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Percutaneous Absorption of Methotrexate: Effect on Epidermal DNA Synthesis in Hairless Mice

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One of the presumed reasons for the lack of clinical activity of topical methotrexate in psoriasis is insufficient percutaneous penetration necessary to inhibit epidermal DNA synthesis. The present study was undertaken to select a vehicle to optimize penetration of methotrexate *in vitro* and to determine the effects of this topical formulation on epidermal DNA synthesis *in vivo* in hairless mouse skin.

Increased penetration of methotrexate was obtained in human skin *in vitro* with Vehicle N compared to water and n-decylmethylsulfoxide vehicles. Repeated topical application of this methotrexate/Vehicle N preparation produced marked epidermal atrophy in treated sites in both normal and hyperproliferative essential fatty acid deficient hairless mouse skin without similar effects at a distant skin site. Local inhibition of epidermal DNA synthesis was also obtained without systemic effects at a distant site. These studies demonstrate that methotrexate in Vehicle N may produce a direct effect on epidermis which may be useful for the topical therapy of psoriasis.

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Methotrexate (MTX) has been used successfully for the systemic treatment of widespread severe psoriasis, but the risk of short-term and long-term effects has precluded the use of this drug for the great majority of patients with minimal psoriasis [1]. Clinical trials of topical methotrexate therapy, in an effort to circumvent systemic toxicity, have been uniformly disappointing [2–5], despite data which suggest that methotrexate acts directly on the psoriatic plaque rather than systemically at a distant site [6,7]. Other studies show that topical methotrexate preparations produce negligible percutaneous penetration of human skin *in vitro* [8] and *in vivo* [9,10] and thus suggest that the drug is not delivered to the proliferative epidermal cells where it is thought to act.

In an effort to develop a topically effective methotrexate preparation for psoriasis, these studies were designed to test various vehicles to enhance methotrexate percutaneous penetration *in vitro* and to evaluate the effect of these topical methotrexate formulations on epidermal DNA synthesis in hairless mice *in vivo*.

MATERIALS AND METHODS

Methotrexate was obtained from Lederle Laboratories, Pearl River, New York, through the courtesy of Dr. J. Birnbaum. Methotrexate (3′,5′,7-^{3}H) sodium (250 mCi/mmole) was obtained from Amersham and prior to use was purified by chromatography on thin-layer cellulose plates in 0.006 M potassium phosphate buffer, pH 6.0. Deoxyuridine (6-^{3}H) (25 Ci/mmole) was obtained from New England Nuclear, n-decylmethylsulfoxide from Cyclo Chemical, and Vehicle N (alcohol 47.5%, water, laureth-4, isopropylalcohol 4%, propylene glycol) from Neutrogena Corporation.

In Vitro Percutaneous Penetration Assay

The percutaneous penetration of methotrexate was measured in glass diffusion cells as previously described [8]. Excised human skin

Percutaneous Penetration of 2% Methotrexate in Human Skin in vitro

Vehicle	Penetration[a]				Drug Content[a,b]			
	24 hr		48 hr		Stratum Corneum		Epidermis	
	μg	%[c]	μg	%[c]	μg	%[c]	μg	%[c]
Water	6 ± 2	0.06	15 ± 5	0.15	9 ± 5	0.09	0.1 ± 0	0.001
C_{10}MSO (2.5%)	12 ± 10	0.12	36 ± 28	0.36	21 ± 5	0.21	0.8 ± 0.3	0.008
Vehicle N	50 ± 11	0.50	124 ± 10	1.24	16 ± 8	0.16	1.5 ± 0.5	0.01

[a] Mean values ± SD for 4 diffusion chambers.
[b] Samples taken after 48 hr incubations.
[c] Percent of applied dose.

obtained from abdominoplasty was used for these studies. 0.5 ml containing 10 μCi methotrexate-^{3}H and cold methotrexate to give a final concentration of 2% in various vehicles was applied to the epidermal surface (0.2 ml/cm^2). A total of 10 mg methotrexate was applied to each diffusion cell. The dermal reservoir contained phosphate buffered saline. Three diffusion cells were run for each vehicle. Diffusion cells were incubated with constant stirring at 28°C. Methotrexate penetration was followed at various time points up to 48 hr by liquid scintillation counting. The test solution was removed after the 48-hr incubation period and the epidermal surface was rinsed 3 times with vehicle in which the drug being tested was dissolved. This process effectively removed all drug which had not penetrated into the stratum corneum. The epidermis was separated by heating the skin for 1 min on a hot plate at 60°C. Methotrexate was extracted from the epidermis and dermis utilizing the technique described by Werkheiser, Zakrzewski, and Nichol [11] which specifically dissociates methotrexate from cellular bound proteins. The stratum corneum was removed by repeated stripping with cellophane tape until the skin glistened.

