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Iontophoretic Assistance of 5-Iodo-2'-Deoxyuridine Penetration into Neonatal Mouse Skin and Effects on DNA Synthesis[1, 2] (39689)

L. P. GANGAROSA, N. H. PARK, AND J. M. HILL

Departments of Oral Biology, Pharmacology, and Cell and Molecular Biology, Medical College of Georgia, Augusta, Georgia 30902

5-Iodo-2'-deoxyuridine (Idoxuridine, IdUrd), an analog of thymidine, inhibits the replication of mammalian cells, bacteria, and DNA-containing viruses (1-3). IdUrd was shown by Kaufman to be effective in herpes simplex eye infection (4), and the effective topical therapy of herpes simplex keratitis with IdUrd has been verified (5-7). Therefore, the usefulness of this drug in ophthalmology has led to its acceptance as an antiviral agent. Some reports have claimed that IdUrd is effective in animals for the therapy of cutaneous lesions caused by herpes simplex virus (8, 9), but another report claimed lack of efficacy in skin lesions due to the poor transport of the compound in aqueous solution through skin barriers (10). IdUrd in an adherent vehicle was also tried for herpes simplex labialis with equivocal results (11), again suggesting poor penetration.

Since iontophoresis is a method for assuring penetration of charged drugs into surface tissues, this method might overcome the problem of IdUrd penetration into skin. Iontophoresis has been used in medical and dental fields to increase the penetration of drugs (12-18). The experiments presented here were performed to observe the effect of iontophoresis on penetration of IdUrd into neonatal mouse skin and to determine the effect of iontophoresis of IdUrd upon thymidine (TdR) metabolism in this rapidly proliferating tissue.

Materials and methods. Animals. Albino female Swiss-Webster-NCI, outbred, neonatal mice 2 to 3 g body weight and 2-3 days of age were used.

Radioactive materials and chemical. [^{125}I]IdUrd was purchased from New England Nuclear Co. (sp act, >200 Ci/mmole). [^{3}H]TdR was obtained from Schwarz/Mann Bioresearch (sp act, 15.6 Ci/mmole). IdUrd in mannitol (50:50) was kindly supplied by Calbiochem and, when it was dissolved in demineralized-redistilled water at 0.125%, the solution had a pH of 9.2, osmolarity of 10 mosm, and Na content of 2.5 mequiv/liter.

Iontophoresis. The trunk of the mouse was wrapped with a cloth saturated with 0.3 ml of 0.125% IdUrd solution for topical application. For iontophoresis of IdUrd, a strip of aluminum foil was wrapped around the cloth and connected to an appropriate electrode, while the return electrode was connected to the tail of the mouse. The amount of current applied was 0.5 mA for 10 min (EMF = 11 V). To observe the IdUrd penetration into skin (Series I experiment), 0.125% IdUrd solution containing 5.0 μCi of [^{125}I]IdUrd/0.3 ml was applied to the mouse. Mice were divided into four groups as follows: Group A: topical application of IdUrd; Group B: anodal(+) iontophoresis of IdUrd (anode was connected to the aluminum foil); Group C: cathodal(−) iontophoresis of IdUrd (cathode was connected to the aluminum foil); Group D: injection of IdUrd (375 μg of IdUrd containing 5 μCi of [^{125}I]IdUrd was injected ip). Each group included eight mice and was divided into two subgroups in order to obtain two periods (15 and 300 min) after IdUrd administration. After drug administration, the mouse's skin was thoroughly washed. At the end of these times, the mice were sacrificed. A second series of experiments (Series II) was performed to observe the effect of IdUrd iontophoresis on TdR metabolism in skin. Each mouse was injected ip with 1.0 μCi of [^{3}H]TdR at 10 min after IdUrd administration. Series II mice

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were divided into five groups: Group E: control (no IdUrd administration); Group F: topical application of IdUrd; Group G: anodal(+) iontophoresis of IdUrd; Group H: injection of IdUrd (375 μg of IdUrd was injected ip); Group I: electrical control (anodal(+) iontophoresis of saline solution). Each group except group I included 12 mice and was divided into three subgroups to obtain three periods (15, 30, and 300 min) after [^{3}H]TdR injection. The mice were sacrificed at the end of these times. Group I contained only four mice sacrificed at 300 min.

