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Antiviral medications and corneal wound healing*

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Summary

Masked controlled rabbit studies were done to determine the toxic effects on corneal wound healing of the antiviral ointments 0.5% idoxuridine, 3% Ara A, and 3% acyclovir, and the antiviral drops 0.1% idoxuridine, 3% Ara AMP, and 1% trifluridine. Ara A, acyclovir, trifluridine and idoxuridine drops had no significant effect on the rate of closure of epithelial wounds. Idoxuridine ointment given 5 times a day significantly retarded the rate of epithelial wound closure, but not when given 4 times a day. Only Ara AMP caused a retardation of epithelial healing and an actual increase in the defect after 4 days of treatment. Histopathologically all drugs, except acyclovir, showed a toxic effect on the regenerating epithelium.

All drugs, except acyclovir, showed retarded stromal wound healing with reduced bursting strength and collagen content. Ara AMP had increased bursting strength and collagen content possibly because of greater inflammation. Acyclovir, in comparison to all the other medications studied, appeared to have minimal to no toxic effects on experimental epithelial and stromal wound healing, and on this basis is the agent of choice for use in herpes simplex stromal keratitis with ulceration and as a prophylactic agent for long-term use after penetrating keratoplasty.

corneal wound healing; antiviral medication

Introduction

There have been numerous clinical studies documenting the epithelial toxicity of

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currently marketed topical antiviral agents (idoxuridine, Ara A, and trifluridine) in the United States on both a short- and long-term basis [1–4]. In addition, acyclovir, a highly selective antiviral agent [5–10], has been studied in several short-term, controlled clinical trials for herpes simplex (HSV) keratitis. Surprisingly it was found to cause mild epithelial toxicity [5–10]. Considering the limitations of these human studies, investigators have noted difficulty in separating the short-term epithelial toxicity of an antiviral agent and HSV epithelial disease. Also, no conclusions as to the effect of these antiviral agents on stromal wound healing can be made from such clinical studies. This question is most important with regard to the prophylactic long-term use of an antiviral agent after penetrating keratoplasty.

Since 1974 our laboratory has studied the effect on corneal epithelial and stromal wound healing of all 3 topical antiviral agents marketed in the U.S.A., as well as acyclovir and Ara AMP [11–13]. From these studies direct conclusions as to the short-term epithelial toxicity and effect on stromal wound healing of these agents can be made without having the toxic drug effects obscured by active herpetic epithelial disease.

Materials and Methods

One-hundred-eight male New Zealand white rabbits weighing between 2 and 3 kg were used. Antiviral ointments used were 0.5% idoxuridine (IDU), 3% Ara A, and 3% acyclovir (ACV) in a petrolatum base. Placebo controls in the ointment studies received petrolatum base alone. Antiviral drops used were 0.1% idoxuridine, 3% Ara AMP, and 1% trifluridine (TFT). Placebo controls in the drop studies received physiologic saline drops. The concentrations of all medications were chosen because of their standard clinical use.

Epithelial wound healing

Five and 10 mm diameter epithelial defects were examined in the initial IDU and Ara A ointment study. Subsequent studies were based on 8.5 mm epithelial defects. After general anesthesia was achieved with intravenous pentobarbital and topical anesthesia with 0.5% proparacaine using sterile technique, a central epithelial defect varying in diameter as described above was made by marking the cornea with a trephine of the appropriate diameter under the operating microscope. The epithelium was then removed within the circumscribed area with a No. 15 Bard Parker blade. Completion of the epithelial removal was checked by staining with either 1% methylene blue or 2% fluorescein. The treatment groups were divided into 3 series of experiments:

Series I:
- A) IDU 0.5% ung 4×/day
- B) Ara A 3% ung 4×/day
- C) petrolatum control 4×/day

Gentamicin 0.3% was instilled once daily.

Series II:
- A) IDU 0.1% gtts 8×/day
- B) Ara AMP 3.0% gtts 8×/day

C) TFT 1.0% gtts 8×/day
D) physiologic saline control 8×/day
Chloramphenicol 0.5% was instilled twice daily.

Series III: A) IDU 0.5% ung 5×/day
B) ACV 3.0% ung 5×/day
C) petrolatum control 5×/day
Chloramphenicol 0.5% was instilled twice daily.

Treatment was continued for 7 days. Assignments of masked coded drugs were made so that no animal received the same drug to both eyes (balanced incomplete block design). Fluorescein or methylene blue-stained epithelial defects were photographed on a daily basis after they had been examined at the slit lamp for rate of epithelial closure and the quality of the regenerating epithelium. The photographs were then enlarged and the area of the defects measured with a planimeter establishing the rate of closure. The eyes were examined by 2 independent observers on a daily basis and masked scored for the quality of epithelium using the following scoring system:
0, no visible abnormality
0.5, intraepithelial edema visible with difficulty only by retroillumination
1.0, edema readily apparent by retroillumination, but not by direct illumination
2.0, edema apparent by direct illumination
3.0, epithelium grossly thickened and cloudy
4.0, epithelium thickened and cloudy with a rough and irregular surface.
In the 2nd and 3rd series of experiments, conjunctival injection, stromal edema, and iritis were also graded on a 0 to 4+ basis. All animals were killed with intravenous pentobarbital after 7 days of treatment.

