Experimental ulcerative herpetic keratitis. II. Influence of topical corticosteroid in immunised rabbits

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SUMMARY Since the great majority of patients possess immune responses to herpes simplex virus (HSV), the influence of a topical anti-inflammatory corticosteroid (0.1% clobetasone butyrate) on ulcerative herpetic keratitis was studied in rabbits with a previous HSV skin infection (immunised) and compared with that in normal rabbits. Corticosteroid treatment had a much greater ulceration-exacerbating effect in immunised than in normal animals. On day 7 the mean area of ulceration in immunised rabbits was 3 times greater in treated eyes. 0.01% clobetasone butyrate treatment had less effect on immunised rabbits; 0.001% had no effect. It is concluded that the immunised rabbit provides a useful experimental model for studying the relationship between concentration of topical anti-inflammatory agents and enhancement of herpetic ulceration.

It is known that the administration of anti-inflammatory corticosteroids in ulcerative herpetic keratitis can result in severe exacerbation of disease with the development of geographic or amoeboid ulcers and an increase in the incidence of subsequent complications.^{1 2}

Similar aggravation of herpetic corneal ulceration has been demonstrated in rabbits treated with drugs of this group.³⁻⁶ These studies employed normal rabbits which had had no previous contact with herpes simplex virus (HSV), while by contrast the majority of the human population have immune responses to the virus. Since a major effect of corticosteroids is to inhibit immune reactions,⁷ the use of rabbits with a previous HSV skin infection ('immunised') should more closely represent the clinical disease.

The aims of this investigation were to study the effect of a topically administered corticosteroid on ulcerative herpetic keratitis in normal and in immunised rabbits, and to examine the relationship between corticosteroid concentration and degree of enhancement of ulceration. Clobetasone butyrate was used because it is effective in various ocular inflammatory conditions and produces relatively little rise in

intraocular pressure in susceptible subjects.^{9 10}

Material and methods

The details of virus and rabbits used, and the methods of immunisation of rabbits, corneal inoculation of virus, and assessment of corneal disease have been described.⁸ The ranges of virus concentration used were 0.4 to 26.1×10^5 PFU/ml for normal and 3.9 to 250.0×10^5 PFU/ml for immunised animals (PFU = plaque-forming units).

Clobetasone butyrate or placebo treatment was given 5 times daily at 1½ hour intervals, beginning on the day before corneal inoculation of virus. One eye of each rabbit received clobetasone butyrate and the other placebo, and the allocation of treatment was unknown to the observer. The standard concentration of the preparation was 0.1%, and the vehicle was used as placebo and diluent. Experiments were continued until the extent of corneal ulceration became severe, or the placebo-treated eye had recovered from ulcerative disease.

The effects of 0.1% clobetasone butyrate on normal and immunised rabbits (4 animals in each group) were compared. The effects of 0.01% and 0.001% clobetasone butyrate were studied in immunised rabbits only (5 animals in each group).

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Results

EFFECT OF 0.1% CLOBETASONE BUTYRATE IN NORMAL AND IN IMMUNISED RABBITS

The corneal infectious dose for 50% of inoculations (CID_{50}) measured at 2 days after infection revealed no difference between placebo- and clobetasone-butyrate-treated eyes, either in normal or in immunised rabbits (Table 1, Fig. 1).

In normal rabbits ulcerative disease showed a tendency towards increased severity in the corticosteroid-treated eyes on day 7, the mean

Table 1 Mean CID_{50} values in placebo- and clobetasone-butyrate-treated corneas of the 4 groups of rabbits

Rabbits	Treatment		CID_{50} (PFU/ml $\times 10^5$)	
	Control	Experimental	Control	Experimental
Normal	Placebo	0.1% CB	9.9	5.1
Immunised	Placebo	0.1% CB	104.2	141.3
Immunised	Placebo	0.01% CB	94.1	104.2
Immunised	Placebo	0.001% CB	97.5	147.7

CB=clobetasone butyrate.

Fig. 1 Virus titration in placebo- (—) and 0.1% clobetasone-butyrate-treated (---) corneae in immunised rabbits.

