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# Antiviral Activity in the Rabbit Cornea of Adenine Arabinoside, Ara-A 5′ Monophosphate, and Hypoxanthine Arabinoside; and Interactions with Adenosine Deaminase Inhibitor

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SUMMARY

The multiple microtrephination technique was used in the rabbit cornea to compare the activity against herpes simplex virus (HSV) of adenine arabinoside (ara-A), ara-A 5′monophosphate (ara-AMP) and hypoxanthine arabinoside (ara-Hx), and to determine the effect of addition of adenosine deaminase inhibitor (ADAI) to each. The greatest antiviral activity was shown by ara-AMP, and the least by ara-Hx. ADAI increased the activity of ara-A but had no effect on ara-AMP or ara-Hx.

Ara-A was first shown to have a significant effect against HSV (de Garilhe & De Rudder, 1964) and other DNA viruses in cell culture (Schabel, 1968), in 1964, and it has subsequently gained wide acceptance in the treatment of HSV keratitis in man (Jones *et al.* 1974; McGill *et al.* 1975). It suffers, however, from the limitation of very low water solubility (0·45 mg/ml), which precludes good ocular delivery and it has an extremely low lipid solubility, which indicates poor prospects for cell membrane penetration. *In vitro* it is deaminated to ara-Hx which has a greater water solubility than ara-A (4·5 mg/ml) but an extremely low lipid solubility. Ara-Hx has only one tenth the antiviral activity of ara-A *in vitro* (Miller *et al.* 1968), and one-fifth of its antiviral activity *in vivo* (Pavan-Langston, Langston & Geary, 1974).

Adenosine deaminase occurs widely in living systems, but there is no data on its presence in the cornea. The use of an inhibitor of adenosine deaminase in some cell culture systems increases the efficacy of ara-A approx. 40-fold, but the deaminase inhibitor itself has no antiviral action (Connor *et al.* 1974).

Ara-AMP is a derivative of ara-A which is highly water soluble, but which has a very low solubility in lipids. *In vivo* it has an antiviral effect equivalent to that of the parent compound, and it is metabolized more slowly than ara-A (B. J. Sloan and C. A. Miller, unpublished results).

We have compared the antiviral activities of ara-A, ara-AMP and ara-Hx, and determined the effect of addition of ADAI to each by using the multiple microinoculation technique (Jones & Al-Hussaini, 1963). Under anaesthesia the corneae of Dutch rabbits were inoculated on sixteen sites each with four serial decimal dilutions of the pH 8 strain of HSV, giving four sites per dilution. The cell-free pool of HSV gave a titre of $1{\cdot}34 \times 10^7$ p.f.u./ml in Vero cell monolayers, and was serially diluted with Eagle's minimum essential medium supplemented with 5 % foetal bovine serum, 200 μmol/ml of glutamine, 100 μg/ml of vancomycin and 50 μg/ml of streptomycin.

The eyes were examined 48 h after inoculation, using a microscope, and after the application of 1 % rose Bengal, which stains plaques of HSV-replicating corneal epithelial cells (Jones & Patterson, 1967). The 50 % corneal infectivity titre ($CID_{50}$) was calculated by the method of Reed & Muench (1938). In the absence of treatment there was good

agreement between right and left eyes in any one animal (s.d = 0·35), but inter-animal variation was greater (s.d. = 0·75).

Antiviral efficiacies were measured by comparing infectivity titres in treated and control eyes, or by comparing two different treatments in groups of three or more rabbits. Treatment was begun 2 h after inoculation, and was given five times a day, on a coded basis where possible. The various antivirals were made up in an ointment base. The deaminase inhibitor was dissolved in liquid paraffin for addition to the ointment. Equal gravimetric weights of the antiviral were used; this tends to bias results against ara-AMP since its mol. wt. is a third greater than that of ara-A, and comparisons by ara-A equivalents are thus more meaningful.

Table 1. *Antiviral effect of various concentrations of ara-A ointment applied 5 times a day (1½ to 2 hourly) against HSV infection of the rabbit cornea, commencing 2 h after inoculation*

| Concentration of ara-A ointment (%) | Number of rabbits | Corneal infectivity titre (log $CID_{50}$) | | Antiviral effect (reduction in titre) | |
|---|---|---|---|---|---|
| | | Control | Treated | | |
| 0·25 | 4 | >3 | 2·82 | >0·18 | |
| 0·5 | 4 | 2·64 | 2·25 | 0·39 | |
| 0·75 | 3 | 2·51 | 1·79 | 0·72 | |
| 1 | 4 | 2·07 | 0·73 | 1·34 | Mean 1·44 |
| 1 | 4 | 2·61 | 1·01 | 1·60 | |
| 1 | 2 | 2·06 | 0·74 | 1·32 | |
| 2 | 4 | 2·78 | 0·00* | >2·78 | |
| 3 | 5 | 3·83 | 0·00* | >3·83 | |

* Too few lesions to give a 50% end point.