Eyes in each treatment group with varying epithelial changes were selected for histologic examination. Neutral 10% formalin was dropped on the corneas immediately after killing the animals. After enucleation the whole eye was fixed in the same formalin solution. After paraffin processing 6 μm sections were cut and stained with hematoxylin and eosin for microscopic examination.

Stromal wound healing

After general anesthesia with intravenous pentobarbital and topical anesthesia with 0.5% proparacaine using sterile technique, a full thickness central corneal button (2 mm in Series I and II and 1.5 mm in Series III) was trephined under the operating microscope and excised with Vannas scissors. After a plasmoid aqueous plug had formed several minutes later, the antibiotic solution or ointment along with atropine 1% drops were instilled twice daily throughout the study. The treatment regimen for each series was the same as in the epithelial wound healing studies, but was begun 3 days later after it was confirmed in each eye at the slit lamp with fluorescein that a firm fibrin plug had formed and was epithelialized. During the treatment period observations by loupe or slit lamp were made on a daily or alternate-day basis to confirm that there were no leaks from the corneal wound, and that the anterior chamber was deep and no synechiae had formed.

At 3 weeks all the fibrin plugs were replaced by scar tissue clinically. The animals were killed at this time with intravenous pentobarbital. In Series I and II experiments,

wound strength was then ascertained by inserting a 25-gauge needle into the anterior chamber at the limbus and increasing the intraocular pressure by 5 lb/inch^2 at 5-s intervals until the wound ruptured. Subsequently in all series the corneal wound buttons were excised using the same trephine as for the initial wounds. The buttons were lyophilized, weighed, and then assayed for hydroxyproline as a measure of collagen content. Both the wound model and the hydroxyproline assay techniques have been previously described [14,15]. A modified Student's *t*-test for comparison of sample means was used for statistical analysis.

Results

Series I

Neither IDU or Ara A ointments were found to retard significantly the rate of epithelial closure of either large (10 mm) or small (5 mm) epithelial defects (Fig. 1). Despite the equal healing rate, the quality of the regenerating epithelium was significantly worse in the IDU-treated eyes in comparison to the Ara A or control eyes ($P < 0.01$) (Fig. 2). This correlated with the more severe histologic abnormalities seen in the IDU-treated eyes (Fig. 3). The control regenerating epithelium showed compact basal cells with more flattened wing cells. The majority of the Ara A-treated eyes were graded 1–2+ with mild to moderate intra- and occasionally intercellular edema. In contrast the majority of the IDU-treated eyes were graded 3–4+ with epithelial irregularity, edema, and slough.

The mean bursting strength of penetrating stromal wounds for the IDU, Ara A and control groups was 23.6 ± 5.7, 24.6 ± 3.8, and 41.2 ± 3.4 lb/inch^2, respectively. The IDU and Ara A groups had significantly lower bursting strength compared to the control group ($P < 0.02$ for both groups). The mean hydroxyproline per button, reflecting collagen content, for the IDU, ara A, and control groups was 3.81 ± 0.77, 4.60 ± 0.62, and 6.64 ± 0.52 μg, respectively. The collagen content was significantly

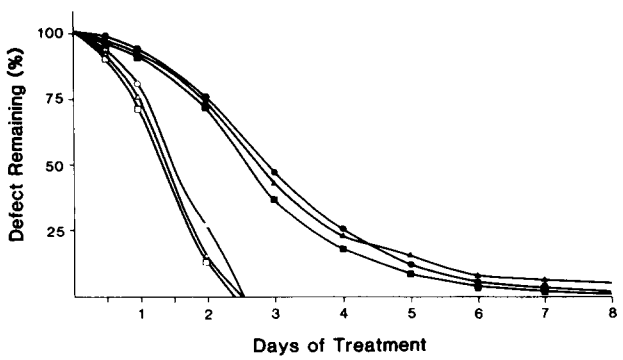

Fig. 1. Rate of healing of standardized 5 mm corneal epithelial defects in rabbits treated with IDU (△), Ara A (□), and control (○). Rate of healing of standardized 10 mm corneal epithelial defects treated with IDU (●), Ara A (▲), and control (■). Each point represents mean of treatment group population on designated days of therapy. (Courtesy Arch. Ophthalmol. (1974).)