Homogenates. After sacrifice, the skin of the trunk was removed, weighed and homogenized in 10 ml of 5% citric acid–8% sucrose solution per gram wet weight of tissue and processed according to the methods described by Hill *et al.* (19). Briefly, an aliquot of homogenate was mixed with 0.5 *N* $HClO_4$, and this mixture was centrifuged. The supernatant was designated as first acid-soluble fraction. This fraction was neutralized with 4 *N* KOH before separation of metabolites.

Separation of IdUrd, I^-, TdR, and thymidine monophosphate (TMP). IdUrd, TdR, and TMP were separated by thin-layer chromatography from the first acid-soluble fraction using a modification of the method described by Garrett *et al.* (20). Free I^- was separated from the acid soluble fractions with methyl mercuric bromide and toluene (21). The ^{125}I radioactivity of IdUrd or I^- was determined in a Beckman gamma counter, and the ^{3}H radioactivity of TdR and TMP was determined using a Beckman liquid scintillation counter.

Radioactivity of DNA. After washing with 0.5 *N* $HClO_4$, the homogenates were hydrolyzed and centrifuged. The superantants were used for the DNA assays using the diphenylamine reaction (22), and radioactivities were determined. Alcohol–ether washes and alkaline hydrolysis were performed on several samples before $HClO_4$ hydrolysis; DNA content and radioactivity determinations gave the same values as direct hydrolysis by $HClO_4$ indicating no TdR incorporation into RNA or protein during this period.

Results. Table I shows the uptake of [^{125}I]IdUrd into acid-soluble fraction of the neonatal mouse skin at 15 min after [^{125}I]IdUrd administration. Both anodal(+) and cathodal(−) iontophoresis resulted in significantly increased uptake of [^{125}I]IdUrd (185 and 113%, respectively) into neonatal mouse skin compared to topical application. Since the [^{125}I]IdUrd solution contained a small amount (less than 4%) of the free $^{125}I^-$ and, furthermore, IdUrd is rapidly metabolized to inorganic iodide and uracil, the amount of free $^{125}I^-$ in the skin acid-soluble fraction was measured. Cathodal iontophoresis significantly increased the radioactivity of $^{125}I^-$ in the skin acid-soluble fraction (295%) compared to topical application, whereas anodal iontophoresis resulted in a slight but significant decrease (29%) 15 min after [^{125}I]IdUrd administration. These results indicate that at 15 min after IdUrd administration by iontophoresis, most of the I^- in the skin acid-soluble fraction may be due to penetration of I^- in the IdUrd solution rather than the metabolism of IdUrd. The highest skin levels of IdUrd and I^- occurred when ip injection was used which would be expected since the animal received

TABLE I. RADIOACTIVITY OF [^{125}I]IdUrd AND FREE $^{125}I^-$ IN SKIN ACID-SOLUBLE FRACTION AFTER ADMINISTRATION OF [^{125}I]IdUrd TO NEONATAL MICE.[a]

Method of application	Radioactivity (cpm/mg of wet tissue ± SE)					
	[^{125}I]IdUrd		$^{125}I^-$		Total ^{125}I (before tlc)	
	(cpm)	(Δ)	(cpm)	(Δ)	(cpm)	(Δ)
Topical	60 ± 9		21 ± 1		82 ± 6	
Iontophoresis (+)	171[b] ± 23	+185%	15[b] ± 2	−29%	218[b] ± 13	+154%
Iontophoresis (−)	128[b] ± 16	+113%	83[b] ± 10	+295%	221[b] ± 29	+168%
Injection (ip)	685[b] ± 93	+1042%	281[b] ± 47	+1238%	1420[b] ± 278	+1639%

[a] Each determinant contains data from four mice. Skin samples were taken 15 min after application.
[b] Significantly different ($P < 0.05$) from topical application. Total radioactivity was measured from acid-soluble fraction before thin-layer chromatography (tlc).

all the drug by this method, whereas the other methods delivered only a fraction of the dose.

