cells) in 2.5 hr was counted across the entire cornea on one histological section and was considered representative. The results are expressed as labeled cells per 100 basal corneal epithelial cells.

The conjunctival epithelial mitotic rate was determined by counting the number of thymidine labeled cells per 60 basal epithelial cells. This number (60 basal epithelial cells) was chosen because it was the average number of basal epithelial cells per field on the microscope at ×400 magnification.

Each sample was counted by two investigators, one of whom did not know the nature of the treatment for the sample. Values are expressed as averages bracketed by the standard errors of the mean with the number of eyes counted in parentheses. The P values were calculated using the Student t-test.

Results. Appearance: All control, wounded corneas healed within 3 days, with no regressions observed, while 40% of the 5-FU treated wounded corneas still had small (2–4 mm) epithelial defects at 4 days. Those eyes with epithelial defects were white and quiet with no clinical signs of inflammation. Nonwounded eyes, both saline and 5-FU treated, showed no epithelial abnormalities at any time (Fig. 1).

Histologically, the corneal epithelium was markedly thinner in wounded eyes treated with 5-FU than in wounded eyes treated with saline, even if the eyes were healed. Nonwounded corneas showed normal corneal epithelial morphology at 4 days. None of the eyes showed any evidence of inflammatory reaction (Fig. 1).

Corneal and conjunctival epithelial mitotic rate: The normal corneal epithelial mitotic rate of 1.0
appplied. This system may, therefore, provide an animal model for persistent epithelial defect in association with mitotic deficiency, produced in a noninflammed eye.

Forty percent of the wounded eyes treated with 5-FU for 4 days showed delayed wound healing and persistence of the epithelial defects while the 5-FU was continued. Preliminary work has shown that epithelial defects persist for as long as the 5-FU is applied. This system may, therefore, provide an animal model for persistent epithelial defect in association with mitotic deficiency, produced in a noninflammed eye.

Topical 5-FU decreased dramatically the mitotic rate of both corneal and conjunctival epithelial in both wounded and nonwounded eyes. This finding is consistent with the known mechanisms of action of 5-FU in inhibiting synthesis of DNA and its effect on retinal pigment epithelial and fibrocyte mitoses. Thus, 5-FU has serious toxic effects on the ocular surface epithelium which must be considered carefully when this drug is used in treatment of glaucoma.

Key words: mitotic rate, ocular surface epithelium, epithelial defect, 5-FU

Discussion. We present here the normal resting mitotic rates for the rabbit corneal and conjunctival epithelia. Our value of 1.0 labeled cells per 100 basal corneal epithelial cells is similar to that reported earlier for the rat corneal epithelial mitotic rate and for human conjunctival epithelium. Our values define the ocular surface epithelial mitotic rates in the rabbit and serve as internal controls for these studies.

Epithelial wound healing occurs by a cell sliding followed by a mitotic surge, but the exact time of the surge of mitotic activity remains undefined. This work demonstrates clearly that 4 days following a 10-mm diameter central corneal epithelial wound, the rabbit corneal epithelial mitotic rate is markedly elevated, providing a reliable means to achieve an increased rate of corneal epithelial mitosis. Furthermore, it shows that sliding of cells is, in some cases, sufficient to cover a 10-mm defect, since the defect was closed in six of 10 in eyes treated with 5-FU and having very low mitotic rates.

The normal conjunctival epithelial mitotic rate was 1.8 ± 0.4 (n = 4) labeled cells per 60 basal cells per 2.5 hr incubation. The same value was found in wounded, saline treated controls. The conjunctival epithelial mitotic rate was dramatically decreased in both the wounded and nonwounded eyes treated with 5-FU (P < 0.001) (Fig. 3).

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References