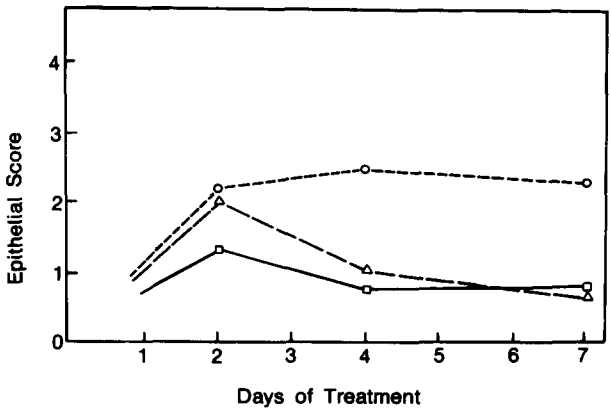

Fig. 2. Quality of regenerating corneal epithelium treated with IDU (○), Ara A (△), and control (□), as scored by slit-lamp examination. Each point represents mean score of treatment group population on designated days of therapy. (Courtesy Arch. Ophthalmol. (1974).)

lower in both drug-treated groups (IDU, $P < 0.01$; Ara A, $P < 0.05$). The lower bursting strength and reduced collagen content in both treated groups closely correlated with each other (Fig. 4).

Series II

Neither IDU nor TFT drops were found to retard significantly the rate of epithelial closure of 8.5 mm epithelial defects (Fig. 5). All epithelial wounds were completely healed in these 2 groups and the control group by day 4 of treatment. In contrast, all Ara AMP-treated eyes had epithelial defects which were actually enlarging by day 4 of treatment. The quality of the regenerating epithelium was significantly worse in the IDU- and TFT-treated groups in comparison to the control group ($P < 0.05$) (Fig. 6). There was no significant difference between the IDU and TFT groups ($P < 0.10$). The Ara AMP group had the most impressive toxic effects on the regenerating epithelium ($P < 0.05$). Unlike the other drug-treated groups, the scores were progressively worse with each day of therapy. The degree of conjunctival injection, stromal edema, and iritis correlated with the quality of regenerating epithelium for all groups.

Histopathologically, as in the first series, the slit-lamp appearance correlated with the severity of the pathologic changes (Fig. 7). Both the IDU and TFT groups showed varying degrees of intra- and interepithelial edema with irregularity from grade 2–3+, as seen in the first series. The Ara AMP group had considerable loss of epithelium, and, unlike all other treated groups in all 3 series, marked stromal vascularization and edema was noted.

The mean bursting strength of penetrating stromal wounds for the IDU, TFT, Ara AMP, and control groups was 29.3 ± 9.8, 26.6 ± 17.0, 45.2 ± 8.2, and 37.1 ± 9.2 p.s.i., respectively. The IDU group had a significantly lower bursting strength than the control group ($P < 0.05$). Although the TFT group had a lower bursting strength, the difference was not significant ($P < 0.10$). The bursting strength was significantly increased in the Ara AMP group ($P < 0.05$). The hydroxyproline per button for the