Table II shows the incorporation of [^{125}I]IdUrd into neonatal mouse skin DNA. At 15 min after [^{125}I]IdUrd administration, both cathodal(−) and anodal(+) iontophoresis significantly increased the incorporation of [^{125}I]IdUrd into DNA (621 and 286%, respectively) compared to topical application. Also at 300 min, [^{125}I]IdUrd incorporation into DNA was significantly increased by cathodal and anodal iontophoresis (109 and 69%, respectively).

Table III shows the effects of various types of IdUrd treatment on TdR metabolism 300 min after IdUrd administration. The radioactivity of [^{3}H]TdR in the skin acid-soluble fraction was significantly increased in the groups pretreated with IdUrd iontophoresis or ip injection compared to either control or topical application, whereas iontophoresis of saline did not alter the radioactivity of [^{3}H]TdR compared to the untreated group. On the other hand, the radioactivity of [^{3}H]TMP was significantly decreased by IdUrd. These results indicate the inhibitory effect of IdUrd on thymidine kinase. Iontophoresis itself (saline control) did not alter [^{3}H]TMP radioactivity compared to the untreated group. At 15 or 30 min after IdUrd administration (data not shown), there were no significant differences between iontophoresis and the topical application group.

In Fig. 1, the incorporation of tritiated thymidine into neonatal mouse skin DNA is indicated. The control group (top line), which received only [^{3}H]TdR, exhibited a linear increase in specific activity of DNA with time. The mice treated with IdUrd topically had an initial depression of incorporation at 15 min, with slight depression at 30 min, and recovery almost to the level of the control at 300 min. The mice treated by iontophoresis of IdUrd exhibited depressed incorporation of TdR into DNA, resembling that seen in the ip treated group (bottom line) especially at the 300-min time. The same inhibition was noted for the ionto-

TABLE II. INCORPORATION OF [^{125}I]IdUrd INTO NEONATAL MOUSE SKIN DNA.[a]

Method of application	Radioactivity (cpm/mg of DNA ± SE)			
	Labeling time			
	15 min		300 min	
	(cpm)	(Δ)	(cpm)	(Δ)
Topical	216 ± 35		763 ± 121	
Iontophoresis (+)	833[b] ± 116	+286%	1,290[b] ± 123	+69%
Iontophoresis (−)	1,588[b] ± 408	+621%	1,596[b] ± 198	+109%
Injection (ip)	12,863[b] ± 1823	+5855%	69,199[b] ± 855	+8969%

[a] Each determinant contains data from four mice.
[b] Significantly different ($P < 0.05$) from topical application.

TABLE III. EFFECT OF IdUrd ON [^{3}H]TdR AND [^{3}H]TMP RADIOACTIVITIES IN SKIN ACID-SOLUBLE FRACTION OF NEONATAL MICE.[a]

Method of IdUrd application	Radioactivity (dpm/mg of wet tissue ± SE)			
	[^{3}H]TdR		[^{3}H]TMP	
	(dpm)	(Δ)	(dpm)	(Δ)
Untreated controls	175 ± 5		110 ± 5	
Topical	230[b] ± 5	+31%	65[b] ± 5	−41%
Iontophoresis(+)	425[b,c] ± 5	+143%	45[b] ± 10	−59%
ip Injection (ip)	870[b,c] ± 5	+397%	40[b,c] ± 5	−65%
Iontophoresis(+) of saline	180 ± 5	+3%	120 ± 5	+9%

[a] Each determinant contains data from four mice. Skin samples were taken 300 min after application.
[b] Significantly different ($P < 0.05$) from untreated group.
[c] Significantly different ($P < 0.05$) from topical application group.

