Pharmacology, Tolerance, and Antiviral Activity of Vidarabine Monophosphate in Humans

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Vidarabine (adenine arabinoside) is a purine nucleoside useful in humans for therapy of herpes simplex virus encephalitis and herpes zoster virus infections in immunocompromised patients. However, the potential usefulness of vidarabine is limited by its poor solubility, which requires continuous infusion in relatively large volumes of intravenous fluid. Vidarabine 5'-monophosphate is highly soluble and has the advantage that it can be administered intermittently intramuscularly or intravenously. In a clinical, pharmacokinetic study, plasma levels and urinary excretion of vidarabine 5'-monophosphate were determined after intravenous and intramuscular administration in 29 immunosuppressed patients with herpes simplex or zoster virus infections at dosages of 15 to 30 mg/kg per day administered for 5 days. As determined by high-pressure liquid chromatography, vidarabine 5'-monophosphate was metabolized in a fashion comparable to the metabolism of vidarabine and its major metabolite in plasma was arabinosyl hypoxanthine. After administration, 40 to 50% of the vidarabine 5'-monophosphate was recovered from the urine as arabinosyl hypoxanthine, and 3 to 4% was recovered as vidarabine. Determinations of areas under the curve for arabinosyl hypoxanthine were not statistically different by dosage for intramuscular or intravenous routes of administration. At all dosages studied, viral clearance appeared to occur with therapy. The advantage of increased solubility will lead to controlled clinical investigations in which vidarabine 5'-monophosphate is administered by intramuscular or intravenous routes against targeted human herpesvirus infections.

The development of vidarabine (vira-A) as an antiviral agent has stimulated the introduction of promising compounds for clinical evaluation. As new therapeutic agents are developed, pharmacokinetic and tolerance studies must precede drug efficacy evaluations. Ideally, these studies can provide information regarding the optimal route of drug administration, plasma levels at defined dosages, half-life, metabolism, clearance, and degree of penetration into cerebrospinal fluid. In addition, preliminary data regarding viral clearance and toxicity are obtained.

An orderly approach to the study of antiviral agents has been followed in the development of vira-A for clinical evaluation (6, 9). vira-A is a purine nucleoside which, when given intravenously, is deaminated to arabinosyl hypoxanthine (ara-Hx), and this latter compound is the principle metabolite in plasma and urine (2, 3). Because of both poor solubility and intramuscular absorption, vira-A requires large fluid volumes for intravenous administration and therefore must be given over prolonged periods (8 to 12 h). In herpes simplex virus encephalitis, obligate fluid load may prejudice patient care.

The phosphorylated ester of vira-A, vidarabine 5'-monophosphate (vira-MP), is water soluble and consequently may be a potentially more useful antiviral agent. The in vitro inhibitory activity of this drug against the herpesviruses is similar to that of vira-A (8). The half-life after single-dose administration of radioactively labeled vira-MP is 3.5 to 6.2 h and this drug is metabolized to ara-Hx (A. J. Glazko, personal communication). Because tolerance and detailed pharmacokinetics were not ascertained in this early study, we performed a dose-escalating, pharmacokinetic, tolerance, uncontrolled virological study of vira-MP in humans after administration of this drug by intramuscular and intravenous routes.

MATERIALS AND METHODS

From September 1977 through February 1979, we studied patients immunosuppressed by underlying diseases or drug(s) or both who had active, culture-proven mucocutaneous herpes simplex or zoster virus infections. These patients were not eligible for ongoing National Institute of Allergy and Infectious Diseases controlled antiviral investigations. After we obtained

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informed consent, patients received vira-MP either intravenously over 30 min or intramuscularly at dosages of 15, 20, 25, or 30 mg/kg per day divided four times daily for 5 days. The drug was kindly supplied by Warner-Lambert/Parke, Davis & Co., Ann Arbor, Mich., as 200 mg of vira-MP per ml in 5-ml vials.

**Specimen collection.** Plasma specimens were obtained before therapy and at 0.5, 1, 3, 6, and 12 h after drug administration on days 1, 3, and 5. Additional specimens were obtained at 24 and 48 h after therapy. To prevent ex vivo deamination of vira-MP, 20 μg of the adenosine deaminase inhibitor 2-deoxycoformycin was added to each 1 ml of blood. Collected specimens were centrifuged immediately, and the plasma was frozen. Urine was collected over four consecutive 6-h intervals on days 1, 3, and 5. In addition, two consecutive 24-h urine collections were obtained after completion of therapy. The total volume of each collection was recorded, and 40-ml portions were frozen pending assays for drug and metabolites.

