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Iontophoretic Application of Ara-AMP (9-β-D-Arabinofuranosyladenine-5'-Monophosphate) into Adult Mouse Skin[1] (39929)

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Introduction. 9-β-D-Arabinofuranosyladenine (ara-A) appears to be an effective (1-4) and relatively nontoxic (2, 5) antiviral agent for the management of some infections caused by herpes simplex viruses (HSV). However, ara-A has several disadvantages as an antiviral agent. First, the water solubility is extremely low. Second, *in vivo,* ara-A is rapidly metabolized to the less effective ara-hypoxanthine (ara-Hx) or to noneffective metabolites, such as adenine (6, 7). Third, ara-A must be metabolized to ara-AMP, the 5'-phosphorylated form of ara-A, which is the substrate for formation of ara-ATP, the active metabolite for antiviral action (7). The transformation of ara-A to ara-AMP is very slow (7). Ara-AMP was synthesized in order to remedy the disadvantages of ara-A. It was found to be active *in vitro* (8, 9) and in animal models (10, 11). Unfortunately, the transport of ara-AMP across the cell membrane is limited, since it contains a highly charged phosphate group. Slow absorption of ara-AMP is a disadvantage for the management of surface HSV infections, such as herpes labialis, herpes keratitis, or herpes vaginitis. Iontophoresis can be employed to overcome this disadvantage of ara-AMP. Iontophoresis is a method of choice to assure penetration of charged chemicals through surface tissues (12-18). In this publication, we report the increased penetration into the adult mouse skin of ara-AMP and its subsequent metabolism and distribution.

Materials and methods. Animals and materisls. Albino male Swiss-Webster-NCI outbred adult mice of 35-40 g body weight were used. [^{3}H]Ara-AMP (sp act, 5054 mCi/mmole) and ara-AMP were kindly supplied by Parke-Davis & Co. For chromatography PEI-cellulose F plates were purchased from Brinkmann Instruments. The MedTherm Electro-Medicator Model AEI (kindly supplied by MedTherm Corporation, Huntsville, Ala.) was used as a direct current iontophoresor.

Iontophoresis. The dorsal trunk hair of the mouse was removed by Surgex hair remover cream following anesthesia with sodium pentobarbital (50 mg/kg, ip injection). Experiments were carried out 24 hr after application of the hair remover so that the slight inflammation of skin sometimes caused by hair remover was avoided. The anesthetic was given again and 0.2 ml of a 0.1% ara-AMP (200 μg) solution containing 5 μCi of [^{3}H]ara-AMP was applied to the depilitated skin through a cotton wick applicator (surface area, 38 mm^2). For cathodal (−) iontophoresis, the applicator saturated with ara-AMP was connected to the negative pole (cathode) of an iontophoresis apparatus and the return electrode (anode) was connected to the tail. The amount of current applied was 0.5 mA for 10 min (this required about 6 V). Anodal (+) iontophoresis of ara-AMP was also performed in a manner similar to cathodal (−) iontophoresis except for reversal of the electrodes. For topical application, the procedures were the same except that no electrical current was applied. Animals were sacrificed at 10 min after completing the administration of ara-AMP. Two-tenth milliliter of 2% ara-AMP (4.0 mg) containing 50 μCi of [^{3}H]ara-AMP was applied to dorsal skin by cathodal (−) ionotophoresis and the metabolism of ara-AMP was studied. Animals were sacrificed at 0 (immediately after finishing the iontophoresis), 1, 2, 4, 15, and 24 hr.

Preparation of skin acid-soluble fraction and separation of ara-AMP and its metabolites. After sacrifice, the skin which was connected to the electrode was removed by punch biopsy. Also, the skin which was not