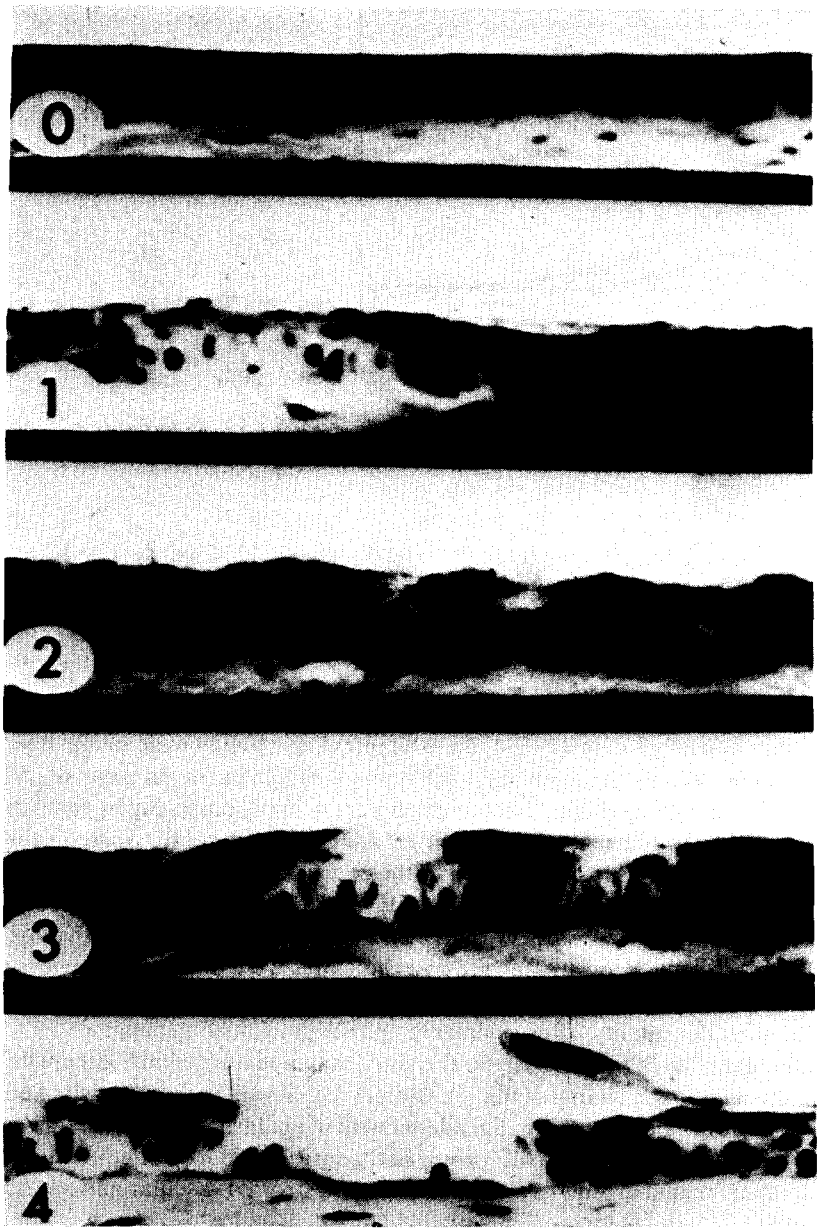

Fig. 3. Histologic changes associated with a slit-lamp score of 0–4+. Sections were taken from peripheral margin of the healing epithelial wound after 7 days of treatment. (Courtesy Arch. Ophthalmol. (1974).)

IDU, TFT, Ara AMP, and control groups was 7.9 ± 3.5, 8.7 ± 4.2, 13.9 ± 2.3, $11.1 \pm 3.8\,\mu g$, respectively. Correlating with the bursting strength data, there was a significant decrease in collagen content in the IDU group ($P < 0.05$), but no difference in the TFT group ($P < 0.10$).

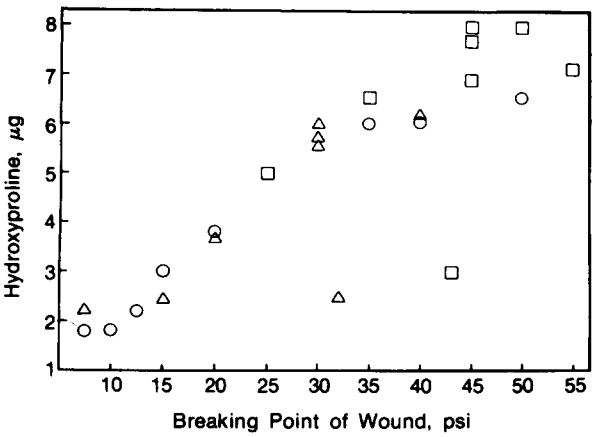

Fig. 4. Amount of hydroxyproline in each button treated with IDU (○), Ara A (△), and control (□), plotted against breaking point of that button. Note correlation between wound strength and collagen (hydroxyproline) content. (Courtesy Arch. Ophthalmol. (1974).)

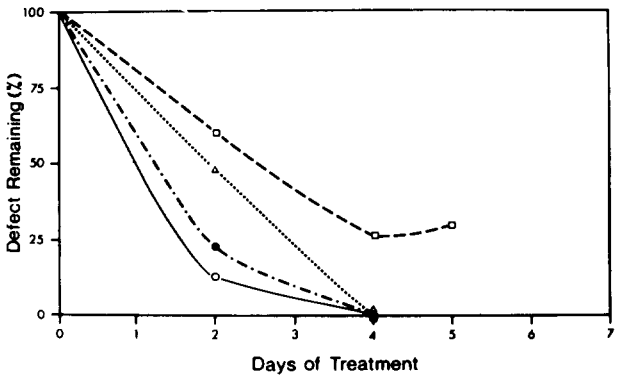

Fig. 5. Rate of healing of standardized 8.5 mm corneal epithelial defects in rabbits treated with IDU (●), TFT (△), Ara AMP (□), and control (○). Each point represents mean of treatment group population on designated days of treatment. (Courtesy Arch. Ophthalmol. (1977).)

Series III

Unlike in Series I, IDU ointment significantly retarded the rate of epithelial closure, in this case with an 8.5 mm epithelial defect ($P < 0.05$) (Fig. 8). There was no significant difference in the epithelial healing rate between the ACV and control groups. As in Series I, the quality of the regenerating epithelium was significantly worse in the IDU group ($P < 0.01$) (Fig. 9). Unlike all the other antiviral medications tested, ACV had no significant toxic effect on the regenerating epithelium clinically. This lack of epithelial toxicity for ACV was also observed histopathologically. No difference in the appearance of the regenerating epithelium could be seen between the ACV and the control group when read in a masked fashion (Fig. 10). In contrast, as previously noted, IDU-treated eyes showed varying degrees of toxicity ranging from