Table 2. *Antiviral effect of various concentrations of ara-AMP ointment applied 5 times a day (1½ to 2 hourly) against HSV infection of the rabbit cornea, commencing 2 h after inoculation*

| Concentration of ara-AMP ointment (%) | 'Ara-A equivalent' concentration (%) | Number of rabbits | Corneal infectivity titre (log $CID_{50}$) | | Antiviral effect (reduction in titre) |
|---|---|---|---|---|---|
| | | | Control | Treated | |
| 0·25 | 0·17 | 4 | >3 | 0·94 | >2·06 |
| 0·5 | 0·35 | 6 | 2·52 | 0·71 | 1·81 |
| 1 | 0·69 | 6 | 1·83 | 0·00* | >1·83 |
| 1 | 0·69 | 4 | 1·91 | 0·00* | >1·91 |
| 2 | 1·38 | 3 | 3·00 | No lesions | >3·00 |
| 3 | 2·07 | 5 | 2·73 | No lesions | >2·73 |
| 1% aqueous drops | 0·69 | 3 | 1·92 | 0·30 | 1·92 |

* Too few lesions to give a 50% end point.

The dose/response relationship for ara-A was approximately linear, but eyes receiving 2% or 3% ara-A produced too few lesions for the 50% infectivity titre to be determined (Table 1).

The antiviral effect of ara-Hx was markedly inferior to that of ara-A; the reduction in infectivity titres for 1%, 2% and 3% ara-Hx being 0·60, 1·08 and 1·71 respectively.

The antiviral effect of ara-AMP was strikingly superior to that of ara-A (Table 2), and

there was complete inhibition of lesions in eyes receiving 2 % or 3 % ara-AMP, which has never occurred previously using other antivirals in this experimental system.

Addition of deaminase inhibitor consistently had no significant effect upon the efficacies of ara-Hx or ara-AMP; it produced a mean increase in infectivity titre of 0·06 for ara-Hx and 0·03 for ara-AMP. Addition of ADAI to ara-A, however, increased its antiviral efficacy (as measured by infectivity titre) by a mean of 0·94 in seven experiments (twenty-three rabbits). There was somewhat greater than usual right/left variation in these experiments, perhaps indicating inter-animal variation in deaminase activity.

These results, expressed as mean reduction in infectivity titres for the various antivirals, give a ratio of 1:2·5:3·5 for 1 % ara-Hx, 1 % ara-A and 1 % ara-A+ADAI, or 1:2·3:3·3 when expressed as ara-A equivalents. The efficacy of 1 % ara-AMP was so great as to be beyond precise measurement by this method.

It is thus established that the corneal epithelium of the rabbit has an ara-A deaminase function, the blocking of which materially enhances the antiviral activity of that drug – although to an extent less than that expected from results of *in vitro* studies (Connor *et al.* 1974): if this deaminase function were completely effective, and if ara-A and ara-Hx penetrate the cell equally well, one would expect to find that the antiviral efficacies of the two drugs in this model would be identical, which they were not. In this system the addition of ADAI did not enhance the activity of ara-AMP. This suggests that ara-AMP enters the cells as ara-AMP and also that the dephosphorylase activity at the surface of the corneal epithelial cells is low. Moreover, the activity of ara-AMP was greater than that of ara-A +ADAI. This may be due to greater delivery of ara-AMP to the corneal epithelium as a result of its greater water solubility.

These factors, combined with the lower toxicity of ara-AMP (B. J. Sloan and C. A. Miller, unpublished results), suggest a considerably improved potential for topical and perhaps systemic antiviral therapy, when compared with what has been possible with the grudgingly soluble parent compound ara-A. In further experiments we have also shown greater antiviral activity in the corneal epithelium against the pH 8 strain of HSV from 1 % ara-AMP drops than from 1 % trifluorothymidine drops.

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*Department of Clinical Ophthalmology*
*Institute of Ophthalmology*
*University of London*
*Moorfields Eye Hospital*
*London EC1 2PD*

M. G. FALCON
B. R. JONES

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