Pharmacokinetics of E-5-(2-Bromovinyl)-2'-Deoxyuridine in Mice

E. de Clercq, J. Descamps, P. de Somer, P. J. Barr, A. S. Jones, and R. T. Walker

Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium and Department of Chemistry, University of Birmingham, Birmingham B15 2TT, Great Britain

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The pharmacokinetics of the newly developed anti-herpes agent, E-5-(2-bromovinyl)-2'-deoxyuridine, was compared with that of the standard anti-herpes drug 5-iodo-2'-deoxyuridine. Both compounds were administered to mice at 100 mg/kg by either the intraperitoneal, subcutaneous, or oral route. The active blood drug levels achieved by E-5-(2-bromovinyl)-2'-deoxyuridine were considerably higher than those attained by 5-iodo-2'-deoxyuridine (serum peak concentrations: 40 to 100 and 4 to 10 µg/ml, respectively). Active blood drug levels could still be found 320 min after oral administration of E-5-(2-bromovinyl)-2'-deoxyuridine.

Recently we have described two new 5-substituted 2'-deoxyuridines (dUrd), E-5-(2-bromovinyl)-dUrd and E-5-(2-iodovinyl)-dUrd, which proved highly effective and selective in inhibiting replication of herpes simplex virus type 1 (HSV-1), in both cell culture and mice (2). In primary rabbit kidney (PRK) and human skin fibroblast (HSF) cell cultures, E-5-(2-bromovinyl)-dUrd inhibited the cytopathic effect of HSV-1 (KOS strain) and various other HSV-1 strains at a concentration of 0.004 to 0.02 µg/ml. The minimal inhibitory concentration (MIC) of 5-iodo-dUrd (IDU, idoxuridine), the drug that has been most regularly used in the treatment of (localized) herpesvirus infections (1), for HSV-1 was on the average 20 times higher than that of E-5-(2-bromovinyl)-dUrd (2).

In view of the extremely low concentration at which E-5-(2-bromovinyl)-dUrd inhibited HSV-1 (KOS) replication in cell culture, it seemed possible to monitor drug levels in mice that were given E-5-(2-bromovinyl)-dUrd by measuring the antiviral activity of the serum and tissue homogenate samples of these animals.

To examine the pharmacokinetics (absorption, clearance, and tissue distribution) of E-5-(2-bromovinyl)-dUrd and 5-iodo-dUrd in mice, single doses of 1 mg of either compound (dissolved in 0.2 ml of Dulbecco phosphate-buffered saline) were administered intraperitoneally, subcutaneously, or orally to 20-day-old NMRI mice weighing approximately 10 to 12 g (similar small-size mice have been used previously in our in vivo antiviral studies [2]). The mice were sacrificed at 20, 40, 80, 160, or 320 min after drug administration, at which time blood and brain, lung, and liver tissue samples were removed. The tissues were homogenized in Potter-Elvehjem tubes after addition of 9 volumes of Eagle minimal essential medium, and the serum samples and 10% tissue homogenates were assayed for anti-herpesvirus activity by use of a cytopathic effect inhibition assay in HSF microtiter trays. The first dilution assayed for the serum and homogenate samples was uniformly 1:10. HSV-1 (KOS) served as the challenge virus. Concentrations of E-5-(2-bromovinyl)-dUrd and 5-iodo-dUrd in the serum and brain, lung, and liver tissue samples were calculated from the MICs of the compounds when assayed in parallel with the serum and tissue samples. These MICs, which corresponded to the doses of compound that were required to reduce viral cytopathic effect by 50%, were 0.01 to 0.02 and 0.2 µg/ml for E-5-(2-bromovinyl)-dUrd and 5-iodo-dUrd, respectively. Thus, for E-5-(2-bromovinyl)-dUrd, the minimal detectable concentrations could be estimated at 0.1 to 0.2 µg/ml of serum and 1 to 2 µg/g of tissue. For 5-iodo-dUrd these values were 2 and 20 µg, respectively.

For 5-iodo-dUrd, the drug levels achieved in brain, lung, and liver tissue fell below the minimal detectable levels (Table 1). Low levels of antiviral activity, amounting to approximately 2 to 10 µg of 5-iodo-dUrd per ml, were detected in blood at 20 to 40 min after either intraperitoneal, subcutaneous, or oral administration of 5-iodo-dUrd (Table 1). These results are consistent with previously reported data for suckling mice treated intraperitoneally with 5-iodo-dUrd at 200 mg/kg, in that blood drug levels could only be detected within 1 h after drug administration and no effective drug levels were achieved in brain tissue (3).