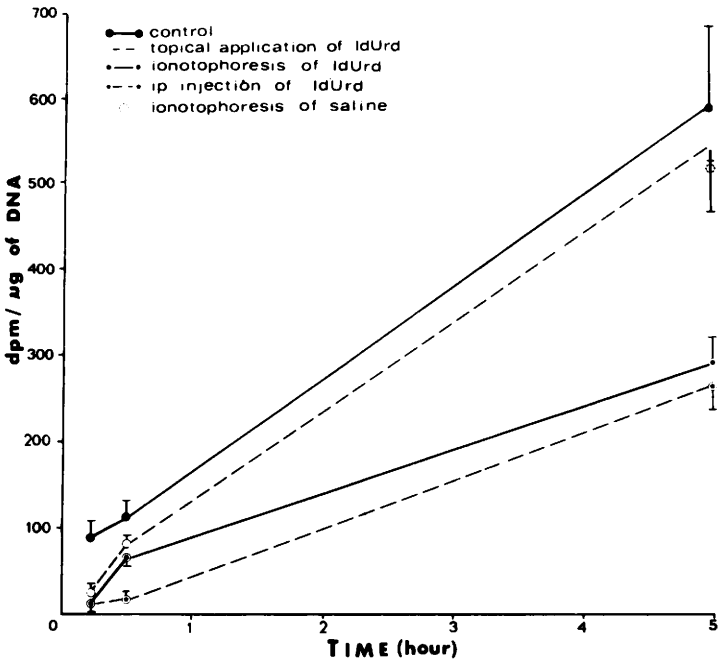

FIG. 1. DNA specific radioactivity of [^{3}H]thymidine in neonatal mice skin after application of IdUrd.

phoresis and the ip group at 300 min when we repeated the experiment (data not shown).

Discussion. The medical and dental literatures indicated that electrically charged drugs can be most effectively introduced into surface tissues by means of iontophoresis. Iontophoresis is used in the pilocarpine diagnostic test for cystic fibrosis (12) and for administration of vasodilators for peripheral vascular disease (13). Comeau *et al.* (14) reported local anesthesia of the ear by iontophoresis. Gangarosa (15) also reported successful local anesthesia of oral mucosa with lidocaine and epinephrine iontophoresis. Furthermore, fluoride iontophoresis appears effective for the treatment of exposed hypersensitive dentin (16–18). Based on these reports, the authors decided to study whether the antiviral drug, IdUrd, could be assisted in its penetration by iontophoresis. Although IdUrd does not possess an obvious ionic site, the replacement of the methyl group in position 5 of TdR with iodide alters the electron configuration of the pyrimidine moiety because of possible inductive and resonance effects of the halogen (3) which might result in the formation of polar moieties in solution. *In vitro* conductivity studies performed by us indicate that the IdUrd:mannitol solution has a low conductivity which is acceptable for application by iontophoresis (23).

The results of iontophoresis of IdUrd in mice indicate that penetration of IdUrd into skin can be increased by this method. As shown in the pharmacokinetic study of [^{125}I]IdUrd penetration into skin, cathodal or anodal iontophoresis effectively increased the penetration of IdUrd into neonatal mouse skin. Theoretically, in iontophoretic application, only one electrode should assist the penetration of an ionic drug, i.e., the penetration of negative ions will be assisted by cathodal iontophoresis while positive ions will be assisted by anodal iontophoresis. Since both cathodal and anodal iontophoresis assist IdUrd penetration, we believe that electro-osmosis (24) might be involved as an important factor in the penetration of IdUrd into skin. Preliminary results in our laboratory (data not shown) support the electro-osmotic hypothesis; we found that nonionic substances, in NaCl solution, can be assisted in their penetration by either cathodal or anodal iontophoresis. Also our IdUrd:mannitol solutions contained a small amount of sodium ion (see

methods section).