**Determination of vira-MP and metabolites.** Plasma and urine levels of vira-MP, vira-A, and ara-Hx were assayed in the laboratories of Warner-Lambert/Parke, Davis & Co. (A. Kinkel) by high-pressure liquid chromatography, using an Aminex A-28 anion exchange column (2 mm [inside diameter] by 15 cm) and a 0.15 to 0.2 M acetate–0.25 M borate buffer (pH 8.0 to 8.35). The pH and buffer ionic strength were varied to remove interfering peaks. The assay precision, as determined by coefficient of variation averages, was 3.0% for urine and 3.7% for plasma specimens, as previously reported (1).

The areas under the plasma level time curves (AUC) for 0 to 6 h on days 1, 3, and 5 for ara-Hx were calculated by using the trapezoidal rule. Mean steady-state plasma levels were determined from the mean steady-state AUC obtained from the data on days 3 and 5. The plasma half-life of ara-Hx was determined by linear regression analysis of the logarithm of the ara-Hx concentration versus time after administration. For statistical assessment of AUC determinations, we used a Student’s t test comparison of means.

**Clinical evaluation.** Patients were examined daily to determine progression of disease. For zoster, pain, presence or absence of erythema, number of new vesicles, and extent of pustulation and scabbing were monitored daily. For herpes simplex virus infections, patients were examined daily for progression of old lesions and presence of new ones.

**Virology.** For patients with herpes zoster virus infections, the most recently formed vesicles were aspirated daily at bedside and inoculated into triplicate tubes of discontinuous human foreskin fibroblasts. Specimens were transported immediately to the virology laboratory and examined at least three times each for typical cytopathic effects. Cultures were observed for a minimum of 4 weeks before being discarded as negative.

For patients with mucocutaneous herpes simplex virus infections, the base of the most recent lesion was swabbed at 3-day intervals; each swab was bathed in 2 ml of standard tissue culture medium and transported promptly to the laboratory, and serial 10-fold dilutions of the lesion wash were inoculated into a line of continuous BSC-1 cells. Cells were observed three times a week for typical cytopathic effects. Viral typing was performed by immunofluorescence, as previously reported (5).

**Toxicity monitoring.** Physicians examined patients daily for evidence of adverse reactions to the drug, including pain at the injection site, nausea, vomiting, diarrheas, and central nervous system dysfunction. Serial blood and urine specimens were obtained at the time of enrollment, on days 3 and 7, and then weekly for 1 month to monitor for laboratory toxicity. Complete blood counts with differential, platelets, reticuloocyte count, and assessments of liver and renal function were performed. In addition to the previously documented evaluations, weekly weights were determined. Creatinine clearance and estimated renal plasma flow as determined by radionucleotide scanning were recorded for all patients before and after therapy.

**RESULTS**

**Study population.** A total of 29 patients entered the study, and pharmacokinetic, drug tolerance, and viral clearance data were obtained for all; 13 patients were renal transplant recipients, and the remaining 16 were immunosuppressed by drugs or underlying diseases. In the latter group, seven had lymphoproliferative malignancies, six had solid tumors, and the remaining three were receiving high doses of corticosteroids for other conditions. A total of 21 patients had varicella-zoster virus infections (8 disseminated and 13 localized), and 8 patients had progressive mucocutaneous herpes simplex virus infections.

