Efficacy of acycloguanosine against herpetic ulcers in rabbit cornea

HIROSHI SHIOTA, SUMIKO INOUE, AND SHINTA YAMANE

From the Department of Ophthalmology, Tokushima University School of Medicine, Tokushima, Japan

SUMMARY The effect of a new antiherpetic compound of very low toxicity called acycloguanosine (Wellcome 248U) on herpetic ulcers in rabbit cornea was studied by the Corneal Epithelial Lesion Therapeutic Assay (CELTA). The therapeutic effect of 3% acycloguanosine ointment on dendritic ulcers was equal to that of 0.5% 5-iodo-2'-deoxyuridine (IDU) ointment. No toxic symptoms could be detected by slit-lamp on 4 days' treatment with this concentration of acycloguanosine. Because of its selective action on virus only, its extremely low toxicity in animals, and its availability for systemic administration, acycloguanosine seems to be an ideal antiviral compound for use in the treatment not only of herpetic keratitis but also of other herpetic diseases in man.

Herpes simplex virus (HSV) is one of the main infectious causes of eye disease in industrialised countries. IDU, first shown to be effective by Kaufman *et al.* (1962), has been the main drug used in the treatment of herpetic keratitis. However, cases that fail to respond to IDU or that develop allergic or toxic signs have been reported (Pavan-Langston and Dohlman, 1972; McGill *et al.*, 1974; Hyndiuk *et al.*, 1975), and so other antivirals are required. Adenine arabinoside (ara-A) and trifluorothymidine (F₃T) appeared to be suitable alternatives several years ago and they have in fact proved effective against ulcerative herpetic keratitis (Pavan-Langston and Dohlman, 1972; Wellings *et al.*, 1972).

However, ara-A is scarcely soluble in water and has little effect on deep herpetic keratitis by topical application, while F₃T, though more soluble and effective than ara-A or IDU, is rather unstable and very expensive. Furthermore these 3 compounds all affect the replication of normal cells, causing some degree of toxicity. Thus more suitable antivirals are required for the treatment of HSV infections.

A possible candidate for this purpose is a guanine derivative called acycloguanosine (Wellcome 248U) (Fig. 1). This drug has marked antiviral activity on HSV and extremely low toxicity, so that it may be administered systemically (Schaeffer *et al.*, 1978; Kaufman, 1978). In this study the efficacy of acycloguanosine against HSV was verified and the therapeutic effect of the drug against ulcerative

Fig. 1 Structure of acycloguanosine (248U)

herpetic keratitis in rabbit cornea was compared with that of IDU by the Corneal Epithelial Lesion Therapeutic Assay (CELTA), a method which gives an indication of the action of antiviral drugs in large-scale clinical trials (Shiota, 1979).

Methods and materials

Pigmented rabbits weighing between 2.0 and 3.0 kg were used. For inoculation of virus a modification of the multiple microtrephination method of Jones and Al-Hussaini (1963) was used. Rabbits were anaesthetised with intravenous pentobarbital sodium (30 mg/kg) and given a retrobulbar injection of

Correspondence to Hiroshi Shiota, MD, Department of Ophthalmology, Tokushima University School of Medicine, Kuramoto-cho, Tokushima City 770, Japan

Fig. 2 (a) Plan of inoculation. (b) Method of scoring

0.5 ml of 1% procaine. One eye was closed with Cellophane tape while the other was proptosed and inoculated with HSV. For inoculation a suspension of the RE strain of HSV (type 1) with a titre of 2.27×10^6 plaque-forming units/ml was drawn into a fine glass capillary tube of 1.0 mm internal diameter, and the upper end of the capillary was plugged with Plasticine.