For E-5-(2-bromovinyl)-dUrd, the drug levels attained in the serum were considerably higher
Table 1. Blood and tissue distribution of E-5-(2-bromovinyl)-dUrd and 5-iodo-dUrd in mice

| Drug                  | Compound concn* | Time (min) | Intraperitoneal | Subcutaneous: serum | Oral: serum |
|-----------------------|-----------------|------------|-----------------|---------------------|-------------|
|                       | Serum (µg/ml)   | Brain (µg/g) | Lung (µg/g) | Liver (µg/g)     | serum (µg/ml) |
| E-5-(2-bromovinyl)-dUrd | 0   | <0.1 | <1 | <1 | <20 | 0.1 | 0.2 |
|                       | 20  | 30   | 4.5 | 20 | 60 | 40-100 | 40-60 |
|                       | 40  | 20-30 | 1.5 | 6 | 60 | 20-40 | 20-40 |
|                       | 80  | 2-3   | 1 | 6 | 60 | 2-10 | 10-20 |
|                       | 160 | 0.2-0.3 | <1 | 2 | 30 | 0.3-0.6 | 2-4 |
|                       | 320 | <0.1 | <1 | <1 | 30 | <0.1 | 1.0 |
| 5-iodo-dUrd           | 0   | <2   | — | — | — | <2 | <2 |
|                       | 20  | 4    | — | — | — | 10 | 2-4 |
|                       | 40  | 2    | — | — | — | 2 | 2-3 |
|                       | 80  | <2   | — | — | — | <2 | <2 |
|                       | 160 | <2   | — | — | — | <2 | <2 |
|                       | 320 | <2   | — | — | — | <2 | <2 |

* All data represent average values for three separate experiments. In each experiment drug levels were determined for the pooled serum and tissue samples from three mice per group. Where appropriate, range of values is indicated.

For brain and lung tissue the minimal detectable level of 5-iodo-dUrd was 20 µg/g; for liver tissue the minimal detectable level of 5-iodo-dUrd was 200 µg/g, since liver tissue homogenate exhibited an anti-herpes activity on its own when assayed at a dilution lower than 1:100.

The pharmacokinetics of E-5-(2-bromovinyl)-dUrd appears to be quite similar to that reported previously for 9-(2-hydroxyethoxymethyl)-guanine (acycloguanosine) (4). Both compounds are readily absorbed upon oral administration, and, when given orally, they are removed much more slowly from the blood than after subcutaneous administration. For both E-5-(2-bromovinyl)-dUrd and acycloguanosine, the drug levels achieved in the liver are significantly higher and those in the brain are significantly lower than the drug concentrations found in the blood.

than those obtained with 5-iodo-dUrd, regardless of the route by which the drugs were administered (Table 1). Upon oral administration of E-5-(2-bromovinyl)-dUrd, active drug concentrations persisted in the blood for at least 320 min. With E-5-(2-bromovinyl)-dUrd, effective drug levels were also achieved in brain, lung, and liver tissue, although the drug levels attained in the brain were about 10-fold lower than the serum drug levels (Table 1). Thus, E-5-(2-bromovinyl)-dUrd may experience some hindrance in crossing the blood-brain barrier.

The following experiments indicated that the antiviral activity measured in the serum (and tissues) of mice that were given 5-iodo-dUrd or E-5-(2-bromovinyl)-dUrd truly reflected active drug concentrations.

(i) The antiviral activity could be reversed completely by 2'-deoxythymidine; for example, where a serum sample [taken 20 min after subcutaneous administration of E-5-(2-bromovinyl)-dUrd] was effective in suppressing the cytopathic effect of HSV-1 (KOS) in HSF cell cultures up to a dilution of 1:2,000, no inhibition of cytopathic effect, not even at 1:10 serum dilution, was noted after 2'-deoxythymidine (100 µg/ml) had been added to the serum dilutions.

(ii) If serum activity was assessed with HSV-2 (G strain) as the challenge virus, no antiviral activity could be detected. This is not surprising in view of the relatively high MIC of E-5-(2-bromovinyl)-dUrd for HSV-2 (G). In HSF cells this MIC is 4 to 10 µg/ml and thus is 400 to 500 times higher than for HSV-1 (KOS), which implies that with HSV-2 (G) as the challenge virus one would be unable to detect E-5-(2-bromovinyl)-dUrd in serum unless its concentration was higher than 40 to 100 µg/ml.

(iii) Similarly, no activity was observed in serum samples from mice treated with E-5-(2-bromovinyl)-dUrd if vaccinia virus was chosen as the challenge virus. For vaccinia the MIC of E-5-(2-bromovinyl)-dUrd in HSF cells is 10 µg/ml, which corresponds to a minimal detectable level of 100 µg of drug per ml of serum.

(iv) Finally, with vesicular stomatitis virus as the challenge virus no antiviral activity could be demonstrated in the serum of mice treated with either E-5-(2-bromovinyl)-dUrd or 5-iodo-dUrd; in cell culture neither E-5-(2-bromovinyl)-dUrd nor 5-iodo-dUrd inhibits vesicular stomatitis virus replication, even at concentrations up to 200 µg/ml.
dUrd, combined with its extreme potency in inhibiting the replication of HSV-1, may explain why E-5-(2-bromovinyl)-dUrd, when administered intraperitoneally to athymic nude mice infected intracutaneously with HSV-1 (KOS), effectively suppressed the development of herpetic skin lesions and associated mortality (2). The fact that active drug levels could be sustained for several hours after oral administration of E-5-(2-bromovinyl)-dUrd suggests that oral administration may be the route of choice for the application of E-5-(2-bromovinyl)-dUrd in the systemic treatment of HSV infections.

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