Iontophoretic Application of Adenine Arabinoside Monophosphate to Herpes Simplex Virus Type 1-Infected Hairless Mouse Skin

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Several antiviral agents were applied topically or by iontophoresis to hairless mouse skin inoculated with herpes simplex virus type 1 (HSV-1), and the chemotherapeutic effectiveness was evaluated. Topical application of iododeoxyuridine, arabinoside A, and adenine arabinoside monophosphate (ara-AMP) moderately decreased the average lesion score, number of mice with paralysis, and number of mice dying in HSV-1-infected animals. Also, the mean survival time was moderately prolonged by topical application of some antiviral agents. When ara-AMP was applied by cathodal (-) iontophoresis to the HSV-1-infected skin, the average lesion score, number of mice with paralysis, and number of mice dying were greatly decreased. Furthermore, the mean survival time of mice was highly increased by cathodal (-) iontophoresis of ara-AMP. The therapeutic efficacy of ara-AMP iontophoresis was much superior to the topical application of iododeoxyuridine, arabinoside A, and ara-AMP. These data suggest that ara-AMP iontophoresis would be the method of choice for the management of HSV-1 skin lesions in hairless mice.

Iontophoresis is the process of increasing the penetration of a desired ionized substance into surface tissues by the aid of a direct electrical current (12). Pilocarpine iontophoresis is the method of choice for the diagnosis of a cystic fibrosis (11). Iontophoresis of lidocaine and epinephrine has been reported for the local anesthesia of the external ear canal and ear drum (3, 6, 7) and oral mucous membrane (8). Fluoride iontophoresis is very useful for the treatment of ultrasensitive dentin (9, 19, 25). Recently, we have reported the iontophoretic application of several antiviral agents in neonatal mouse skin (10, 13) and adult mouse skin (13, 22). Iontophoretic application greatly increased the penetration of iododeoxyuridine (IUdR) and 9-β-D-arabinofuranosyladenine monophosphate (ara-AMP) into skin (10, 13, 22). We also have shown that ara-AMP and its antiviral metabolites (9-β-D-arabinofuranosyladenine [ara-A] and arahypoxanthine) remain at the application site for a significant period of time (22). Since the transport of ara-AMP across the cell membrane is highly limited (2, 24) and, in contrast to ara-A, ara-AMP produces sustained cytotoxic effects, iontophoretic application of ara-AMP would be an acceptable approach for a local herpes viral lesion.

Herpes simplex viruses (HSV), both types 1 and 2, commonly induce primary or recurrent herpetic lesions at the body surface, i.e., orofacial herpes infections, herpes keratitis, and herpes genitalis (20, 21, 29). Numerous antiviral agents have been developed and applied for the management of these diseases. Since most of the useful antiviral agents are nucleoside or nucleotide analogs, they have cytotoxic effects as well as possible teratogenic, mutagenic, or carcinogenic effects (24). In general, the systemic administration of these antiviral agents should be avoided. However, Whitely et al. (30) have reported the successful control of biopsy-proven HSV encephalitis by systemic administration of ara-A. Topical application of antiviral agents has shown moderately successful results in the eye but less than satisfactory results on skin and mucocutaneous junctions (14, 15). It appears that unsatisfactory results may be due to poor penetration of the antiviral agents into surface tissues. Therefore, iontophoresis of antiviral agents on HSV-infected lesions might be very

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useful for increasing the chemotherapeutic effectiveness of the antiviral agents, especially those which are charged.

Recently, ara-AMP was synthesized to eliminate some of the disadvantages of ara-A, i.e., extremely low water solubility (24) and metabolic instability (1, 5, 24). Even though ara-AMP has been used successfully against HSV in vitro (26, 28) and in animal models (15, 17, 27, 28) the transport of ara-AMP across the cell membrane is highly limited because of its charged phosphate group. This property limits the topical application of ara-AMP to surface herpesvirus infections. Recently, we reported that the penetration of ara-AMP into mouse skin was greatly increased when ara-AMP was applied by cathodal (−) iontophoresis as compared to topical application (22). Also, ara-AMP and its metabolites with antiviral activity were sustained, in high levels, in the skin acid-soluble fraction even at 24 h after ara-AMP administration by cathodal (−) iontophoresis (22). Based on the positive results in pharmacokinetic and metabolic studies, the chemotherapeutic efficacy of ara-AMP iontophoresis on HSV-1-infected hairless mice was evaluated.