**Pharmacokinetic assessment.** The major metabolite of vira-MP in plasma was the hypoxanthine derivative ara-Hx; vira-MP was not detected in plasma or urine specimens. In Fig. 1, plasma levels of ara-Hx are plotted against time for all doses studied. The half-life of ara-Hx ranged from 2.3 to 5.4 h (mean ± standard error of the mean, 3.98 ± 1.9 h) after intravenous administration of vira-MP. Peak plasma levels were achieved 0.5 and 3 h after intravenous and intramuscular administrations, respectively. When found in plasma, vira-A was present at low concentrations, averaging 1.0 μg/ml regardless of dose. There was no statistically significant difference between mean intravenous and intramuscular plasma levels for either vira-A or ara-Hx at any dose studied. In addition, no statistical difference existed for grand mean AUC determinations for route of administration at each dosage. After intravenous administration 88 of 234 specimens (35%) had detectable vira-A levels, in contrast to 112 of 330 (34%) after intramuscular administration. Table 1 shows mean peak plasma levels for vira-A and ara-Hx, as well as AUC determinations at all doses stud-
There was a rank order relationship between dose and achievable plasma level up to 25 mg/kg per day; that is, as the dose of vira-MP increased, so did the resultant plasma level of ara-Hx. Moreover, AUC determinations by dosage increased at statistically significant levels at dosages through 25 mg/kg per day ($P \leq 0.03$, Student's $t$ test) but not when the 25- and 30-mg/kg per day dosages were compared.

Ara-Hx was the major urinary metabolite. Table 2 shows the mean total daily urinary outputs of ara-Hx after intramuscular and intravenous administrations. Recovery of ara-Hx in the urine was somewhat greater after intravenous dosing than when the drug was given intramuscularly, although the difference was not significant.

**Cerebrospinal fluid levels.** Two patients, who received vira-MP intramuscularly at dosages of 15 and 25 mg/kg per day, had cerebrospinal fluid obtained 1 and 2 h, respectively, before receiving medication on day 5 for drug determination. The ara-Hx concentration in this fluid was 2.7 µg/ml, compared with a mean

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**Table 1. Mean peak plasma and AUC determinations**

<table>
<thead>
<tr>
<th>Dose (mg/kg per day)</th>
<th>Route of drug administration</th>
<th>No. of patients studied</th>
<th>Mean peak plasma level (µg/ml)</th>
<th>ara-Hx AUC (µg/ml)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>vira-A</td>
<td>ara-Hx</td>
</tr>
<tr>
<td>15</td>
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<td>2</td>
<td>ND</td>
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<td>1.2</td>
<td>6.3</td>
</tr>
<tr>
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<td>5</td>
<td>1.3</td>
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</tr>
<tr>
<td></td>
<td>i.m.</td>
<td></td>
<td>1.3</td>
<td>8.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td>9.3</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>0.9</td>
<td>9.3</td>
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</tbody>
</table>

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**Table 2. Mean total daily urinary recovery of ara-Hx**

<table>
<thead>
<tr>
<th>Dose (mg/kg per day)</th>
<th>Route</th>
<th>No. of patients studied</th>
<th>Urinary recovery (% of dose)</th>
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<tr>
<td></td>
<td>i.m.</td>
<td>5</td>
<td>32.8</td>
</tr>
</tbody>
</table>

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\[a\] i.v., Intravenous; i.m., intramuscular.

\[b\] All intradose comparisons were not significant by the Student $t$ test.

\[c\] ND, Not detected.
plasma level of 3.25 μg/ml. Two additional patients who received the drug intravenously at dosages of 15 and 30 mg/kg per day had ara-Hx cerebrospinal fluid levels of 0.4 and 1.0 μg/ml 3 days after the last dose of medication.

Plasma levels versus renal function. In renal transplant recipients, both mean plasma levels and AUC determinations at all doses did not differ significantly from levels obtained in study patients with other underlying conditions. Renal function remained stable during the treatment period in both groups, with the exception of three individuals. Two of these three were renal transplant recipients who had increasing serum creatinine levels and both decreasing creatinine clearance and estimated renal plasma flow evaluations during the study period, which were attributed to graft rejection. Both received vira-MP intramuscularly at 30 mg/kg per day, resulting in mean peak plasma ara-Hx levels of 14.4 and 14.8 μg/ml. The third patient received 25 mg/kg per day intravenously and had a mean peak plasma level of 19.2 μg/ml. Both peak plasma levels and AUC determinations exceeded by twofold or more mean levels achieved for the dosage group. All three patients had creatinine clearances equal to or less than 50 ml/min per 1.73 m² and estimated renal plasma flows of 150 to 240 ml/min. In contrast, the remaining 26 patients had creatinine clearances in excess of 90 ml/min per 1.73 m². Thus, patients with seriously impaired renal function had higher plasma and AUC levels of ara-Hx.