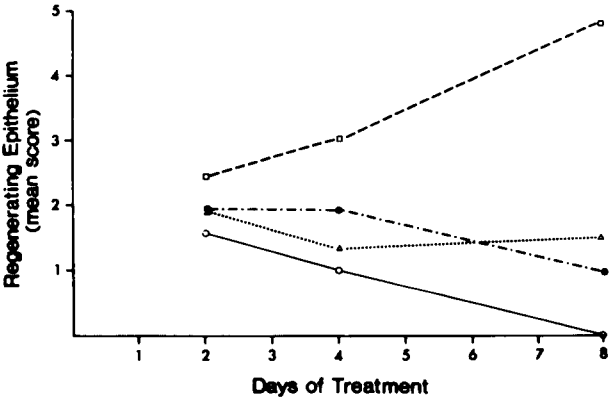

Fig. 6. Quality of regenerating epithelium treated with IDU (●), TFT (△), Ara AMP (□), and control (○), as scored by slit-lamp examination. Each point represents mean score of treatment group population on designated days of treatment. (Courtesy Arch. Ophthalmol. (1977).)

mild intercellular edema and epithelial thinning to frank epithelial slough and polymorphonuclear leukocyte infiltration. The scores of conjunctival injection, stromal edema, and iritis, as in Series II, paralleled the scores of epithelial quality with IDU significantly worse and ACV no different.

The mean hydroxyproline per mg dry weight for the IDU, ACV, and control groups was $3.87 \pm 0.36, 4.85 \pm 1.07, 4.66 \pm 1.37$ μg, respectively. Although the IDU group had a significantly lower collagen content than the ACV group ($P < 0.02$), it did not significantly differ from the control group ($P < 0.20$). Unlike all other antivirals studied where no stromal vascularization was present, there was no significant difference in the collagen content between the ACV and control groups.

Discussion

The ideal topical antiviral agent must not only be active against the viral infection, but must also have minimal toxicity to normal and regenerating corneal epithelium and stromal cellular elements. Toxic effects on the healing epithelium and stroma may be clinically important in the short-term use of these agents in active HSV epithelial keratitis and interstitial keratitis, with or without ulceration. These effects are probably critical in their long-term use for chronic, recurrent HSV stroma keratitis and after penetrating keratoplasty for HSV stromal keratitis.

Numerous clinical studies have described epithelial toxicity, including superficial punctate keratitis, lacrimal punctal and meibomian gland occlusion, and keratinization of the lid margin associated with the short-term use of IDU, Ara A, and TFT [1–3]. With their chronic usage, particularly IDU, irreversible conjunctival cicatrization may ensue [4]. However, there have been no clinical studies directly suggesting that these antivirals retard epithelial wound healing. Closure of such epithelial wounds may be accomplished principally by initial sliding [16]. Growth and division