Fig. 2 Progress of ulcerative disease in placebo- (—) and 0.1% clobetasone-butyrate-treated (---) corneas: normal rabbits (mean area of corneal ulceration $\pm$ SE) Range of virus concentrations inoculated: 0.4 to 26.1×10^5 PFU/ml.

areas of ulceration at that time being $32 \pm 15\%$ (SE) of the cornea in placebo- and $41 \pm 14\%$ in clobetasone-butyrate-treated eyes (Fig. 2).

In immunised rabbits a large difference between placebo and corticosteroid-treated eyes was observed. An increase in area of ulceration in clobetasone-butyrate-treated corneas was first seen on day 4, and a very marked difference was found on day 7, when the mean areas of ulceration were $6 \pm 5\%$ of the cornea in placebo- and $23 \pm 9\%$ in clobetasone-butyrate-treated eyes. Pooled data from 18 eyes showed a statistically significant increase in areas of ulceration in eyes treated with 0.1% clobetasone butyrate ($p < 0.025$; rank sum test). At this stage the healing phase of ulcerative disease had already begun in the placebo-treated eyes, while the area of ulceration in corticosteroid-treated corneas was at least that found on day 4. The form of the ulcers was largely geographic, with relatively regular margins. Two animals had large central epithelial defects, where the relative importance of viral infection and self-inflicted abrasive loss of diseased epithelium was not clear. Between days 7 and 9 a reduction in area of ulceration occurred in both placebo- and corticosteroid-treated eyes (Fig. 3).

Fig. 3 Progress of ulcerative disease in placebo- (—) and 0.1% clobetasone-butyrate-treated (---) corneae; immunised rabbits (mean area of ulceration $\pm$ SE). Range of virus concentrations inoculated: 3.9 to 250.0×10^5 PFU/ml.

EFFECTS OF 0.01% AND 0.001% CLOBETASONE BUTYRATE IN IMMUNISED RABBITS

No differences in CID_{50} were observed between eyes treated with placebo and with 0.01% or 0.001% clobetasone butyrate (Table 1).

The exacerbating effect of 0.01% clobetasone butyrate on ulcerative disease was clearly less than that of the 0.1% preparation. An increase in area of corneal ulceration in the 0.01% clobetasone-butyrate- compared with the placebo-treated eye was seen in 2 of 5 rabbits on day 4 and in 3 of the 4 remaining animals on day 7 (Fig. 4).

Treatment with 0.001% clobetasone butyrate had no discernible effect on the extent of ulceration at any stage of the infection (Fig. 4).

Discussion

It may be concluded that clobetasone butyrate has no effect on initial corneal susceptibility to infection; it produces a considerably greater exacerbation of ulceration in immunised than in normal rabbits; this exacerbation is apparent late in the infection (and delays the initiation of healing); and it is dose-dependent.

The lack of any significant effect in normal animals is at variance with the findings of previous studies,³⁻⁶ and it might be related to the steroid and schedule used or to the termination of this experiment before the beginning of the healing phase.⁸ However, it is clear that a much greater enhancement of ulceration occurred in immunised rabbits, and an experimental model employing animals with previous skin or ocular HSV infection provides a close parallel with human disease.^{8, 11}

In immunised rabbits a gradation in effect was observed from a distinct exacerbation of ulceration with 0.1% clobetasone butyrate to an indiscernible effect with the 0.001% preparation, indicating that the maximum 'safe' concentration in this model lies between 0.01 and 0.001%. Various dilutions of corticosteroid are now used clinically,² and an understanding of the effects of low concentrations on ulcerative herpetic

Fig. 4 Dose response curves for the exacerbating effect of clobetasone butyrate on ulcerative herpetic keratitis in immunised rabbits (mean increase in area of ulceration in corticosteroid- compared with placebo-treated eyes $\pm$ SE).

keratitis could help to decide when antiviral cover is necessary and to select moderate concentrations for general use. It is conceivable that a low concentration of corticosteroid could carry anti-inflammatory activity without risk of enhancement of herpetic disease, since the dose-response relationships are probably different for the 2 effects.¹²

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