MATERIALS AND METHODS

Virus. HSV-1, McKrae strain, obtained from D. Pavan-Langston, Harvard Medical School, Boston, Mass., was used. The virus was propagated in primary rabbit kidney cell cultures and had a titer of approximately 10^7 plaque-forming units assayed on green monkey kidney cell (CV-1) monolayers. Stocks of the same batch of virus were stored at −75°C until used.

Mice. Hairless mice, bred at the Medical College of Georgia vivarium facility, were originally derived from the HRS/Y strain, Jackson Laboratories, Bar Harbor, Maine. Mice weighing 18 ± 2 g were divided into six groups of ten mice each.

Inoculation of HSV-1 to mouse skin and scoring of the lesions. Under ether general anesthesia, the skin over the lumbar vertebrae was scratched with a 26-gauge sterilized needle, making a cross-hatched pattern. Then the viral solution (0.05 ml) was rubbed on the scratched area for 10 s. Preliminary experiments indicated that this method induced 100% infection and death. Mice were examined for 14 days, and the lesions were scored according to the scoring method of Liebmann et al. (18). The viral infection was confirmed by isolation of HSV from the infected skin or brain in selected cases. Infected skin or brain was excised and homogenized in Eagle minimum essential medium, using a completely sterile technique. The homogenates were centrifuged (3,000 rpm, 5 min), the supernatants were inoculated on CV-1 monolayer cells, and the cytopathic effect was observed.

Treatment plans. Ara-A (3% petrolatum base ointment), ara-AMP (10% gel), and ara-AMP powder were obtained from the Warner-Lambert/Parke-Davis Pharmaceutical Research Laboratories (Ann Arbor, Mich.) through the courtesy of T. Petrick and R. Buchanan. IUdR (0.5% petrolatum base ointment) was obtained from Smith, Kline, and French Laboratories (Philadelphia, Pa.). Mice were divided into six groups: (i) control group (infection only); (ii) topical application of 0.5% IUdR; (iii) topical application of 3% ara-A; (iv) topical application of 10% ara-AMP gel; (v) cathodal (−) iontophoresis of a 2% NaCl solution; and (vi) cathodal (−) iontophoresis of a 2% ara-AMP solution.

All treatments were started 24 h after the inoculation of HSV-1, since it has been reported that the therapeutic effectiveness of antiviral agents is highest when administered between 24 and 48 h after inoculation of HSV-1 in hairless mice (16). Iontophoresis of ara-AMP and NaCl was performed once daily for 3 days, whereas topical application of ara-A, IUdR, and ara-AMP was carried out twice daily for 4 days. For cathodal (−) iontophoresis of NaCl or ara-AMP, a cotton wick electrode (surface area, 38 mm²) saturated with NaCl or ara-AMP was applied to the HSV-1-inoculated skin area, and the anode was connected to the tail of the mouse. The amount of electrical current applied was 0.5 mA (electromotive force = 5 V) for 10 min, using the Med Therm Electoro Medicator, model AE1 (Med Therm Corp., Huntsville, Ala.). Iontophoresis of ara-AMP was carried out under pentobarbital anesthesia (50 mg/kg, intraperitoneal injection).

RESULTS

The development of the HSV-1 skin lesion of infected mice was similar to the pattern described by Constantine et al. (4). Most of the skin lesions appeared on day 5 or 6 postinoculation. The lesions enlarged very rapidly and formed a unilateral or bilateral bandlike lesion which subsequently ulcerated. A cytopathic effect from the supernatants of infected tissue homogenates was observed from all lesions tested. Since infected mice became paralyzed and the cytopathic effect was observed from brain homogenates, it was inferred that the mice died of HSV-1 encephalitis.