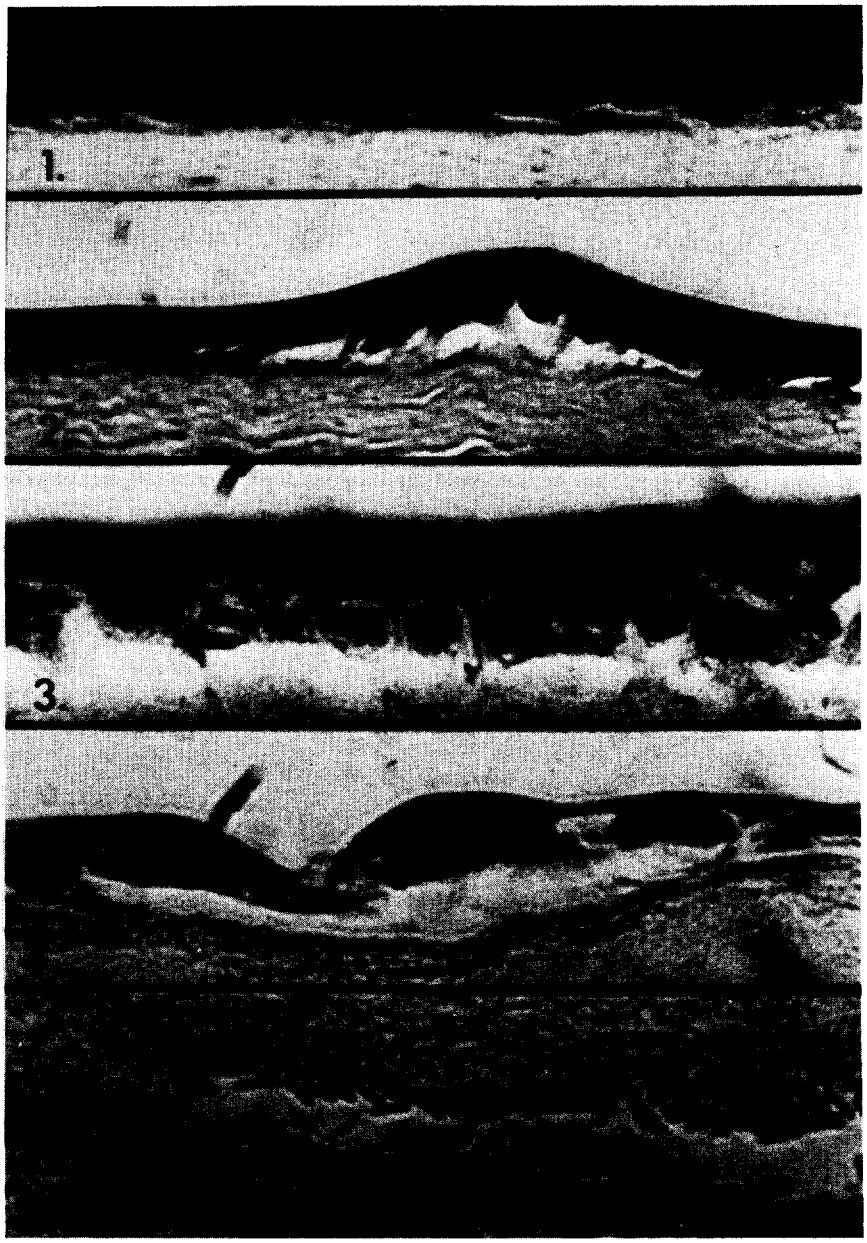

Fig. 7. Histologic changes associated with slit-lamp score of 0–4; 1) control eyes; 2, 3) changes in IDU and TFT-treated eyes showing some epithelial thinning and intracellular edema; 4, 5) typical changes of Ara AMP-treated eyes with marked epithelial thinning and loss and stromal neovascularization. (Courtesy Arch. Ophthalmol. (1977).)

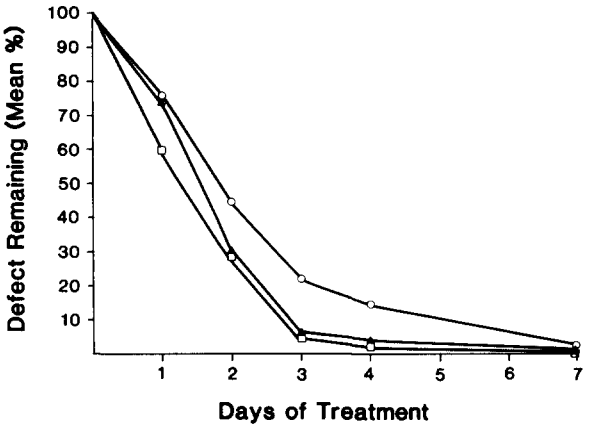

Fig. 8. Rate of healing of standardized 8.5 mm corneal epithelial defects in rabbits treated with IDU (○), ACV (□), and control (▲). Each point represents mean of treatment group population on designated days of treatment. (Courtesy Am. J. Ophthalmol. (1979).)

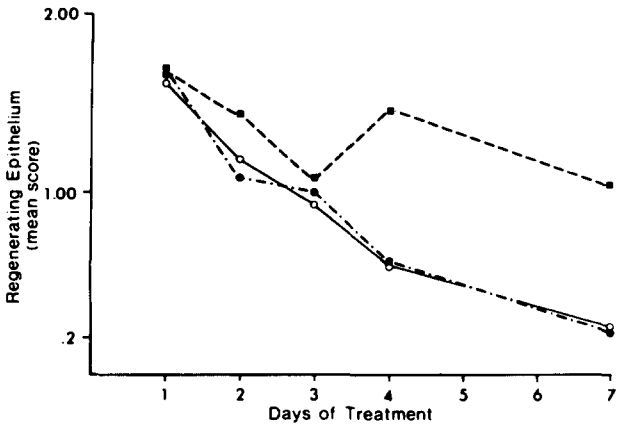

Fig. 9. Quality of regenerating corneal epithelium treated with IDU (■), ACV (●), and control (○), as scored by slit-lamp examination. Each point represents mean score of treatment group population on designated days of treatment. (Courtesy Am. J. Ophthalmol. (1979).)

occur later in the healing process. In such a case it is not surprising that these antiviral agents which affect both cellular and viral DNA synthesis [17] do not retard epithelial wound healing, but damage only proliferating epithelium.

In our experimental studies we have confirmed the clinical experience that IDU, Ara A and TFT have a toxic effect on normal, regenerating rabbit corneal epithelium. Ara A appeared to be less toxic than IDU in our experimental studies, but this was not apparent clinically [1]. Ara AMP's significant therapeutic effect and high solubility makes it an attractive antiviral agent [18], but its toxicity, including stromal vascularization and worsening epithelial erosion, we believe precludes its clinical use.