In Vivo Studies

HRS/J albino hairless mice age 2–3 mo were used for these experiments. For induction of essential fatty acid deficiency, 3-week-old animals were fed an essential fatty acid deficient diet (Teklad) for 60 days.

Topical methotrexate preparations at various concentrations or vehicle control 0.05 ml were applied to the back skin area of 1.5 cm × 3 cm daily × 1 or × 3. Six to 10 mice were used for each treatment group. Tritiated deoxyuridine was utilized as a precursor for assaying the effect of methotrexate on the *de novo* pathway for epidermal DNA synthesis. The incorporation of tritiated deoxyuridine into DNA depends on the normal functioning of dihydrofolate reductase which is blocked by methotrexate. At selected time points after single or repeated topical methotrexate applications, mice were injected IP with tritiated deoxyuridine (50–100 μCi) for measurement of epidermal DNA synthesis in treated back skin to determine the local effect of methotrexate. DNA synthesis was simultaneously measured in untreated abdominal skin of Vehicle N and methotrexate treated animals to determine systemic effects of methotrexate at a distant site. One hour after administration of isotope, animals were sacrificed by cervical dislocation and treated back skin and untreated abdominal skin was obtained. Epidermis was removed after incubating the skin specimens for 1 hr at 37°C in 2 M sodium bromide. DNA was extracted and assayed using the technique described by Halprin [12]. Results of epidermal DNA synthesis were calculated as counts per minute per microgram of DNA. The effects of methotrexate treatment on DNA synthesis on the back and abdomen were expressed as percent of DNA synthesis in Vehicle N treated back skin and untreated abdomen in control animals.

RESULTS

In Vitro Percutaneous Penetration Studies

There was a significant increase in methotrexate penetration at both 24 and 48 hr in Vehicle N as compared with water ($p < 0.01$) and n-decylmethylsulfoxide ($p < 0.01$) (Table). Vehicle N also produced a slight increase in epidermal methotrexate content compared to the other vehicles.

In Vivo Studies

Three consecutive applications of Vehicle N in normal hairless mice control animals unexpectedly produced epidermal hyperplasia characterized by exfoliation, acanthosis, and hypergranulosis (Fig 1*A*). Three daily applications of 2% methotrexate in Vehicle N to both normal (Fig 1) and hyperproliferative essential fatty acid deficient (Fig 2) hairless mouse skin produced marked epidermal atrophy in the areas where it was applied, without similarly affecting untreated abdominal skin.

Three daily applications of 2% methotrexate in Vehicle N suppressed epidermal DNA synthesis 50% and 75% in treated back skin compared to the Vehicle N treated control, at 54 and 72 hr respectively (Fig 3). In this experiment no significant ($p < 0.001$) systemic methotrexate effect was seen in the untreated abdominal skin at 54 hr (6 hr after the last treatment). However, by 72 hr (24 hr after the last treatment) both local and systemic effects were obtained. Four daily applications of methotrexate resulted in total suppression of DNA synthesis in both the back skin and in the untreated abdominal skin (Fig 3).

Three daily applications of 1% methotrexate in Vehicle N also produced inhibition of DNA synthesis in treated skin 6 hr after the last treatment, while lower concentrations had no local or systemic effect (Fig 4). Topical methotrexate (0.5%) in n-decylmethylsulfoxide likewise had no effect on epidermal DNA synthesis in this model (Fig 4).