CELTA METHOD

Trephinations through the corneal epithelium with the capillary tube were made at 25 sites in the cornea (Fig. 2a) as for the corneal epithelial lesion reduction assay (Falcon and Jones, 1977). After inoculation the eye was left open for 30 seconds and then the lids were closed with Cellophane tape. The same procedure was repeated on the other eye. In one group of 4 rabbits the right eyes were treated with 3% acycloguanosine ointment and the left eyes with 0.5% IDU ointment, or vice versa. In another group 4 eyes received no treatment as controls. The treatment was started 48 hours after inoculation and continued every 2 hours during the daytime 5 times a day for 4 days. The eyes were examined

48 hours after inoculation and then every 24 hours with a photo-slit lamp after applying 1% Bengal rose, which stains plaques of HSV-replicating cells in the corneal epithelium (Jones and Patterson, 1967). Each inoculation site was scored 0 to 4 according to the extent of the circumference infected, and 1 or 2 was added if half or the whole of the cornea in the circle was ulcerated. Thus each site gave a score of between 0 and 6 as illustrated in Fig. 2b. The total daily score for each eye was calculated and expressed as a percentage of the score (percentage score) immediately before treatment. The therapeutic efficacies of acycloguanosine and IDU against ulcerative herpetic keratitis were compared by calculating their average per centage scores.

Results

At 48 hours after HSV inoculation large dendritic ulcers extending along almost the whole circumference infected at each site were seen in all eyes as shown in Fig. 3a, and the mean total score per eye was 95.6. In control eyes these ulcers developed steadily, forming wide geographic ulcers with prominent corneal oedema and severe ciliary injection by 6 days after inoculation, as shown in Fig. 3b. In contrast, in eyes treated with 3% acycloguanosine ointment the large dendritic ulcers gradually became smaller. After treatment with acycloguanosine for 3 days these lesions became punctate, and after treatment for 4 days they became minute and some sites did not show any lesions at all (Fig. 3c). Infected eyes were thus nearly or completely cured by 4 days' treatment with acycloguanosine. Treatment with 0.5% IDU ointment caused the same clinical improvement as treatment with acycloguanosine. A representative eye after 4 days' treatment with IDU is shown in Fig. 3d.

These results are summarised in Table 1 and Fig. 4. The antiviral efficacy of 3% acycloguanosine ointment on ulcerative herpetic keratitis appeared to be

Table 1 Percentage scores of severity of herpetic ulcers in rabbit cornea

Days of treatment	3% 248U (n=4)	0.5% IDU (n=4)	Control (n=4)
0	100	100	100
1	82.1 ± 17.0	83.4 ± 12.7	105.5 ± 11.4
2	40.7 ± 7.0	45.5 ± 8.4	83.4 ± 21.9
3	26.7 ± 5.7	25.5 ± 2.3	89.3 ± 27.3
4	20.3 ± 8.7	20.3 ± 5.8	122.2 ± 20.9

Treatment was started 48 hours after HSV inoculation

Fig. 3 Responses of herpetic ulcers in rabbit cornea to acycloguanosine and IDU. (a) Eye before treatment, 48 hours after inoculation. (b) Control eye 6 days after inoculation. (c) Eye treated with 3% acycloguanosine (248U) ointment for 4 days. (d) Eye treated with 0.5% IDU ointment for 4 days

equal to that of 0.5% IDU ointment. No signs of toxicity were detectable by slit lamp during treatment with either drug.

Discussion

We have shown that acycloguanosine was highly effective for treating herpetic ulcers and that the effect of 3% acycloguanosine was similar to that of 0.5% IDU. These findings are in good accord with those of Kaufman (1978). However, Pavan-Langston *et al.* (1978) reported from similar experi-

ments on rabbits that the therapeutic efficacy of 3% acycloguanosine was significantly better than that of 0.5% IDU and 3% ara-A. Kaufman also reported that acycloguanosine was effective against herpetic iritis when given systemically or subconjunctivally (1978). Moreover, Schaeffer *et al.* (1978) found that acycloguanosine was effective against herpetic keratitis in rabbits by topical application, against herpetic encephalitis in mice *per os*, and against cutaneous herpetic infections in guinea-pigs.