The mechanism of action of IdUrd on DNA viruses involves competitive antagonism of TdR to block the metabolic steps for TdR incorporation into DNA (1-3). The studies presented here indicate that iontophoresis of IdUrd may inhibit TdR phosphorylation and incorporation into DNA as effectively as ip injection of IdUrd. This result suggests that the IdUrd may block TdR metabolism including incorporation of thymidine triphosphate into DNA.

Summary. These experiments indicate that iontophoresis of IdUrd into neonatal mouse skin may be as effective as ip administration in blocking TdR metabolism including its incorporation into DNA of the skin. Also, IdUrd appeared to be incorporated into DNA. These results suggest that antiviral chemotherapy in surface tissues may be better accomplished by using iontophoretic assistance of penetration since the drug is delivered specifically at the desired site and the overall dose to the animal is small compared to systemic administration. We would expect that the drug which is administered by iontophoresis would probably act similarly on active virus-producing cells to prevent viral multiplication.

1. Prusoff, W. H., Pharmacol. Rev. **19,** 209 (1967).
2. Prusoff, W. H., and Ward, D. C., Biochem. Pharmacol. **25,** 1233 (1976).
3. Prusoff, W. H., and Goz, B., Fed. Proc. **32,** 1679 (1973).
4. Kaufman, H. E., Proc. Soc. Exp. Bio. Med. **109,** 251 (1961).
5. Kaufman, H. E., Martola, E., and Dohlam, S. H., Arch. Ophthalmol. **68,** 253 (1962).
6. Kaufman, H. E., and Maloney, E. D., Arch. Ophthalmol. **69,** 626 (1962).
7. Luntz, M. H., and MacCallum, F. O., Brit. J. Ophthalmol. **47,** 449 (1963).
8. Hall-Smith, S. P., Corrigan, M. J., and Gilkes, M. J., Brit. Med. J. **2,** 1515 (1962).
9. Dawber, R., Brit. Med. J. **2,** 526 (1974).
10. Juel-Jensen, B. E., and MacCallum, F. O., Brit. Med. J. **2,** 987 (1964).
11. Najjar, T. A., Sleeper, H. R., and Calabresi, P., J. Oral Med. **24,** 53 (1969).
12. Gibson, L. E., and Cooke, R. E., Pediatrics **23,** 545 (1959).
13. Stone, T. W., J. Pharm. Pharmacol. **24,** 318, (1972).
14. Comeau, M., Brummett, R., and Vernon, J., Arch. Otolaryngol. **98,** 114 (1973).
15. Gangarosa, L. P., J. Amer. Dent. Assoc. **88,** 125 (1974).
16. Collins, E. M., Dent, Dig. **68,** 360 (1962).
17. Scott, H. M., J. Dent. Child. **29,** 225 (1962).
18. Murthy, K. S., Talim, S. T., and Singh, I., Oral Surg. Oral Pathol. Oral Med. **36,** 448 (1973).
19. Hill, J. M., AB, G., and Malt, R. A., Biochem. J. **144,** 447 (1974).
20. Garrett, E. R., Chemburkar, P. B., and Suzuki, T., Chem. Pharm. Bull. **13,** 1113 (1965).
21. Hughes, W. L., Brommerfor, S. L., Gitlin, D., Krueger, R. C., Schultze, B., Shah, V., and Peilly, P., Fed. Proc. **23,** 640 (1964).
22. Burton, K., Biochem. J. **62,** 315 (1956).
23. Gangarosa, L. P., Park, N. H., Hill, J. M., Fong, B., and Scott, D. F., Presented at the annual meeting Southeastern Section, Society for Experimental Biology and Medicine (1975).
24. Harris, R., Iontophoresis, Ch. 4 *in* "Therapeutic Electricity and Ultraviolet Radiation" (Sidney Licht ed.), 2nd ed., pp. 156-178. E. Licht (Publisher), New Haven, Conn. (1967).

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