DISCUSSION

Since the topical use of methotrexate in psoriasis has not been successful, one of the theories postulated has been that the therapeutic effect of methotrexate is due to action at a site other than in the skin [4]. No evidence to support this theory has been found. However, since methotrexate can produce the same biologic and biochemical effects in psoriatic epidermis with intralesional [7] and systemic administration [6], it may be inferred that the therapeutic response is mediated by local chemotherapeutic activity in skin. Other factors should be considered as causes for failure of topical antimetabolite therapy, such as inadequate percutaneous absorption or lack of extended tissue-drug contact as would be expected with circulating drug after systemic administration. Insofar as a vehicle is a factor in percutaneous penetration of a drug, we have shown in the present study that Vehicle N substantially enhances methotrexate penetration compared to n-decylmethylsulfoxide and water vehicles. In view of previous data by Weinstein, Goldfaden, and Frost [6] showing that an intradermal dose of methotrexate of 1 μg/0.1 ml results in a 50% inhibition of DNA synthesis in psoriatic cells, it would appear that the epidermal methotrexate concentrations obtained with Vehicle N are approaching levels sufficient for pharmacologic activity in psoriatic epidermis. Although penetration of methotrexate through psoriatic epidermis has not been determined, the inferior barrier of hyperproliferative skin [13] may facilitate increased penetration necessary to produce a therapeutic effect.

Epidermal DNA synthesis in the normal and essential fatty acid deficient hairless mouse has been a useful bioassay for topical chemotherapeutic drug activity in psoriasis [14,15]. Testing of the topical methotrexate/n-decylmethylsulfoxide preparation, previously shown to lack clinical activity in psoriasis [5] had no effect on epidermal DNA synthesis in the hairless mouse model (Fig 4). In this model methotrexate in