Particular attention should be paid to the mode of the selective action of this compound. Acyclo-

Fig. 4 Therapeutic effects of acycloguanosine (248U) and IDU against herpetic ulcers in rabbit cornea: 3% 248U and 0.5% IDU ointment were applied 5 times a day starting 48 hours after HSV inoculation. Four eyes were subjected to each treatment

guanosine is phosphorylated by a virus-specified thymidine kinase and converted to acycloguanosine triphosphate, which inhibits viral DNA polymerase (Elion *et al.*, 1977). Since acycloguanosine is not appreciably phosphorylated by cellular kinase, it does not affect host cells and thus has extremely low toxicity. In addition Schaeffer *et al.* (1978) found that it is not metabolised when given systemically.

From the facts that acycloguanosine has marked antiviral activity but extremely low toxicity, and that it can be administered systemically and is not metabolised on systemic administration, this drug should be ideal for treatment not only of herpetic keratitis but also of other herpetic infections in man.

We are indebted to Wellcome Research Laboratories, Kent, UK, and Professor Barrie R. Jones, University of London,

for provision of 3% 248U ointment; Mr M. Okumoto for provision of the RE strain of HSV; Dr M. Imai, Dr T. Ogawa, and Mrs Y. Endo for technical assistance.

This study was supported in part by a research grant from the Japanese Ministry of Education, Science, and Culture.

References

- Elion, G. B., Furman, P. A., Fyfe, J. A., de Miranda, P., Beauchamp, L., and Schaeffer, H. J. (1977). Selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl)guanine. *Proceedings of the National Academy of Sciences of the USA*, **74**, 5716–5720.
- Falcon, M. G., and Jones, B. R. (1977). Herpes simplex keratitis: animal models to guide the selection and optimal delivery of antiviral chemotherapy. *Journal of Antimicrobial Chemotherapy*, **3**, 83–89.
- Hyndiuk, R. A., Hull, D. S., Schultz, R. O., Chin, G. N., Laibson, P. R., and Krachmer, J. H. (1975). Adenine arabinoside in idoxuridine unresponsive and intolerant herpetic keratitis. *American Journal of Ophthalmology*, **79**, 655–658.
- Jones, B. R., and Al-Hussaini, M. K. (1963). Therapeutic considerations in ocular vaccinia. *Transactions of the Ophthalmological Societies of the United Kingdom*, **83**, 613–631.
- Jones, B. R., and Patterson, A. (1967). Anti-inflammatory drugs and infectious disease: effects of anti-inflammatory drugs on virus replication in the cornea. In *Inflammation*, pp. 143–158. Excerpta Medica: Amsterdam.
- Kaufman, H. E., Nesburn, A. B., and Maloney, E. D. (1962). IDU therapy of herpes simplex. *Archives of Ophthalmology*, **67**, 583–591.
- Kaufman, H. E. (1978). Herpetic keratitis. *Investigative Ophthalmology*, **17**, 941–957.
- McGill, J., Holt-Wilson, A. D., McKinnon, J. R., Williams, H. P., and Jones, B. R. (1974). Some aspects of the clinical use of trifluorothymidine in the treatment of herpetic ulceration of the cornea. *Transactions of the Ophthalmological Societies of the United Kingdom*, **94**, 342–352.
- Pavan-Langston, D., and Dohman, C. H. (1972). A double blind clinical study of adenine arabinoside therapy of viral keratoconjunctivitis. *American Journal of Ophthalmology*, **74**, 81–88.
- Pavan-Langston, D., Campbell, R., and Lass, J. (1978). Acyclic antimetabolite therapy of experimental herpes simplex keratitis. *American Journal of Ophthalmology*, **86**, 618–623.
- Schaeffer, H. J., Beauchamp, L., de Miranda, P., Elion, G. B., Bauer, D. J., and Collins, P. (1978). 9-(2-Hydroxyethoxy methyl)guanine activity against viruses of the herpes group. *Nature*, **272**, 583–585.
- Shiota, H. (1979). Antiviral chemotherapy against herpetic keratitis. In XXIII Concilium Ophthalmologicum Kyoto 1978. Excerpta Medica: Amsterdam. In press.
- Wellings, P. C., Awdry, P. N., Bors, F. H., Jones, B. R., Brown, D. C., and Kaufman, H. E. (1972). Clinical evaluation of trifluorothymidine in the treatment of herpes simplex corneal ulcers. *American Journal of Ophthalmology*, **73**, 932–942.