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connected to the electrode and the liver were removed for further analysis. Tissues were weighed and homogenized, using a Polytron homogenizer, in 0.5 *N* $HClO_4$. After centrifuging, the supernatant, designated as acid-soluble fractions, was neutralized with 4 *N* KOH and lyophilized. After lyophilization, the samples were redissolved with a small amount of distilled water. From the redissolved samples, ara-AMP, ara-A, ara-Hx, and adenine were separated by thin-layer chromatography (tlc). Aliquots were applied 1.5 cm from the bottom of the PEI-cellulose plates. Following application of the samples, standard solutions containing ara-AMP, ara-A, ara-Hx, and adenine were spotted over the samples to serve as markers. The plates were developed by ascending chromatography in distilled water to the top and then air-dried. Using a uv light source to visualize the spots, a pencil line was drawn just below adenine and the plates were then developed in 1.0 *M* sodium formate buffer (pH 3.4) up to the line. The R_f values of ara-AMP, ara-A, ara-Hx, and adenine were 0.2, 0.5, 0.7, and 0.4, respectively. Sample spots were cut out with scissors and placed in scintillation vials. Elution was carried out by shaking the capped vials containing 1.0 ml of 0.7 *M* $MgCl_2$, 20 m*M* Tris–HCl (pH 7.4) at 37° for 1 hr. Radioactivities were determined using a Beckman liquid scintillation counter.

Results. Table I shows the amounts of [^{3}H]ara-AMP, [^{3}H]ara-A, and [^{3}H]ara-Hx in the acid-soluble fraction of adult mouse skin at 10 min after [^{3}H]ara-AMP administration. Anodal (+) iontophoresis slightly increased the radioactivities of [^{3}H]ara-AMP, [^{3}H]ara-A, and [^{3}H]ara-Hx compared to topical application but the increase was not statistically significant. On the other hand, cathodal (−) iontophoresis greatly increased the radioactivities of [^{3}H]ara-AMP, [^{3}H]ara-A, and [^{3}H]ara-Hx (3,125, 7,500, and 10,875%, respectively) compared to topical application. These data indicate that ara-AMP is rapidly metabolized to ara-A and ara-Hx. However, significant amounts of ara-AMP are still present. No radioactivity above background was detected in an untreated skin area or in liver.

Table II shows the time-dependent metabolism of ara-AMP in adult mouse skin when administered by iontophoresis. The concentrations of ara compounds and adenine were calculated using the radioactivity (disintegrations per minute per milligram) found in each compound and the specific activity of [^{3}H]ara-AMP administered. The ara-AMP and ara-A fractions immediately upon completion of iontophoresis had the highest level of radioactivity. Since ara-AMP alone was administered during the 10 min of iontophoresis, ara-AMP must be rapidly metabolized to ara-A. Also, the concentration of adenine was high compared to ara-Hx at all periods. From these data, it appears most of the ara-AMP is metabolized to ara-A, which in turn is mostly metabolized to adenine rather than ara-Hx in adult mouse skin. This result is in agreement with the work of Bennett *et al.* (7). The highest concentrations of ara-AMP and its metabolites were noted immediately after iontophoresis; although they rapidly

TABLE I. CONCENTRATION OF Ara-AMP AND ITS METABOLITES IN ADULT MOUSE SKIN ACID-SOLUBLE FRACTIONS AFTER ADMINISTRATION OF Ara-AMP.[a]

	Concentration (μg/g of wet weight)					
	Ara-AMP	Increase (%)	Ara-A	Increase (%)	Ara-Hx	Increase (%)
Topical application	0.10 ± 0.008		0.73 ± 0.240		0.03 ± 0.015	
Anodal (+) iontophoresis	0.41 ± 0.116	283	1.12 ± 0.542	51	0.19 ± 0.035	450
Cathodal (−) iontophoresis	3.48 ± 0.279[b, c]	3,125	56.14 ± 11.352[b, c]	7,500	3.95 ± 0.774[b, c]	10,875

[a] Each group contains treated areas (38 mm^2) from five mice. [^{3}H]Ara-AMP was applied at a dose of 5 μCi per area (200 μg of Ara-AMP). Skin samples were taken 10 min after application. Concentrations of Ara compounds were calculated using the radioactivity (disintegrations per minute per milligram) found for each compound and the specific activity of [^{3}H]ara-AMP administered.

[b, c] Significantly different ($P < 0.05$) from topical application (*b*) or anodal iontophoresis (*c*).

