

OCULAR TOXICITY OF FLUOROURACIL AFTER VITRECTOMY

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The retinal and corneal toxicity of fluorouracil in the rabbit eye after lensectomy and vitrectomy depended on both the dosage and the frequency of intraocular injection and was reversible at certain dosages. All eyes in Group 1 (1.25 mg of fluorouracil every 12 hours for four days and then every 24 hours for three days) had opaque corneas by three days; these did not clear for four weeks. Histologic studies showed loss of photoreceptor outer segments and loss of ribosomes in all the retinal cells examined. The electroretinographic b-wave decreased to 0% of the baseline value (no b-wave), and did not recover after three weeks. In Group 2 eyes (1.25 mg of fluorouracil every 24 hours for seven days), corneal opacification increased to a maximum after two weeks and gradually decreased by four weeks. The electroretinographic b-wave diminished to 9.6% of the baseline value at two weeks but later recovered to 62.5% of the baseline value at three weeks. Histologic studies showed loss of photoreceptor outer segments and ribosomes at nine days; both returned to near normal after five weeks. Clinical, electrophysiologic, and histologic studies showed no toxicity in Group 3 eyes (0.5 mg of fluorouracil every 24 hours for seven days). This dosage of fluorouracil exerts a significant antiproliferative effect on injected retinal pigment epithelial cells and is well tolerated by the rabbit eye.

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Vitrectomy surgery combined with pharmacologic therapy to inhibit proliferating and contracting epiretinal membranes appears to be a promising approach in the management of proliferative vitreoretinopathy. We devised an experimental model of epiretinal membrane formation in the aphakic rabbit eye after vitrectomy and demonstrated the efficacy of intraocular fluorouracil in inhibiting growth of epiretinal membranes and subsequent traction retinal detachment.^{1,2} Additionally, we deter-

mined the ocular toxicity of various doses of intraocularly administered fluorouracil.

MATERIAL AND METHODS

Animals—We used male and female New Zealand albino rabbits, 2.5 to 3.5 kg each in weight.

Surgical technique—We sedated the animals with intramuscularly administered ketamine HCl (20 mg/kg of body weight) and xylazine HCl (2 mg/kg of body weight), and used 0.3 ml of intramuscularly administered atropine to reduce bronchial secretions. We inserted an intravenous line and maintained anesthesia with sodium pentobarbital (25 mg/ml), administered as needed. We dilated the pupils with 10% phenylephrine, 1% cyclopentolate, and 1% atropine.

To obtain an aphakic eye in which the vitreous had been removed, we performed a lensectomy and vitrectomy as previously described.¹ The eyes were allowed to heal for a minimum of two weeks before the intraocular injection of cells and fluorouracil.

Fluorouracil dosage and administration—We tested a variety of fluorouracil dosage and administration schedules in order to determine the efficacy of fluorouracil in decreasing the incidence of traction retinal detachment produced by the injection of 200,000 or 400,000 retinal pigment epithelial cells on the retinal surface.² Fluorouracil was administered in doses ranging from 5.0 mg every eight hours for two days to 0.5 mg every 24 hours for seven days. The most effective drug dosage schedules (determined clinically by the degree of traction retinal detachment) and the least toxic drug dosage schedules (determined clinically by the degree of corneal edema) were further studied by electrophysiologic and histologic tests. Of the 80 eyes that received intraocular fluorouracil, we have

selected 16 aphakic eyes that underwent vitrectomy and fluorouracil injection and that illustrate the important features of fluorouracil toxicity.

The animals were divided into three groups. In Group 1 (four eyes), the eyes were injected with 1.25 mg of fluorouracil every 12 hours for four days and then every 24 hours for three days. In Group 2 (seven eyes), the eyes were injected with 1.25 mg of fluorouracil every 24 hours for seven days. In Group 3 (five eyes), the eyes were injected with 0.5 mg of fluorouracil every 24 hours for seven days. Ten aphakic control eyes that had undergone vitrectomy received injections of 0.5 ml of balanced salt solution on schedules comparable to those of the fluorouracil-treated eyes.