Clinical and virological assessment. At all dosages studied, the 21 patients with zoster cleared the virus from lesions rapidly after enrollment into the study regardless of the dosage utilized. As Fig. 2 shows, more than 50% of these patients became virus-negative and remained so after 2 days of medication. Time to cessation of new vesicle formation and total postulation paralleled viral clearance. The duration of disease before therapy was 6.3 ± 1.2 days (mean ± standard error of the mean).

Figure 3 shows the quantitative reduction in herpes simplex virus excretion from lesion sites. At all doses studied, a reduction in viral excretion occurred with drug administration; this was particularly so at dosages of 25 and 30 mg/kg per day. As observed in prior studies (1), viral excretion returned in four of eight renal transplant patients approximately 10 days after therapy. Presumably, this is a reflection of persistently impaired host defense.

Cutaneous healing was paralleled in all cases by clinical improvement (loss of pain, progressive scabbing, and complete cutaneous re-epithelialization). Progression of viral disease, dissemination, or new lesion formation did not occur after 2 days of drug therapy. No patient died from the viral infection during the study or in the follow-up period.

Toxicity. (i) Adverse clinical experience. The major adverse experience associated with vira-MP occurred after intramuscular drug administration. Of 19 patients who received the drug by this route, 6 (32%) complained of mild to moderate pain associated with the injection. These six patients received dosages of 25 or 30 mg/kg per day. In no patient did the pain require drug discontinuation. Of 29 patients, 3 (10%) developed central nervous system abnormalities (tremors and jitters in all and myoclonus in one) associated with drug administration, and these patients had mean peak plasma ara-Hx concentrations of 14.4 to 19.2 μg/ml, well above the mean values for patients receiving the same dosages. All had abnormal renal function throughout the study period; two were renal transplant recipients and received the drug intramuscularly, whereas the third received the drug intravenously.

On weekly follow-up, no evidence of late toxicity appeared. Two patients died after the study, one from massive pulmonary emboli unrelated to drug administration and the other from squamous cell carcinoma of the lung. In both patients ara-Hx levels were the same as the levels in patients treated with equivalent dosages.

(ii) Laboratory toxicity. Two patients had more than a 20% reduction from base line of hematocrit. Gastrointestinal bleeding and hemolysis were not found in either patient. One patient became leukopenic during therapy, which was attributed to recent cytoxan therapy. Elevations of serum glutamic oxaloacetic transaminase (SGOT) levels occurred in seven patients (24%) who received vira-MP. In five, the elevation was twice base line, whereas in two there was a fivefold SGOT elevation during therapy. Five of the seven patients received drug
intradurally, and four had elevated creatine phosphokinase levels (MM band). All SGOT abnormalities returned to baseline within 2 weeks after completion of the study.

DISCUSSION

Vira-MP appears to offer a significant advantage over vira-A for therapy of herpesvirus infections in humans. Continuous infusion of the poorly soluble parent compound, vira-A, is both complicated in critically ill patients and requires large volumes of intravenous fluid for adequate drug delivery. Vira-MP may provide an alternative drug for therapy of life-threatening herpesvirus infections in humans if efficacy can be confirmed in controlled investigations. The present study demonstrates that the pharmacokinetics and metabolism of vira-MP are similar to those of the parent compound. Specifically, the principal metabolite detectable in plasma and urine after drug administration is ara-Hx, with small amounts of vira-A present, as demonstrated by high-pressure liquid chromatography. When the total numbers of specimens with detectable vira-A after intravenous and intramuscular administrations are compared, the frequency distribution does not favor one route over the other. Similarly, when AUC determinations of plasma levels of ara-Hx are compared for both routes, there is no significant difference.

Thus, intramuscular administration of an antiviral agent is now possible.

Increasing the dosage of the drug results in a step-wise increase in plasma ara-Hx levels and AUC. This rank order relationship holds true through the 25-mg/kg per day dose level. Once a dosage of 25 mg/kg per day is exceeded, there appears to be no significant increase in the plasma level of the drug or the AUC. At each dose studied, no significant difference existed between intravenous and intramuscular drug concentrations for either vira-A or ara-Hx plasma levels. Based upon AUC determinations and within the given dose ranges that were studied, a dosage which seems reasonable for further investigation without increasing the risk for potential toxicity is 25 mg/kg per day. Parenthetically, decreased excretion of herpes simplex and varicella-zoster viruses in infected patients appeared to occur at this dosage. Exceeding this dosage may result in toxicity, as observed in animal model studies with simians, in which bone marrow and neurological toxicities occurred at doses of more than 35 mg/kg per day (R. A. Buchanan, personal communication).