TABLE II. CONCENTRATION OF Ara-AMP AND ITS METABOLITES IN ADULT MOUSE SKIN ACID-SOLUBLE FRACTIONS AFTER IONTOPHORESIS OF Ara-AMP.[a]

Time (hr)	Concentration (μg/g of wet weight)			
	Ara-AMP	Ara-A	Ara-Hx	Adenine
0	123.3 ± 4.39	207.3 ± 50.99	3.4 ± 0.68	9.0 ± 1.87
1	3.9 ± 0.52	23.2 ± 1.08	0.9 ± 0.27	12.3 ± 1.78
2	2.5 ± 0.12	21.8 ± 1.83	0.7 ± 0.16	17.6 ± 1.49
4	1.3 ± 0.14	14.3 ± 1.11	0.8 ± 0.15	12.7 ± 0.56
15	1.7 ± 0.87	10.8 ± 3.08	0.8 ± 0.16	5.1 ± 1.48
24	1.5 ± 0.53	8.7 ± 2.27	0.7 ± 0.11	4.1 ± 0.36

[a] Each group contains treated areas (38 mm^2) from four mice. [^{3}H]Ara-AMP was applied at a dose of 50 μCi per area (4000 μg of Ara-AMP). Skin samples were taken 0, 1, 4, 15, and 24 hr after application. Concentrations of Ara compounds and adenine were calculated using the radioactivity (disintegrations per minute per milligram) found for each compound and the specific activity of [^{3}H]ara-AMP administered.

diminished, effective antiviral substances were still observed in skin acid-soluble fraction after 24 hr. Only trace amounts of radioactivity were present in the untreated skin area and liver over the whole period (data not shown).

Discussion. HSV are common inhabitants in certain human tissues, infecting mucocutaneous junctions, sex organs, and eyes (19–21). The primary attack of HSV can lead to encephalitis and infections of other organs (20, 22). Furthermore, there is a strong association between the herpesviruses and human malignancy. Fortunately, herpesviruses are particularly susceptible to chemotherapy. Isolated herpesviruses can be killed by various agents, ranging from common vinegar to nucleoside and nucleotide analogs (24). However, the problem is finding an effective agent that can cross tissue barriers and cellular membranes and attack the virus without disrupting normal cellular metabolism. Among the antiviral agents, iododeoxyuridine (IdUrd) is widely used, especially for the treatment of herpetic keratitis (25, 26). Nevertheless, IdUrd may have oncogenic and teratogenic effects (27, 28). Also IdUrd-resistant HSV have been reported (29, 30). Furthermore, the efficacy of IdUrd when administered topically for management of cutaneous infections has been less satisfactory (31, 32). Such disadvantages of IdUrd require the development of new antiviral agents with increased penetrability.

Recently, ara-A has emerged as an effective and relatively less toxic antiviral agent (1–5). As mentioned above (see Introduction), ara-A has several disadvantages which could be avoided by using its phosphorylated form, ara-AMP. However, the transport of ara-AMP across surface tissues is limited because it is ionic. The ionic form is favorable for iontophoresis since the penetration of charged molecules across barriers is greatly increased by applying an electrical current of appropriate polarity.

Iontophoresis is a simple, safe, well-documented method of assuring penetration of charged chemicals through surface tissues (12–18). Iontophoresis has been used in medical and dental fields to increase the penetration of drugs (12–15). Also we already reported an increased penetration after iontophoretic application of IdUrd and phosphonoacetic acid (16, 17) to mouse skin.

As shown above, the penetration of ara-AMP was greatly enhanced by cathodal (−) iontophoresis. After penetration, ara-AMP did not spread to adjacent untreated areas and it was metabolized by known pathways. Most of the ara-AMP was metabolized to ara-A, while ara-A was metabolized mostly to adenine.

The present report indicates that iontophoretic application of ara-AMP is superior to topical application in obtaining high and sustained tissue levels of this drug. We believe that this increased penetration of ara-AMP will occur in viral lesions of HSV-infected skin. Therefore, we believe that iontophoresis of ara-AMP will demonstrate clear therapeutic superiority over topical application in the treatment of HSV surface infection. We are now conducting experi-

ments in which iontophoresis of ara-AMP will be applied to HSV-infected mouse skin. When proven in this animal model, the experiments should be extended to skin lesions of human volunteers.

Summary. Cathodal (−) iontophoresis significantly increased the penetration of ara-AMP through adult mouse skin. After penetration into tissues, most of the ara-AMP was rapidly metabolized to ara-A. Even at 24 hr after ara-AMP administration by cathodal (−) iontophoresis, significant amounts of ara-AMP, ara-A, and ara-Hx were determined in the skin acid-soluble fraction. These experiments indicate that iontophoresis is the method of choice for administration of ara-AMP to surface tissues.

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