Table 1 shows the average lesion score, number of mice with paralysis, number of mice dying, and mean survival time of mice. When IUdR, ara-A, and ara-AMP were applied topically, there was a moderate decrease in the average lesion score (25, 28, and 30%, respectively), number of mice with paralysis (33, 22, and 22%, respectively), and number of mice dying (40, 40, and 30%, respectively). The mean survival time was increased (21, 31, and 26%, respectively).

There appears to be no difference between the control (infection only) group and cathodal (−) iontophoresis of the NaCl group. However, cathodal (−) iontophoresis of ara-AMP greatly decreased the average lesion score (70% compared with the control group). Also, the percentage of mice paralyzed or dying was only 20% in the ara-AMP iontophoresis group. The mean survival
Table 1. Effects of topical application and iontophoretic application of antiviral agents on HSV-1 skin infections in hairless mice

<table>
<thead>
<tr>
<th>Group†</th>
<th>Avg lesion scorea (cumulative maximum)</th>
<th>No. with paralysis</th>
<th>No. dying</th>
<th>Mean survival time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (infection only)</td>
<td>4.0</td>
<td>9/10</td>
<td>10/10</td>
<td>8.5</td>
</tr>
<tr>
<td>Iontophoresis (-) of 2% NaCl</td>
<td>4.0</td>
<td>9/10</td>
<td>10/10</td>
<td>8.8</td>
</tr>
<tr>
<td>Topical application of 10% Ara-AMP</td>
<td>2.8b</td>
<td>7/10b</td>
<td>7/10b</td>
<td>10.7b</td>
</tr>
<tr>
<td>Topical application of 0.5% IUdR</td>
<td>3.0f</td>
<td>6/10f</td>
<td>6/10f</td>
<td>10.3f</td>
</tr>
<tr>
<td>Topical application of 3% Ara-A</td>
<td>2.9f</td>
<td>6/10f</td>
<td>6/10f</td>
<td>11.1f</td>
</tr>
<tr>
<td>Iontophoresis(-) of 2% Ara-AMP</td>
<td>1.2c.e</td>
<td>2/10c.e</td>
<td>2/10c.e</td>
<td>12.9c.e</td>
</tr>
</tbody>
</table>

† Each group contains data from 10 mice.
‡ The lesions were measured at daily intervals for 14 days and scored to 4 as described by Liebermann et al. (18): 0 = no lesion; 0.5 = several discrete punctate lesions or lesion less than 5 mm in length; 1.0 = lesion 5 to 9 mm; 2.0 = lesion 10 to 19 mm; 3.0 lesion = 20 to 29 mm; and 4.0 = lesion over 30 mm in length.
§ Significantly different (P < 0.05) from control group (t test).
¶ Significantly different (P < 0.05) from control group (χ² test).
‖ Significantly different (P < 0.05) from any topical application group (t test).
|| Significantly different (P < 0.05) from any topical application group (χ² test).

DISCUSSION

Topical application of IUdR, ara-A, and ara-AMP moderately improved the skin lesions of hairless mice (Table 1). The electrical control (cathodal [–] iontophoresis of NaCl) did not alter the average lesion score, number of mice paralyzed, number of mice dying, or mean survival time compared with the untreated control (infection only); this indicates that electrical stimulation (0.5 mA, 10 min) does not increase or decrease the infectious process of HSV-1 in the skin of hairless mice. However, cathodal (–) iontophoresis of ara-AMP greatly increased the chemotherapeutic effectiveness, presumably by increasing the penetration of ara-AMP into skin (22). The therapeutic efficacy of ara-AMP iontophoresis was much superior to the topical application of IUdR, ara-A, or ara-AMP. Furthermore, early treatment of the HSV-infected lesion by ara-AMP iontophoresis is a life-saving procedure in hairless mice, preventing central nervous system behavioral changes, encephalitis, and death. These data indicate that ara-AMP iontophoresis might be a method of choice for the management of HSV-1 infections of surface tissues.

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