Experimentally the effect of IDU, Ara A, and TFT on the rate of epithelial wound

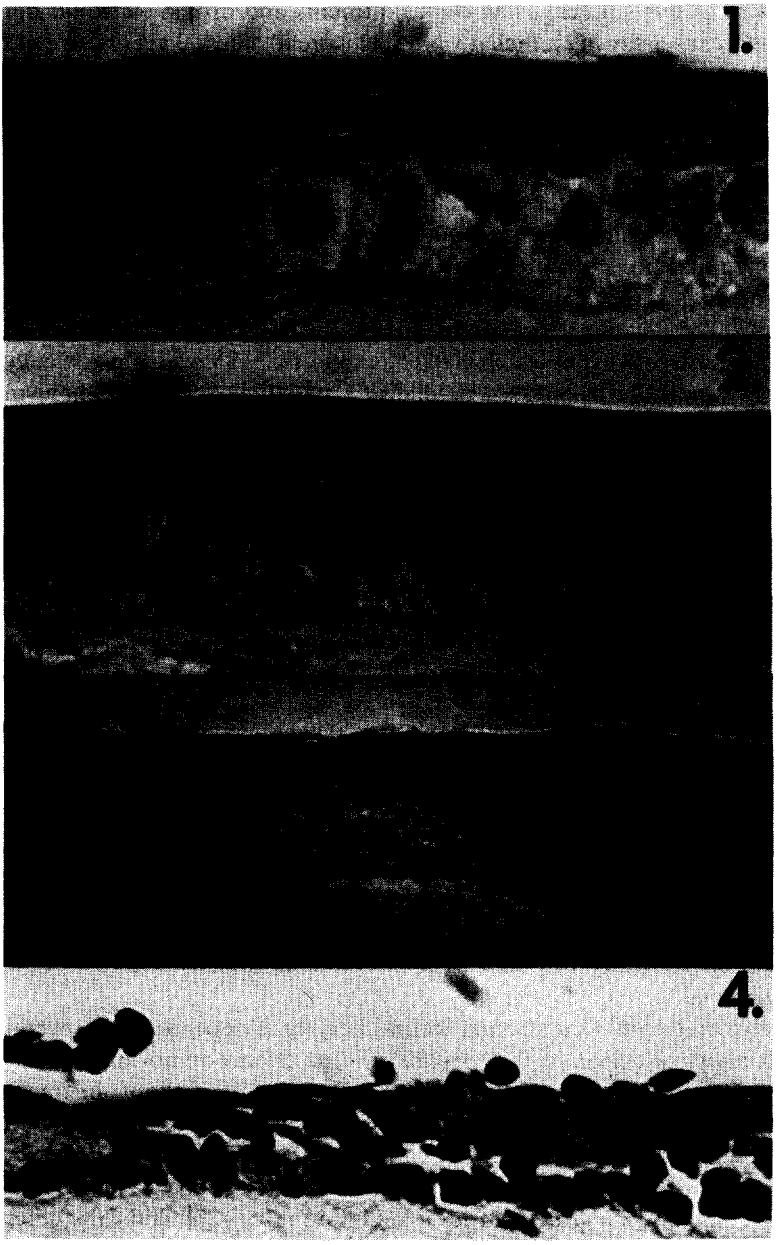

Fig. 10. 1) Central cornea of control epithelial wound showing minimal intracellular edema and no epithelial thinning. 2) Central cornea of ACV-treated epithelial wound showing similar changes as control. 3, 4) Central corneas of IDU-treated epithelial wounds showing marked epithelial thinning with intra- and intercellular edema and polymorphonuclear leukocyte infiltration. (Courtesy Am. J. Ophthalmol. (1979).)

closure is uncertain. In our studies, using both ointments and drops, we could not demonstrate a significant delay in epithelial wound closure for these 3 agents. We did find a significant delay on days 3 and 4 of therapy for IDU ointment in the 3rd series of experiments which was only a relative effect, since both IDU and control eyes were healed by day 7. Such a delay was not seen in the first series of ointment experiments. Other authors have also found no significant delay in epithelial healing with IDU [19–21]; however, a number of authors have found a significant delay [22–25]. The disparity may relate to different experimental conditions. In particular, cell growth and proliferation, in addition to cell migration, may play an important role in the initial healing process of larger epithelial wounds. IDU would then be expected to retard such healing.

Acyclovir is a new antiviral agent which is effective against HSV and is specifically activated by herpes simplex virus-induced thymidine kinase [26]. By this mechanism of action it achieves a selectivity of action, high potency, and lack of toxicity and thus provides a unique advantage over IDU, Ara A, and TFT which do not have this selectivity of action. The lack of toxicity was confirmed in our rabbit studies in which no demonstrable clinical or histologic differences in the regenerating epithelium between ACV and control-treated eyes were seen. As with the other antivirals, there was no difference in the epithelial closure rate.

Surprisingly, superficial punctate keratitis has been noted in double-masked [5–9] and open [10] clinical trials with ACV. Reports have ranged from an occasional occurrence [6] to up to 70% of the patients studied [8]. The punctate keratitis was slight and quickly resolved, however, once treatment was stopped. It also did not progress even if treatment was continued. Several investigators commented that this response may be due to the vehicle in the ointment rather than to the acyclovir [7–9]. Both our experimental study and the clinical studies used the same ointment (acyclovir in a petrolatum base without preservative). The mechanism for the superficial punctate keratitis in the human study remains unresolved. Unlike the experimental conditions with normal epithelium, in the human studies the drug was applied to an abnormal epithelium with active infection. Only with long-term observations when the drug is applied prophylactically will the extent of epithelial toxicity with 3% ACV ointment be determined.

