Antiviral drugs and corneal wound healing. 

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Using a previously described well standardized wound strength model, the fate of different antiviral drugs from corneal wound healing was evaluated. While 1.0 per cent trifluorothymidine and 0.1 per cent cytosine arabinoside were found to cause a significant delay of central corneal wounds, 0.1 per cent iodoaridine (IDU), three drops four times a day for twelve days, resulted in no significant delay in the healing strength of central corneal wounds.

Since their introduction into the field of ophthalmology, antiviral drugs have been used extensively for the treatment of herpes simplex infection of the cornea. Despite some early disagreement, it has been established that idoxuridine (5-ido-2'-deoxyuridine, IDU), in its currently used concentration and dosage, is a valuable drug in the treatment of herpes simplex of the cornea. The effect of IDU (Stoxil) on the healing of penetrating wounds was previously evaluated. Using an early model of our present tensiometer, a marked retardation in the healing of penetrating wounds was found when one drop of 0.1 per cent IDU was applied to the wounded eye every hour for fourteen hours, and the wound strength tested on the thirteenth postoperative day.

It is generally accepted that 1 per cent trifluorothymidine is fully effective in the treatment of herpetic keratitis. In contrast, 0.1 per cent IDU would have to be used at least every two hours around the clock for its antiviral effect to be comparable to that of trifluorothymidine. Since IDU has already been found to markedly retard the healing of penetrating wounds at this concentration and dosage, using essentially the same method for determining the tensile strength, repetition of those studies will add little to the purpose of this study. In contrast, the effect of less intense therapy, such as IDU four times a day, is not known. Of even greater importance is the fact that while IDU is indeed used up to every two hours around the clock for herpetic keratitis, it is seldom used at this concentration when longer duration of treatment is needed, such as covering for concomitant use of corticosteroid, particularly as after corneal transplantation in eyes with previous history of herpetic infections. It is in these eyes where its effect on the gain of tensile strength is of the utmost importance.

A previously described, a well-standardized wound strength model in rabbits was used to compare the effects of IDU, trifluorothymidine, and cytosine arabinoside on the tensile strength of penetrating stromal wounds.

Materials and methods. A total of 42 mature albino rabbits weighing 2 to 3 kilograms (5 to 7 pounds) each were used. The pupils were widely dilated with Neo-synephrine before surgery. Anesthesia was induced by intravenous injection of sodium pentobarbital (Nembutal).

Surgical techniques. Standard lid retractors were used to expose the eye. A 9 to 10 mm. nonpenetrating linear incision was made through the center of the cornea. Two 7-0 silk sutures were placed about 4 mm. apart and looped out of the way.

Table I. Tensile strength of central corneal wounds in rabbits treated with 0.1 per cent IDU twelve days after wounding

<table>
<thead>
<tr>
<th>Total No. of rabbits</th>
<th>Tensile strength (Gm./5.0 mm. wound)</th>
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<tbody>
<tr>
<td></td>
<td>IUD (0.1%)</td>
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<tr>
<td>17</td>
<td>x 178.9</td>
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<tr>
<td>Standard error of the mean</td>
<td></td>
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<tr>
<td>Probability value</td>
<td>± 18.4</td>
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<tr>
<td>P &lt; 0.1</td>
<td>No significant difference</td>
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The section was then completed with a cataract knife and the wound was immediately closed with the two preplaced sutures.

Postoperative care. Ophthalmic Neosporin solution and 4 per cent atropine were applied twice a day postoperatively to both eyes for several days. All animals with anterior synchia, infection, or any other postoperative complication were excluded from the study prior to the actual determination of the tensile strength.

In both eyes, drops were administered immediately after surgery and continued until the twelfth postoperative day when the rabbits were killed. One eye from each rabbit was treated with either trifluorothymidine, IDU, or cytosine arabinoside. The other eye served as an untreated operated control. Three different antimetabolites were investigated: (1) 0.1 per cent 5-iodo-2'-deoxyuridine (IDU) ophthalmic solution, three drops, four times a day between the hours of 8:00 A.M. and 5:00 P.M.; (2) 1 per cent trifluorothymidine solution, three drops four times a day; (3) 0.1 per cent cytosine arabinoside solution, three drops four times a day; and (4) all control eyes received 0.9 per cent saline, three drops, four times a day.

Measurement of tensile strength. The rabbits were killed on the twelfth postoperative day and the eyes were enucleated. A 5 mm. wide strip of cornea (including the two preplaced sutures) was cut out perpendicular to the wound using a special knife with two parallel razor blades and the tensile strength of the wound was measured, as detailed in a previous report. In all cases, tensile strength measurements were done without knowledge of which treatment the cornea had received.

Results. IDU 0.1 per cent. Table I summarizes the results obtained on 17 rabbits in which one eye was treated with 0.1 per cent IDU four times a day and the control eye was treated with 0.9 per cent saline solution. A comparison of the average tensile strength of central penetrating wounds shows no significant difference between the tensile strength of the 0.1 per cent IDU-treated eyes and the control eyes.

Trifluorothymidine 1 per cent. The effect of 1 per cent trifluorothymidine on the tensile strength of central penetrating 12-day-old wounds is shown in Table II. As can be seen, there is a significant delay in the tensile strength gain of the wounds treated with 1 per cent trifluorothymidine.