Drug administration technique—After sedating the rabbit, we performed a fluid-gas exchange as described previously,¹ using a 50% mixture of sulfur hexafluoride and air. Two hours later, the appropriate concentration of the drug in 0.05 ml of balanced salt solution was injected through the clear cornea into the gas-filled eye (initial injections) or the fluid-filled eye (later injections) with a tuberculin syringe attached to a 30-gauge needle.

Electrophysiologic tests—Eyes used for toxicity studies underwent a pars plana lensectomy and vitrectomy and were then allowed to heal for a minimum of three weeks; this permitted the electroretinogram to stabilize. Two consistent baseline recordings were required before an eye was included in the toxicity study. The eyes underwent a fluid-gas exchange followed two hours later by the initial fluorouracil injection. Electroretinograms were performed eight days after the initial drug injection and then at seven-day intervals until the end of the experiment. We made periodic fundus observations to insure that there were no gross abnormalities.

Each animal was sedated as described and the pupils dilated with 10% phenylephrine, 1% cyclopentolate, and 1% atropine. We placed the rabbits inside a Ganzfeld stimulator with the eye 20 cm away from the flash unit of a photostimulator. A self-adhering contact lens electrode was applied to the cornea to receive the corneal potentials and the eye was light-adapted for five minutes. Three electroretinograms were obtained at increasing light intensities. We calculated the average of 16 flashes at each intensity with a digital signal averager and plotted the resulting average waveform on graph paper. The eye was then dark-adapted for 15 minutes and the test procedure repeated. Finally, the eye was again light-adapted and the test procedure repeated. Graphic recordings were measured and waveform amplitudes calculated from an in-line calibration pulse. We used maximal b-wave amplitudes for evaluation of the results.

Two control eyes that had already undergone lensectomy and vitrectomy had baseline electroretinograms followed by injection of 0.05 ml of balanced salt solution through the clear cornea for seven days. Electroretinograms were performed weekly for three weeks after the start of the intraocular balanced salt injections.

Clinical evaluation—We examined the eyes periodically with a penlight and slit lamp and binocular indirect ophthalmoscopy. Corneal opacification was graded from 0 (clear) to 4 (opaque).

Histologic studies—We enucleated eyes from day 9 through day 35 after intraocular fluorouracil injection. Several eyes were examined at each time. We removed the corneas and placed the eyes in a phosphate-buffered, 1% glutaraldehyde, 1% paraformaldehyde mixture (pH 7.1) overnight. The next morning, the specimens were rinsed in several changes of phosphate buffer for one hour.

The tissue was postfixed in 2% osmium tetroxide, washed in distilled water, dehydrated in a graded ethanol series, placed in propylene oxide, and then embedded in Araldite 6005. We studied the tissue by light and electron microscopy.

RESULTS

Clinical toxicity—All eyes in Group 1 (1.25 mg of fluorouracil every 12 hours for four days, then every 24 hours for three days) developed opaque corneas by three days with an average corneal opacity score at two weeks of 4.0. Additionally, vascularization of the corneas was moderately severe. There was little or no decrease in corneal opacification four weeks after fluorouracil injection.

Eyes in Group 2 (1.25 mg of fluorouracil every 24 hours for seven days) remained clear for five days after fluorouracil injection. Two weeks after fluorouracil injection, the average corneal opacity score was 4.0, and it was impossible to visualize the fundus. Three weeks after fluorouracil injection, corneal opacity had decreased to an average score of 2.5, allowing a view of the fundus with indirect ophthalmoscopy in some cases. By four weeks corneal opacification had decreased to an average score of 1.5.

Animals in Group 3 (0.5 mg of fluorouracil every 24 hours for seven days) had an average corneal opacity score of 0.5 after one week; this did not differ from control eyes receiving balanced salt injection. Two weeks after injection, no corneal opacification was noted in either the treated or the control eyes.