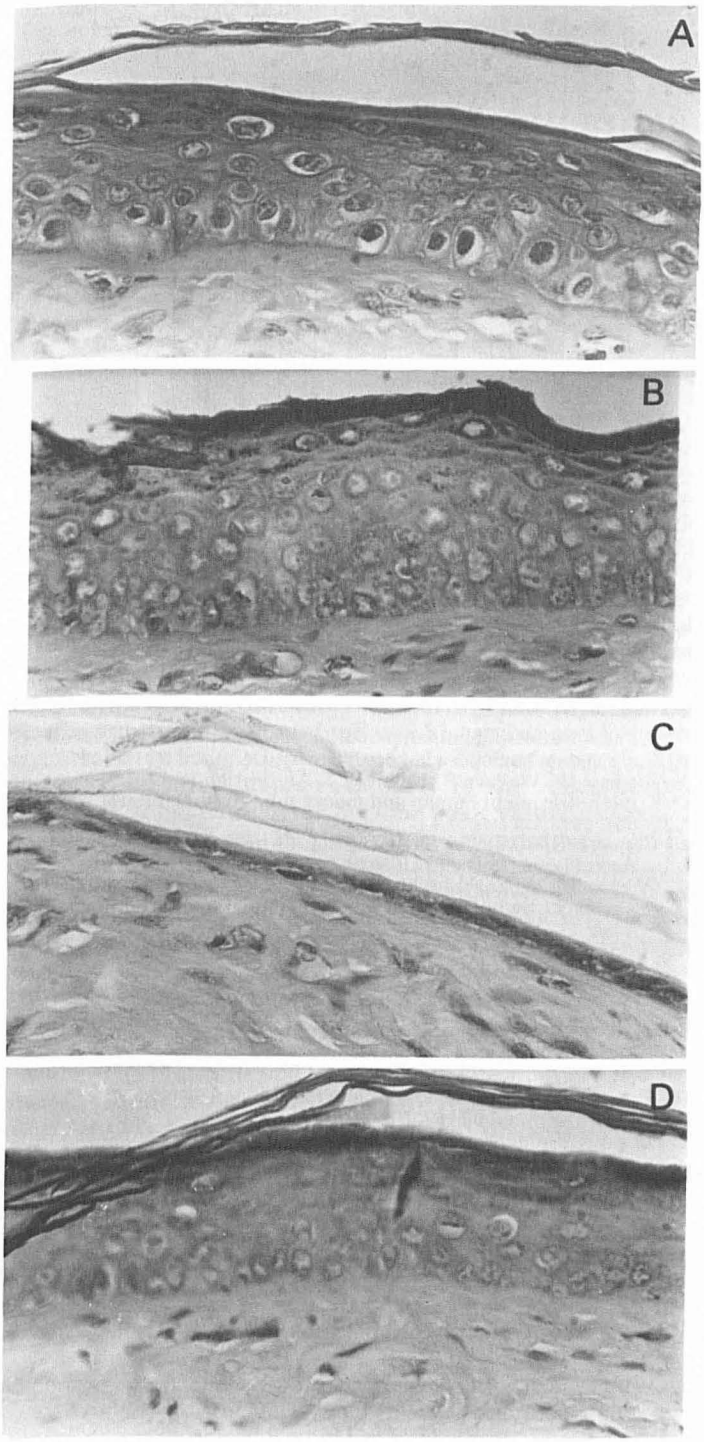

FIG 1. Effects of topical 2% methotrexate in normal hairless mice in Vehicle N on treated back and untreated abdomen skin. Methotrexate or Vehicle N applied daily × 3. Vehicle N control: (*A*) treated back; (*B*) untreated abdomen. Two percent methotrexate: (*C*) treated back; (*D*) untreated abdomen.