Paired statistics shows a significant difference between the tensile strength of the control and the trifluorothymidine-treated wounds with 99 per cent confidence (P < 0.005).

Cytosine arabinoside. The results obtained on rabbits in which one eye was treated with 0.1 per cent cytosine arabinoside four times a day (from 8:00 A.M. to 5:00 P.M.) and the control eye treated with 0.9 per cent normal saline are shown in Table III. A 50 per cent decrease in tensile strength was found to be produced by the application of 0.1 per cent cytosine arabinoside four times a day for 12 days. As expected, paired statistics show a significant difference between the cytosine arabinoside-treated wounds and the control with 99 per cent confidence (P < 0.005).

Discussion. A comparison of the different antiviral drugs revealed some similarities and some differences. IDU is certainly a valuable drug in the treatment of herpes simplex of the cornea. However, some other antimetabolites, such as trifluorothymidine or cytosine arabinoside, are needed for the treatment of IDU-resistant strains. The low solubility and retarded corneal penetration of IDU puts it at a disadvantage in rabbits and in effective treatment of stromal herpes. Trifluorothymidine as a nontoxic, highly soluble drug, with rapid penetration through the cornea, offers some ideal properties for an antiviral drug. Cytosine arabinoside, while it has been proved effective against some IDU-resistant strains of herpes virus, is no longer used for topical antiviral studies because of its toxicity to rabbit and human epithelial cells.
The fact that IDU at frequent application impairs corneal wound healing resulted in the general belief that it would delay wound healing even when used only a few times a day. This misconception has resulted in withholding IDU postoperatively in patients with previous history of herpetic keratitis, in which corticosteroids had to be used to reduce inflammation. Since both toxicity and therapeutic effect follow dose-response relationships and the effect of therapeutic and toxic ranges may differ, it may be possible to arrive at a toxic level, particularly when a drug like an antimetabolite is used. On the other hand, it is also possible to arrive at a regimen which provides the desired therapeutic effect and the least toxic side effect. For example, when treating herpetic keratitis, particularly in cases where only epithelial lesions are present, stromal wound healing is of little importance. In contrast, if epithelial healing is delayed, a significant contraindication for the use of this medication would result. However, IDU has never been found to significantly decrease epithelial wound healing. Its effect in retarding stromal wound healing in this clinical situation where no stromal wounds are present somehow seems less important. Of even greater importance is the fact that it is in cases where stromal wounds are present such as after cataract extraction or penetrating keratoplasty in eyes with previous herpetic infections or chronic use of corticosteroids where the application of IDU four times a day may prevent herpetic infections. On the basis of this study, it appears that the present strength of IDU drug, 0.1 per cent, is just strong enough to be used for prevention of herpetic keratitis in such eyes without impairing corneal wound healing.

The fact that trifluorothymidine has a more striking effect on wound healing is not surprising since it is so much more effective than IDU. It may be necessary to consider this effect in its clinical use, however. After all, it should be expected that any drug which has an effect on viral DNA synthesis might also affect the healing process.

From the Department of Ophthalmology, College of Medicine, University of Florida, Gainesville. Supported by a grant from the John A. Hartford Foundation and in part by United States Public Health Grants Nos. EY01112-02, EY01098-02, and EY52868 (Dr. A. R. Gasset) from the National Eye Institute. Submitted for publication Oct. 16, 1974. Reprint requests: Dr. A. R. Gasset, Department of Ophthalmology, College of Medicine, University of Florida, Gainesville, Fla. 32610.

REFERENCES

Kinetics of corneal epithelial regeneration.
II. Epidermal growth factor and topical corticosteroids. PATRICK C. HO* AND JAMES H. ELLIOTT.

The kinetics of rabbit corneal epithelium regeneration were studied to determine if topical corticosteroid has an adverse effect on corneal epithelial wound healing, and if epidermal growth factor (EGF) can abrogate any adverse effect of topical corticosteroid. Healing of standardized 7 mm. central corneal epithelial wounds was determined by serial standardized color photography of the fluorescein-stained defects and planimetry of the projected photographs. It has been found that topical application of 16 drops per day of vehicle or Decadron decreased the epithelial healing rate as compared to saline drops four times daily. Decadron 0.1 per cent given hourly (16 drops daily) was no more detrimental to corneal epithelial healing rate than the vehicle similarly applied. EGF exhibited no capacity to alter the corneal epithelial healing rate when hourly drops of either the vehicle or Decadron 0.1 per cent were given. Under the conditions of these experiments, no adverse effect on corneal epithelial healing rate could be attributed to Decadron 0.1 per cent.

Epidermal growth factor (EGF), first isolated by Cohen from mice submaxillary glands, has been shown to enhance the healing of experimental corneal epithelial wounds. Recently, in a kinetic study of corneal epithelial regeneration and EGF, Ho and colleagues have quantitated this accelerating effect of EGF on corneal epithelium regeneration. The present studies are undertaken, employing the same quantitative kinetic technique used previously to determine if topical corticosteroids have an adverse effect on corneal epithelial regeneration, and to determine if EGF can abrogate any adverse effect of topical corticosteroids on corneal epithelial regeneration.

Material and method.
Epidermal growth factor.* EGF was isolated from the submaxillary glands of adult male mice and purified by the new procedure of Savage and

*EGF was kindly supplied by Dr. S. Cohen at Vanderbilt University School of Medicine.