Electrophysiologic tests—There was no significant change from baseline in the average maximal b-wave amplitudes between control eyes and eyes in Group 3 (Figs. 1 and 2). Eyes in Group 1 retained only 2% of their baseline b-wave amplitude eight days after intraocular fluorouracil injection; this later diminished to 0% of the baseline amplitude (no b-wave)

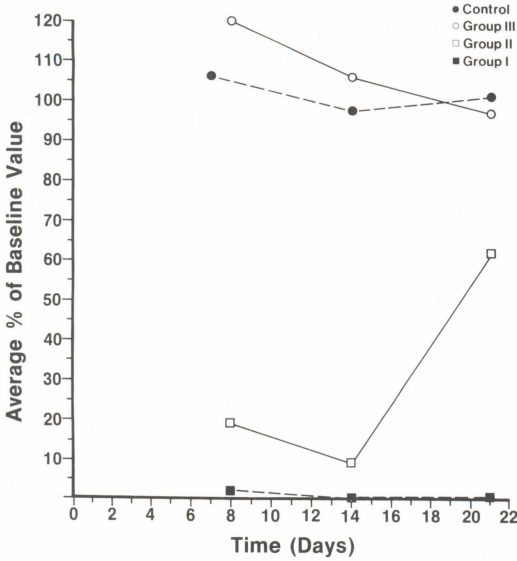


Fig. 1 (Stern and associates). Electroretinographic b-wave amplitudes as an average percentage of baseline value in control and fluorouracil-treated eyes. Group 3 eyes and control eyes show no significant changes in b-wave amplitudes after intraocular fluorouracil injection. Group 2 eyes show reversible loss of b-wave amplitudes whereas Group 1 eyes show no recovery three weeks after intraocular fluorouracil injection.

at two weeks and remained at that level when tested after three weeks. Eyes in Group 2 showed a decrease in the b-wave amplitude to 19.7% of the baseline value eight days after beginning intraocular injection of fluorouracil; this diminished to 9.6% of the baseline value at two weeks but later recovered to 62.5% of the baseline b-wave amplitude three weeks after beginning intraocular fluorouracil injection (Figs. 1 and 2). One eye in Group 2 was tested five weeks after fluorouracil injection and showed complete recovery of the b-wave amplitude (Fig. 3).

Histologic studies—Nine days after intraocular fluorouracil injection, eyes in Groups 1 and 2 showed loss of photoreceptor outer segments and ribosomes in all of the cells examined (Figs. 4 to 6). The basal border of the corneal endothelial cells was vacuolated. By three weeks

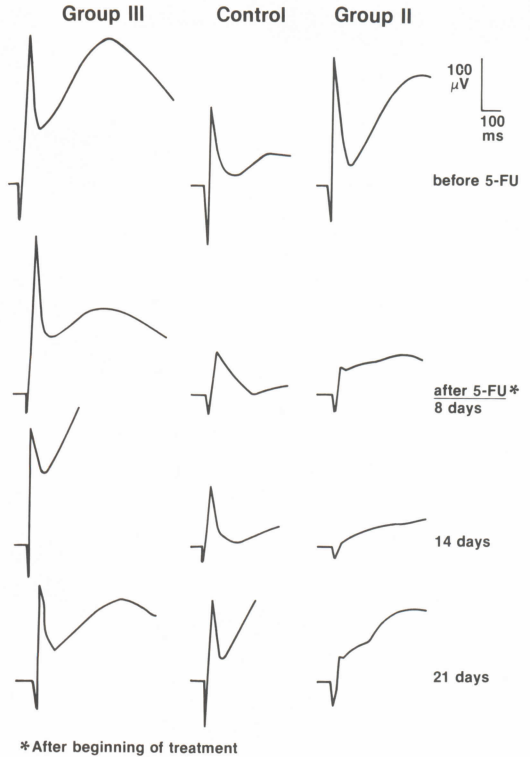


Fig. 2 (Stern and associates). Typical electroretinographic waveforms in control, Group 2, and Group 3 rabbit eyes. The Group 3 eye shows little change, whereas there is complete loss of the b-wave at 14 days followed by partial recovery at 21 days in the Group 2 eye.

