

Pyrimidine analogues in the treatment of experimental herpes infections

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THE investigation of a new anti-microbial drug usually progresses from the test-tube to successful trials in animals followed by a cautious clinical appraisal, and the study of 5-iodo-2-deoxyuridine (IUdR), following this course, led to an accepted treatment for herpetic keratitis. In the treatment of the brain infections the order has been reversed and clinical use of pyrimidine analogues has preceded the accumulation of any satisfactory evidence from animal experiments. I propose to assess the evidence available in the literature and from our own work.

Two general matters should be clarified before turning to details. First the obvious, but frequently neglected point, that to be useful a drug must affect the course of an established infection and experiments in which the drug is given before, or at the same time, as the infecting virus, have little relevance. Second, the question of the 'blood-brain barrier' and whether it is permeable to pyrimidine analogues. Clarkson, Oppelt & Byvoet (1967) studied the passage of IUdR from plasma to cerebrospinal fluid of dogs, but they ignored the tissue of the cerebrum in which the virus replicates. Even if given the knowledge that the drug does penetrate herpes infected brain, it would still be necessary to find out whether it influences the course of the disease. At the present, experimental results have to be interpreted without any direct information on the penetration of brain by iodo-deoxyuridine or cytosine-araboside.

5-Iodo-2'-deoxyuridine (IUdR)

The first reported animal experiment (Kaufman, 1962) demonstrated that IUdR was truly therapeutic for keratitis, i.e. it promoted healing of the cornea when applied 2 or 5 days after infection and many subsequent experiments have confirmed his results. In the course of such experiments Jawetz *et al.* (1965) made an observation which may have application to all treatment with IUdR. These workers found that the virus content of a rabbit cornea after 4 days' treatment was between 0.1 and 0.001 of that of control corneas, but when treatment stopped after 7 days the virus content rose above that of the controls on that day, finally falling to an undetectable amount 1 or 2 days later.

In a study of IUdR therapy of dermal herpes in guinea-pigs, Tomlinson & MacCallum (1968) treated the animals, beginning 48 hr after infection, with 120 mg/kg, subcutaneously for 5 days. Of twelve lesions assayed at the end of treatment two contained no virus and the content of the others ranged from 10 to 10³ TCD. This finding, like that of Jawetz *et al.* (1965), suggests that treatment with IUdR is not alone sufficient to eliminate herpes virus from a lesion, and host defences must also operate.

Kaufman (1963) reported briefly, without technical details, that intrathecal IUdR did not prevent rabbits from developing encephalitis when the cornea was infected with a neurotropic strain of herpes simplex virus. He also said that intraperitoneal IUdR did not reduce the death-rate of mice infected intracerebrally. As the dose of drug was not stated, the significance of that work cannot be assessed.

In an experiment with six guinea-pigs infected intracerebrally with 10^{5.6} PFU of virus, Tokumaru (1967) had four survivors following treatment with IUdR 100 mg/kg for 5 days whereas all control animals died.

In our own experiments (Tomlinson & MacCallum, 1970) guinea-pigs were infected intracerebrally with 10, 10³ or 10⁴ TCD of herpes simplex virus, which had been maintained by passage in mouse brain. Time of death of the control animals varied little with the infecting dose. Groups of four pigs were treated by injecting a 0.5% solution of IUdR intraperitoneally to give each day: either a single dose of 100 IUdR mg/kg; or three doses of 33 mg/kg; or three doses of 100 mg/kg. All pigs were treated for 5 days, different groups starting 1, 2 or 4 days after infection. The number of animals was too small to demonstrate a difference between the treatment schedules, so the results (Table 1) are grouped by day of starting treatment. All untreated animals died on or before the seventh day. Of thirty-two treated pigs six survived and seventeen lived longer than the controls, but the six survivors were each on a different treatment schedule.

These results show that IUdR was therapeutic even when given on day 4 to pigs expected to die on days 5-7 and we may deduce that the drug penetrated

TABLE 1. Guinea-pigs infected intracerebrally and treated with intraperitoneal IUdR

No. of animals	Treated* on day	Days to death		Survivors
		Range	Mean	
8	1	8-11	9.1	1
12†	2	7-12	9.1	2
12†	4	5-12	7.0	3
28	Untreated	5-7	5.8	0

*Dosage: see text.

†Three groups of four animals on different treatment schedules.

the blood-brain barrier. The effect was erratic and in no dosage-schedule/start-of-treatment group did more than one pig out of four survive.

1-β-D-arabinofuranosylcytosine (cytarabine)

A solution of cytarabine was shown by Underwood (1962) to be effective against herpes virus in the rabbit eye. Kaufman & Moloney (1963) extended the observations to show that cytarabine was as effective as IUdR at the same weight/volume concentrate but slightly less effective at equal molarities.