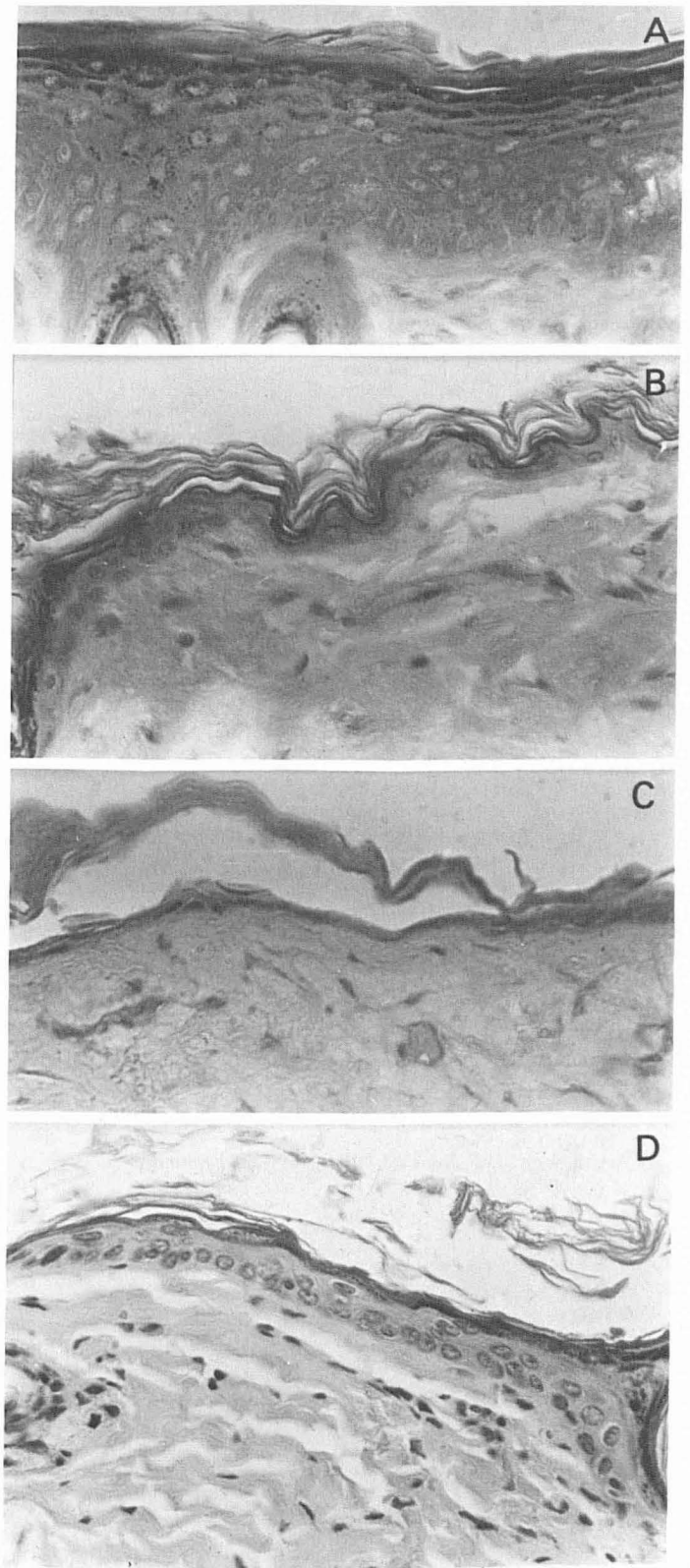

FIG 2. Effects of topical 2% methotrexate in Vehicle N on treated back and untreated abdomen skin in essential fatty acid deficient hairless mice. Methotrexate or Vehicle N applied daily × 3. Vehicle N control: (*A*) treated back; (*B*) untreated abdomen. Two percent methotrexate: (*C*) treated back; (*D*) untreated abdomen.

Vehicle N inhibited DNA synthesis in treated skin, without a significant effect on distant skin, 6 hr after the third application. A single drug application was ineffective. This may reflect either the slow time course of penetration or the extended time period of tissue-drug contact necessary to achieve a biochemical effect. Twenty-four hours after the third application, DNA synthesis was inhibited at both the treated and distant skin sites. This presumably was due to the entry of methotrexate into the systemic circulation at levels sufficient to inhibit DNA synthesis at a distant site.

Ideally, further studies will reveal an appropriate dose schedule for topical use of methotrexate in Vehicle N for psoriatic

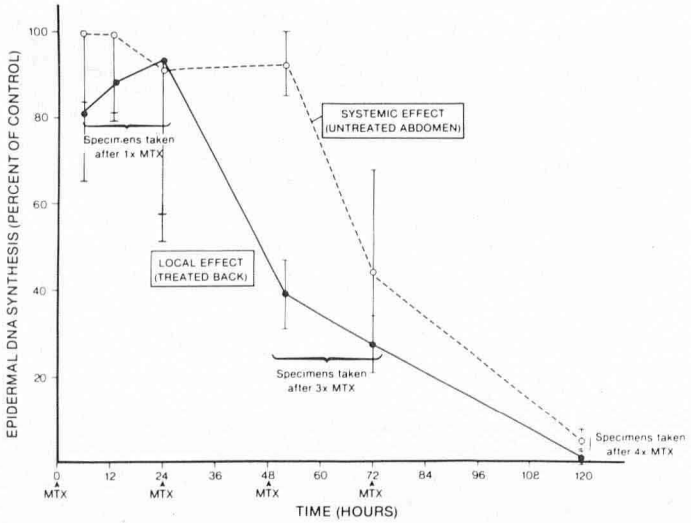

FIG 3. Local and systemic effects of topical 2% methotrexate in Vehicle N on epidermal DNA synthesis in normal hairless mice. Drugs or Vehicle N was applied to the back skin as indicated. Results are expressed as percent of DNA synthesis in control animals treated with Vehicle N.

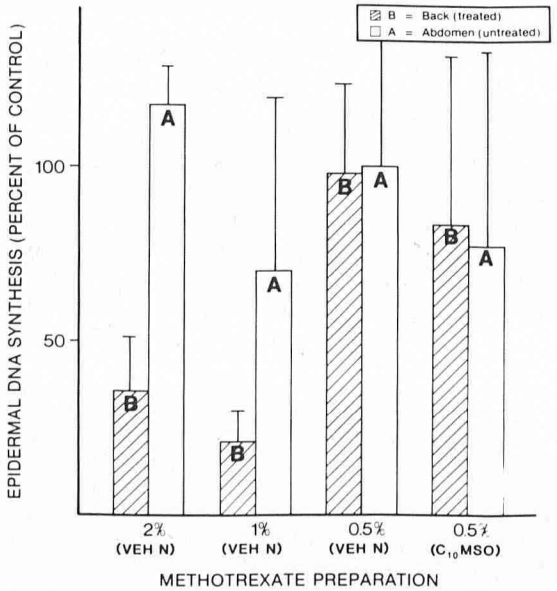

FIG 4. Effect of methotrexate concentration in Vehicle N and n-decylmethylsulfoxide on epidermal DNA synthesis in normal hairless mice. Drugs or vehicles were applied daily × 3. Skin specimens were analyzed for DNA synthesis 6 hr after the last application.

patients such that a local therapeutic effect can be achieved without risk of systemic toxicity.

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