in Group 2 eyes photoreceptor outer segments were present, although truncated, ribosomes were present, and the corneal endothelial cells had very little basal vacuolation. In Group 2 five weeks after the first fluorouracil injection, the photoreceptor outer segments were almost normal in length, ribosomes were present in normal amounts, and the corneal endothelial cells appeared to be normal (Figs. 7 to 9). After five weeks the Group 1 eyes had truncated photoreceptor outer segments, ribosomes were again present, and the corneal endothelium remained slightly vacuolated along the basal border.

Eyes in Group 3 did not differ from the

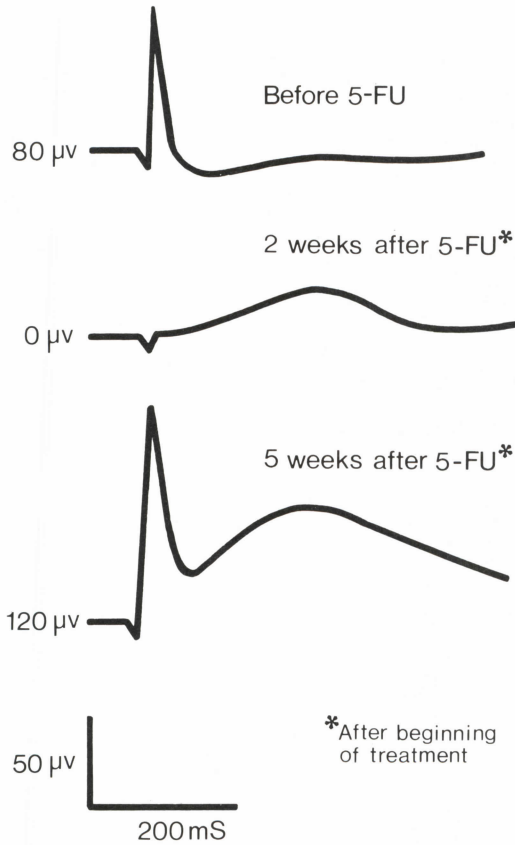


Fig. 3 (Stern and associates). Typical electroretinographic waveforms show complete recovery of the b-wave in a rabbit eye in Group 2 at five weeks.

controls at any time after intraocular fluorouracil injection.

DISCUSSION

In the experimental aphakic eye that has undergone vitrectomy and injection of 200,000 tissue-cultured retinal pigment epithelial cells, 0.5 mg of fluorouracil injected every 24 hours for seven days is the dosage necessary to produce a significant effect on cellular proliferation and traction retinal detachment² without significant toxicity. Previous studies have demonstrated that combined lensectomy and vitrectomy increases the circulation of low-viscosity fluid in the vitreous cavity and increases the access of intravitre-

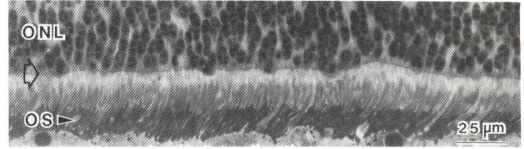


Fig. 4 (Stern and associates). Rabbit retina after lensectomy and vitrectomy. The photoreceptor outer segments (OS, arrowhead) the outer limiting membrane (arrow), and the outer nuclear layer (ONL) appear to be normal ($\times 530$).

ally administered drugs to the outflow channels in the anterior chamber.³ An important implication of the increased clearance of an intraocularly administered drug after vitrectomy and lensectomy is the necessity for repeated injection of the drug or sustained release of the injected drug.