Evidence for the activity of cytarabine on herpes virus in the brain is, however, scanty. Renis, Hollowell & Underwood (1967) infected mice intracerebrally with 40 LD₅₀ of herpes simplex virus and 3 hr later injected 0.9 mg of cytarabine into the brain. This treatment either prolonged life or increased the number of survivors (it is not clear from the text which occurred) and either way the effect was prophylactic rather than therapeutic.

Hall (1968) infected rabbit eyes with a strain of herpes which produced neurological symptoms in 11 days and death from encephalitis in 21 days. Animals treated with an unspecified dose of cytarabine, which was said to be too high and to be toxic, showed no 'evidence of herpes simplex'.

We have tried to demonstrate an effect on herpes encephalitis in guinea-pigs. Some animals were infected intracerebrally, and, in an attempt to produce a less fulminating encephalitis, some were infected on the skin of the back or of the orbital region whence the virus travelled naturally to the brain. From the available evidence it seemed that 5 mg/kg/day for 5 days was the maximum dose likely to be given to a human patient, so this amount and 10 mg/kg were the doses used—the latter in the hope of demonstrating an antiviral effect even at the cost of some toxicity. The cytarabine was given intraperitoneally or, in one experiment, by intracardiac injection. From fifty-two animals, in twelve groups with different combinations of route of infection and treatment schedule, there were no survivors and in no group was the mean time to death significantly longer than in the appropriate control group. After

5 days treatment with 10 mg/kg by intracardiac injection the brains of six animals had a mean virus content of 10⁶ TCD—no less than that of control animals. The dose cannot be increased as even 10 mg/kg was toxic.

We conclude that cytarabine is either rapidly deaminated in the guinea-pig as in the mouse, or it is without effect on herpes encephalitis.

9-β-D-arabinofuranosyl-adenine (adenine arabinoside)

This compound was first synthesized as a potential anticancer drug and was then shown by Privat de Gariilhe & de Rudder (1964) to inhibit the multiplication of herpes and vaccinia viruses in tissue cultures. Further studies *in vitro* by Miller *et al.* (1968) showed that adenine arabinoside was at equal concentrations about as potent as IUdR in a plaque-reduction test.

Adenine arabinoside is only slightly soluble in water—0.05% at 25°C and 0.18% at 37°C, as compared with the 0.5% concentration of IUdR which can be achieved at pH 8.5-9.0. In most of the animal experiments discussed below, the drug was administered as a suspension of fine particles and it is not clear what proportion did dissolve in body fluids and what remained undissolved and ineffective. Using such suspensions the acute oral toxicity for mice was found to be greater than 7950 mg/kg and the LD₅₀ for a single intraperitoneal injection 4700 mg/kg, death being due to peritonitis (Kurtz *et al.*, 1968). When the drug was included in the diet for 28 days mice with a daily intake of 465 mg or more per kg suffered liver damage and irreversible weight loss, but at lower doses weight was regained when the drug was withdrawn. It would appear, therefore, that the drug is much less toxic than IUdR or cytarabine.

To test the *in vivo* activity of adenine arabinoside, Sidwell *et al.* (1968) used hamsters infected on the cornea with a strain of virus which produced signs of encephalitis in 3-4 days and death in 9 days. An ointment containing 33%, or greater, of adenine arabinoside significantly increased the number of survivors, but, because the drug was first applied only 4 hr after infection, its use was more prophylactic than therapeutic.

In the treatment of mice infected intracerebrally with 32 LD₅₀ of herpes virus adenine arabinoside was remarkably effective. Starting 24 hr after infection, seven daily intraperitoneal doses of 1000 mg/kg saved 50% of the mice; with twice daily oral doses of 1250 mg/kg for 4 days 60% survived. Treatment with a total of 2000-4000 mg/kg in several subcutaneous injections during the course of a single day also saved 50-70% of the mice (Sloan *et al.*, 1968). They also showed that hypoxanthine arabinoside, to which adenine arabinoside is degraded in the

body, was as active as the drug. Few results were presented for treatment initiated more than 24 hr after infection.

In the doses given adenine arabinoside was effective, but the injection of large amounts of solid material intraperitoneally, or even subcutaneously, is not suitable for human patients, and given in true solution adenine arabinoside would present an even greater fluid load than does IUdR therapy. To find a suitable presentation of this drug appears to be a major problem.

Our own experiments with adenine arabinoside are only beginning and I have nothing to add.

For several reasons the performance of a drug in an animal model is not necessarily a guide to its effectiveness in human disease. Herpes simplex virus is not a natural parasite of mice, guinea-pigs or rabbits and it usually produces more serious disease in them than in man. The metabolism and excretion of the drugs may be different in animals and man. Lastly, in a serious disease like encephalitis supportive therapy and good nursing have an important role, but these cannot be given to animals.

The conclusion is that from the published evidence of animal models the case for chemotherapy of herpes encephalitis is, at best, 'not proven'.

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