The effect of antiviral agents on stromal wound healing is dependent on several variables: the selectivity of action of the antiviral and its effects on normal cellular division; the penetration of the drug into the stroma with or without an intact epithelium; and the differences in stromal healing vs. epithelial healing. The differential selectivity of each antiviral has already been described here.

Although in an in vitro system IDU significantly penetrates the cornea along with detectable inactive metabolites, 2'-deoxyuridine and iodouracil [27], clinical studies have shown only detectable levels of iodouracil and no unmetabolized drug in the aqueous, regardless of the epithelial status [28]. Ara A in the same in vitro system penetrated the cornea at a slower rate than IDU, but improved with epithelial removal [27]. Clinically only trace amounts of Ara A and its metabolite, hypoxanthine arabinoside, were present in the human aqueous [28]. The poor penetration of IDU and Ara A is predictable on the basis of their poor biphasic solubility.

Of the more soluble antivirals, Ara AMP penetrates more readily [29], but because of significant topical toxicity, its usage is limited. Because of its more favorable biphasic solubility, TFT would predictably penetrate the cornea to a greater degree than IDU and Ara A. Although one penetration study in normal corneas did not find any detectable levels in the aqueous of TFT [30], both in vitro [27] and clinical studies in patients with abnormal corneas [31] have confirmed its excellent penetration into the aqueous. Acyclovir recently was shown to have excellent penetration into the aqueous through the normal cornea prior to cataract extraction [30]. One would predict excellent penetration through the abnormal cornea, similar to TFT.

Stromal wound healing differs from epithelial wound healing in that cell division and growth, rather than cell migration, are predominant [16, 19, 21,32]. Therefore, it would be expected that antiviral agents with a less selective effect on normal cellular DNA synthesis, such as IDU, Ara A and TFT, would have a greater detrimental effect on such healing, whereas acyclovir would have minimal to no adverse effect.

Considering all 3 variables affecting stromal wound healing, the effect of IDU and Ara A is unpredictable because of their limited penetration, whereas TFT has a greater adverse potential because of its excellent penetration. Despite its excellent penetration, ACV would predictably have minimal or no effect because of its high selectivity for only viral DNA synthesis.

Evaluating the effects of antiviral agents on stromal wound healing is difficult clinically. Most of the patients which are at risk either have HSV stromal keratitis with or without active stromal ulceration and require topical corticosteroids in addition to an antiviral agent, or are on topical corticosteroids and a prophylactic antiviral following penetrating keratoplasty. With the use of a topical corticosteroid, it is impossible to sort out the possible toxic effect of an antiviral on stromal wound healing.

Experimental studies would appear to be helpful in resolving this question, but there is a considerable difference of opinion. The evaluation of stromal wound healing has been examined by various methods, including bursting or tensile strength [11,12,19], collagen content [11–13], tritiated thymidine uptake [32,33], and fibroblast proliferation [34]. Different dosage regimens of the antiviral agents and types of stromal wounds (penetrating vs. non-penetrating; linear vs. circular; surgical incision vs. freezing) have been employed.

In our studies using 2 and 1.5 mm penetrating wounds, we found a significant decrease in collagen content and bursting strength after 3 weeks of treatment with IDU drops and ointment, and Ara A. Although TFT decreased bursting strength and collagen content in our stromal wound model, the difference from control was not significant. This result was somewhat surprising, given TFT's excellent corneal penetration. There was a difference in results for the IDU ointment between the Series I and III studies. In Series I there was a significant decrease in collagen content, as well as bursting strength when a 2 mm wound was employed and the drug was given 4 times a day. In Series III there was a decrease in collagen content when a 1.5 mm wound was employed and the drug given 5 times per day. A smaller wound, a different drug regimen, and a different hydroxyproline measurement (per mg dry weight rather than per button) were used. All these factors may account for the different results. We

believe, however, that all 3 agents significantly retard stromal wound healing.

Only IDU has been extensively studied by numerous investigators on various models of stromal wound healing. Agreeing with our results, some investigators have concluded that IDU retards stromal wound healing [19,21,32,34.]. Other investigators have not observed any adverse effects [33,35–38], probably due to different experimental methods and dosage of medication. Of the more soluble antiviral agents, TFT was shown by one other group to retard stromal healing [33].

To our knowledge, acyclovir is unique among the topical antiviral agents in its lack of effect on stromal wound healing [13]. Given its high selectivity, excellent penetration, and lack of effect on stromal wound healing, acyclovir appears to be the agent of choice for use in HSV stromal keratitis with ulceration and as a propylactic agent for long-term use after penetrating keratoplasty.

Acknowledgements

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