Ocular toxicity of intraocular fluorouracil increased with increased dosages as well as frequency of drug administration and was reversible at certain dosages. Clinically, corneal opacification appeared after one week in Group 1 eyes and did not improve during a four-week period. The electroretinographic b-waves in Group 1 eyes remained flat and histologic studies of the retina indicated a loss of photoreceptor outer segments and ribosomes in all retinal cells. This did not change until five weeks after beginning intraocular fluorouracil injection. In Group 2, the frequency of administration of fluorouracil was less than that in Group

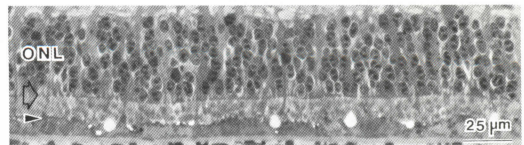


Fig. 5 (Stern and associates). Rabbit retina nine days after beginning intraocular fluorouracil administration (1.25 mg of fluorouracil every 24 hours for the first seven days). The photoreceptor outer segments are absent (arrowhead indicates where they are in a normal retina). The outer limiting membrane (arrow) and outer nuclear layer (ONL) appear to be normal ($\times 530$).

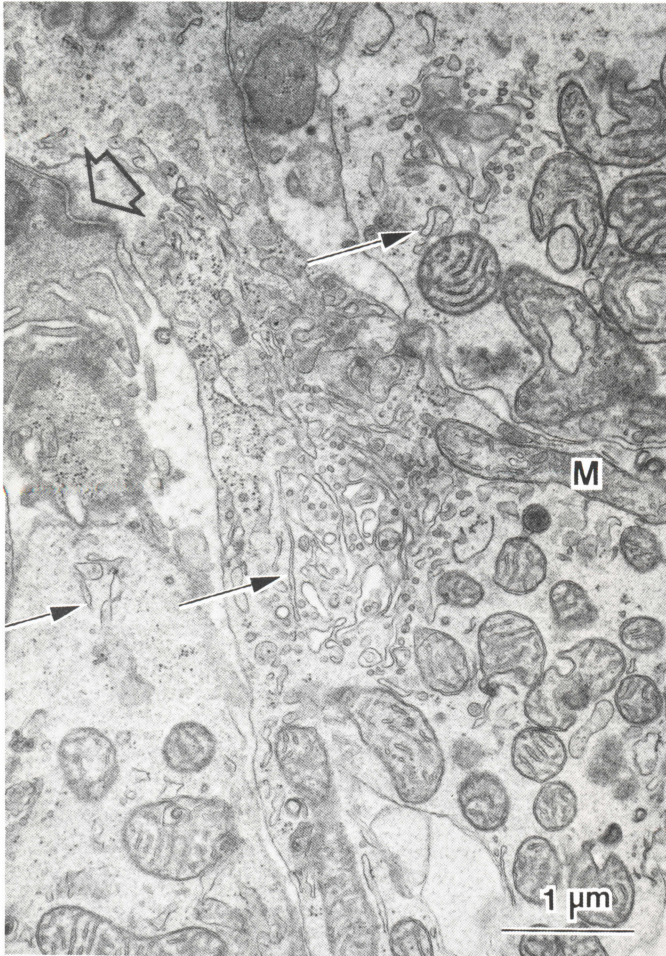


Fig. 6 (Stern and associates). Photoreceptor myoid region of a rabbit retina nine days after beginning intraocular fluorouracil (1.25 mg of fluorouracil every 24 hours for the first seven days). There are few free ribosomes or ribosomes associated with endoplasmic reticulum in this region (arrows). The outer limiting membrane (open arrow) and mitochondria (M) are normal ($\times 17,500$).

1 although the total dosage of fluorouracil administered remained the same. In Group 2, corneal opacification decreased by 50% and both electrophysiologic and retinal histologic studies showed that fluorouracil toxicity was reversible within three weeks at this dosage. By decreasing the dosage of fluorouracil to 0.5 mg daily for seven days, the toxic effects of fluorouracil on the corneal endothelium and the retina were eliminated.