After parenteral administration of vira-MP, drug excretion is similar to the excretion observed after administration of the parent compound, vira-A. For both drugs, the principal route of clearance is via the kidneys, and both vira-A and ara-Hx are recovered in the urine.
Nearly 45% of the administered drug is recovered as ara-Hx, and approximately 5% is recovered as vira-A. Excretion after intravenous dosing is somewhat greater for all dosages studied. Not all drug administered is quantitatively recovered in the urine. This discrepancy varies between 40 and 60% according to the individual patient. From prior studies with vira-A, it is apparent that the drug can be further metabolized from ara-Hx to hypoxanthine, which is excreted in the urine and cannot be quantitated by this assay (2). Preliminary unpublished data suggest that the same phenomenon occurs after the administration of vira-MP (Buchanan, personal communication). The inability to recover all of the drug places limitations on pharmacokinetic interpretations.

At all dosages of vira-MP studied, viral clearance and resolution of clinical disease appeared to occur with therapy; however, efficacy cannot be determined by a study of this design. As noted previously with vira-A therapy, administration of vira-MP does not result in plasma levels of vira-A or ara-Hx which inhibit viral replication in vitro (8). The intracellular concentrations of the drug and metabolites would have to be determined to resolve this discrepancy.

Of special importance in regard to the potential use of vira-MP for herpesvirus infections of the central nervous system is penetration of the drug across the blood-brain barrier. In this study four patients had documented cerebrospinal fluid levels of ara-Hx very similar to those obtained after the administration of vira-A (1). These cerebrospinal fluid levels were approximately two-thirds of the simultaneously determined plasma level.

When plasma levels of ara-Hx were compared in renal transplant recipients and patients with other diseases, no significant difference was present for mean peak levels at any dose. However, when patients were evaluated according to renal function, irrespective of underlying medical conditions, elevated plasma levels of ara-Hx were found in those individuals with deteriorating or poor renal function (creatinine clearance, ≤50 ml/min per 1.73 m²). Of the three patients with decreased creatinine clearances and estimated renal plasma flows, all had peak ara-Hx and AUC levels well in excess of those found in other patients studied at the same dosage. It was these three patients who had evidence of neurological abnormalities, which were possibly related to vira-MP administration. A similar adverse experience has been noted with vira-A therapy of patients with chronic hepatitis B infections (4). However, all three patients had other medical complications, such as cytomegaloviremia and pneumonia in one, multiple pulmonary emboli with hypoxemia and tubercous sclerosis in another, and end-stage metastatic carcinoma with CO₂ narcosis in the third. Similar neurological toxicity has been observed for inexplicable reasons with extremely high doses of vira-A administered intramuscularly to rhesus monkeys (4). Thus, caution must be exercised when employing either vira-A or vira-MP in patients whose renal function is marginal or deteriorating. In these studies renal function warranting caution was a creatinine clearance of less than 50 ml/min per 1.73 m² or an estimated renal plasma flow of less than 300 ml/min or both.

The major laboratory abnormality associated with vira-MP administration appears to be elevation of SGOT levels. As expected, this abnormality was most evident after intramuscular administration of the drug (17%), but it was also observed infrequently after intravenous administration (7%). Tissue breakdown at the injection site probably accounted for the higher frequency of SGOT elevations in patients who received the drug intramuscularly. All abnormalities resolved spontaneously within 2 weeks after the last dose of drug.

Vira-MP represents a new and potentially useful antiviral agent. The studies performed to date have shown that this drug appears to be relatively nontoxic and can be administered both intravenously and intramuscularly at dosages of up to 25 mg/kg per day. There is no evidence from the present study of progressive drug accumulation. As with vira-A, vira-MP and its metabolites are rapidly cleared by the kidneys. Based upon the knowledge gained from pharmacological studies of the parent compound, vira-A, supplemented by single-dose radioactive studies, vira-MP represents an important second generation of antiviral agents. Controlled investigations of the use of this drug against herpes simplex encephalitis and varicella-zoster virus infections should now be undertaken.

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LITERATURE CITED


