Acycloguanosine: antiviral activity in the rabbit cornea

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SUMMARY We describe experiments, using the multiple microinoculation technique, to produce superficial herpes simplex keratitis in the rabbit cornea, which showed a potent antiviral effect of acycloguanosine.

Although they are usually effective for the treatment of epithelial herpes simplex keratitis, idoxuridine (IDU), adenine arabinoside (ara-A), and trifluorothymidine (F₃T) have limitations of solubilities and toxicity which render them inadequate for the management of deep herpetic disease. Acycloguanosine (Wellcome 248U) shows promise of overcoming these limitations. It is soluble in water (as the sodium salt), and has low toxicities from both topical ocular and systemic administration in several animal species (Schaeffer *et al.*, 1978; Tucker *et al.*, 1978) and man. It has potent antiviral activity against herpes simplex virus (HSV) types I and II, both in vitro and in vivo (Elion *et al.*, 1977; Bauer *et al.*, 1979).

As a preliminary to our first clinical trial of 248U, which has now been completed (Jones *et al.*, 1979), and in order to compare data from our own laboratory with those of others (Bauer *et al.*, 1979) we investigated the efficacy and tolerability of 248U in our rabbit corneal lesion inhibition system (Falcon and Jones, 1977a).

Methods

Details of the multiple microinoculation technique of Jones and Al-Hussaini, and of its modification to produce a corneal epithelial lesion inhibition assay, are published elsewhere (Jones and Al-Hussaini, 1963; Falcon and Jones, 1977a). In summary, 25 circular sites are inoculated on each eye of anaesthetised adult Dutch rabbits by means of a capillary tube of 1 mm diameter filled with pH 8 strain of HSV (of 1.5×10^6 plaque-forming units/ml). Treatment generally begins 2 hours after inoculation and is administered 5 times daily. 48 hours after inoculation each site is scored 0 to 4 according to

Fig. 1 Dose/response curves for 248U, ara-A and ara-AMP ointments. Treatment was given 5 times daily, starting 2 hours after inoculation. Eyes scored 48 hours after inoculation

the number of sites infected, and a corneal epithelial lesion inhibition assay (CELIA) is produced by comparing treated with control eyes in groups of 3 or 4 rabbits (which gives statistically valid results). The advantage of this lesion-inhibition system is that the lesions are small and discrete at the time of scoring, which provides rapid, accurate, and repeatable results from the minimum of animals, and the experiment can be terminated before the eyes have become uncomfortable.

Results

Experiments were performed to give a dose/response curve for 248U ointment. This was compared with ara-A ointment, and ara-A 5' monophosphate (ara-AMP) ointment, which had been shown previously to give greater lesion inhibition than any other antiviral tested in this system (Falcon and Jones, 1977b). It was also compared with standard 0.5% IDU ointment and with 1% F₃T drops (Fig. 1).

We had shown previously that a single dose of

ara-AMP given directly after inoculation had an almost catastrophic lesion-inhibiting effect, whereas IDU, ara-A, or F₃T had little effect (Falcon and Jones, 1977b). 248U was employed in the same system and likewise had very little effect (Fig. 2).

In experiments to investigate the effect of systemic 248U, 50 mg/kg of 248U was given intravenously twice daily, the injections starting 2 hours after inoculation, and this experiment was continued for 72 hours. At 48 hours there was a small difference in scores between treated and control animals, and this had increased very considerably by 72 hours (Fig. 3).

Although this model, and particularly the scoring system, were derived primarily for lesion-inhibition experiments, we attempted to use them to assess therapy of established lesions: treatment began 48 hours after inoculation and was given 5 times daily. Scoring was carried out immediately before the beginning of the treatment and subsequently after 3 and 5 more days. We compared control, oculentum IDU 0.5%, oculentum ara-A 1%, oculentum ara-AMP 1%, guttae ara-AMP 1%, guttae F₃T 1%, and guttae 248U 1%. It proved impossible to produce meaningful scores, since the lesions 48

Table 1 Number of eyes healed (out of 3 in each group), 3 and 5 days after start of antiviral treatment, which was given 5 times daily, starting 48 hours after inoculation

Antiviral	Number healed	
	At 3 days	At 5 days
Control	0	0
Oculentum IDU 0.5%	0	0
Oculentum Ara A 1%	0	0
Oculentum Ara AMP 1%	0	0*
Guttae F ₃ T 1%	2	3
Guttae 248U 1%	3	3

* Severe toxic epitheliopathy at this stage

hours from inoculation had already spread away from the inoculation sites. We therefore merely scored each eye as 'healed' or 'unhealed' (Table 1).

Discussion

Fig. 1 shows that the dose/response curves for 248U and ara-A are very similar, and it shows that the efficacy of 1% and 2% 248U ointment, as measured by lesion inhibition, is comparable to that of 1% F₃T drops. The dose/response curve for ara-AMP indicates a substantially superior antiviral effect, but this has been shown to be due to the unique and profound lesion-inhibiting effect of ara-AMP when given shortly after inoculation (Falcon and Jones, 1977b) (Fig. 2). ara-AMP has proved too toxic for practicable clinical application to the eye.

These lesion-inhibiting experiments thus indicate a useful potential for topical 248U, which is supported by our experiments in the treatment of established lesions (Table 1), though these have emphasised the limitations of this method.

Our experiments with intravenous 248U therapy demonstrate an encouraging antiviral effect in the cornea, although, as might be expected, this is delayed in comparison with the effect of topical treatment. Experiments are now in progress to establish a model of deep herpetic keratitis and to investigate the role of subconjunctival and systemic 248U therapy for it. These routes may prove to be the most effective means of achieving a good intraocular level of the drug, since very low aqueous levels of 248U were obtained after its application to the cornea, whether or not the epithelium was breached (Bauer, personal communication). The water solubility and low toxicity of 248U should provide no barriers to such therapy and indeed offer possibilities for safe and effective therapy of herpes virus infections throughout the body.

Fig. 2 Lesion inhibition from single doses of various antivirals. Treatment was given once immediately after inoculation, and the eyes were scored 48 hours later

Fig. 3 Lesion inhibition 48 and 72 hours after inoculation: 50 mg/kg of 248U was given intravenously twice daily, starting 2 hours after inoculation

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