Although there was no gross corneal toxicity in the Group 3 eyes by the 1 to 4 grading system, corneal pachymetry and endothelial cell counts might detect more subtle corneal toxicity. The regenerative

ability of the rabbit endothelium should also be recognized when interpreting these results. In particular, the improvement in corneal clarity in Group 2 by four

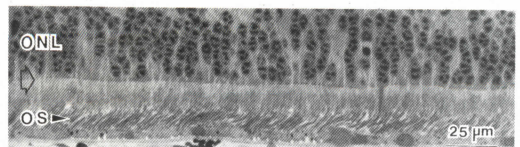


Fig. 7 (Stern and associates). Rabbit retina 28 days after intraocular fluorouracil administration (1.25 mg of fluorouracil every 24 hours for the first seven days). The photoreceptor outer segments (OS) are present. The outer limiting membrane (arrow) and outer nuclear layer (ONL) appear to be normal ($\times 530$).



Fig. 8 (Stern and associates). Photoreceptor myoid region of a rabbit retina 28 days after intraocular fluorouracil administration (1.25 mg of fluorouracil every 24 hours for the first seven days). Many free ribosomes and ribosomes associated with the endoplasmic reticulum (arrows) are present. The outer limiting membrane (open arrow) and mitochondria (M) are normal ($\times 17,500$).

weeks after injection may have been the result of reversible toxicity or rabbit endothelial cell regeneration.

Fluorouracil exerts a variety of actions on the cellular metabolic pathways, explaining both its inhibition of rapidly proliferating retinal pigment epithelial cells and its toxicity to the cornea and retina. Fluorouracil inhibits the synthesis of DNA by irreversibly binding with the enzyme thymidylate synthetase.⁴ Because thymidine is unavailable for DNA synthesis, cell death results. Interference with DNA synthesis is most obvious in dividing cells and fluorouracil has been shown to be more toxic to proliferating

cells than to nonproliferating cells.⁵ We have shown that tissue-cultured retinal pigment epithelial cells transplanted to the retinal surface proliferate rapidly for 24 to 48 hours; after this time the rate of proliferation regresses to a sustained low level.^{1,2}

Fluorouracil interferes not only with DNA synthesis but also with RNA synthesis and function by substituting for uracil and thus interfering with the formation of ribosomes that are composed primarily of RNA.^{6,7} We visualized this effect by electron microscopy of eyes in Groups 1 and 2 in which free ribosomes and ribosomes associated with the endoplasmic reticu-



Fig. 9 (Stern and associates). Corneal endothelium five weeks after beginning intraocular fluorouracil administration (1.25 mg of fluorouracil once a day for the first seven days). The Golgi complexes (arrows) appear to be normal, as do the numerous free ribosomes, polysomes, and ribosomes associated with the endoplasmic reticulum (arrowheads). No basal vacuolation is present along Descemet's membrane (DM). CE, nucleus of a corneal endothelial cell; AC, anterior chamber ($\times 32,500$).

lum disappeared in all retinal cells after one week of intraocular fluorouracil injection. The gradual reappearance of ribosomes observed in Groups 1 and 2 after cessation of fluorouracil therapy has also been noted in other biologic systems after discontinuation of fluorouracil therapy.⁷ The reappearance of ribosomes was correlated chronologically with the reappearance of the b-wave of the electroretinogram, although we have no evidence that there was a functional correlation between the two. Fluorouracil has also been noted to affect messenger RNA, transfer RNA, and soluble RNA and to interfere with protein synthesis.⁸ Interference with protein synthesis in the photoreceptors may account for the loss of the outer segments which are in a dynamic state of addition and loss.

We demonstrated that it is possible to inhibit in part epiretinal membrane formation and traction retinal detachment in the aphakic eye that has undergone vitrectomy without apparent toxicity to the retina or cornea. Because of the increased clearance of fluorouracil from the aphakic eye that has undergone vitrectomy, repeated injection of fluorouracil is necessary to achieve a therapeutic effect. Administration of the drug in this manner, however, results in peak levels of intraoc-

ular fluorouracil after each injection. The development of sustained-release vehicles for fluorouracil and other antiproliferative and anticontractile drugs may add to the therapeutic value of these drugs.

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