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The Use of *N,N*-Diethyl-*m*-Toluamide to Enhance Dermal and Transdermal Delivery of Drugs

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Abstract □ A dermal penetration enhancer has been found which improves the dermal delivery of a wide variety of drugs and at the same time has a history of low toxicity for human dermal application. *N,N*-Diethyl-*m*-toluamide (I) has been shown to improve the delivery of many drugs through hairless mouse skin in an *in vitro* diffusion cell model. A topically applied steroid, hydrocortisone, has been used to demonstrate the *in vivo* effectiveness of I on human skin. The degree of pallor produced on human skin by the corticosteroids was used as a measure of the relative delivery of hydrocortisone from formulations with and without I.

Keyphrases □ *N,N*-Diethyl-*m*-toluamide—enhancement of dermal and transdermal drug delivery, hydrocortisone □ Hydrocortisone—enhancement of *N,N*-diethyl-*m*-toluamide on drug delivery, dermal and transdermal delivery □ Delivery, drug—dermal and transdermal, *N,N*-diethyl-*m*-toluamide enhancement, hydrocortisone

Improved dermal delivery of drugs has been the focus of pharmaceutical research worldwide for many years. The goal in most cases has been to find a substance of low toxicity which is nonirritating and will deliver a wide variety of compounds effectively.

Efforts to improve dermal delivery of drugs have included traditional formulation approaches and studies on the effects of surfactants, fatty acids, and glycols (1, 2). Although these approaches attained some degree of success, in no case was the enhancement of drug delivery spectacular. In contrast, dimethyl sulfoxide has been shown to greatly enhance dermal and transdermal delivery of a wide variety of drugs (3–11). Unfortunately, the use of this substance has been limited in humans to the treatment of interstitial cystitis by intravesical instillation. Dimethylacetamide has been found to enhance the delivery of a number of drugs for the treatment of skin diseases (12–14). However, the lack of long-term safety information has limited the use of this compound.

In the present study, the effects of *N,N*-diethyl-*m*-toluamide (I) on skin permeability was examined for a wide variety of drugs. Formulations of I have been used extensively as insect repellents. The compound was first reported to be an effective insect repellent in 1954 (15) and has been applied *ad libitum* to the skin in concentrations ranging from 10 to 100%. Despite prolonged and widespread use in humans, major side effects due to the penetration enhancer itself have not been encountered.

EXPERIMENTAL

Reagents and Drugs—Hydrocortisone[1], hydrocortisone acetate[1], hydrocortisone 17-butyrate[2], and hydrocortisone 17-valerate[3] were among the compounds used. Also used were dibucaine[1], benzocaine[4], indomethacin[1], ibuprofen[5], erythromycin[6], tetracycline hydrochloride[7], griseofulvin[8], mycophenolic acid[9], and methyl salicylate[10]. Triethanolamine salicylate was prepared by adding equal molar amounts of

[1] Sigma Chemical Co.
[2] Analysis calculated: C, 70.24; H, 8.16. Analysis found: C, 70.20; H, 8.12. Assay by HPLC to contain <2% hydrocortisone-21-butyrate.
[3] Analysis calculated: C, 69.96; H, 8.52. Analysis found: C, 69.69; H, 8.50.
[4] ICN Pharmaceuticals, Inc.
[5] Industrie Chemicle Farmaceutiche Italiane SpA.
[6] Assay 959 μg/mg, Sigma Chemical Co.
[7] Solid supplied with Topicycline.
[8] Ayerst Laboratories.
[9] Eli Lilly and Co.
[10] Matheson Coleman and Bell.

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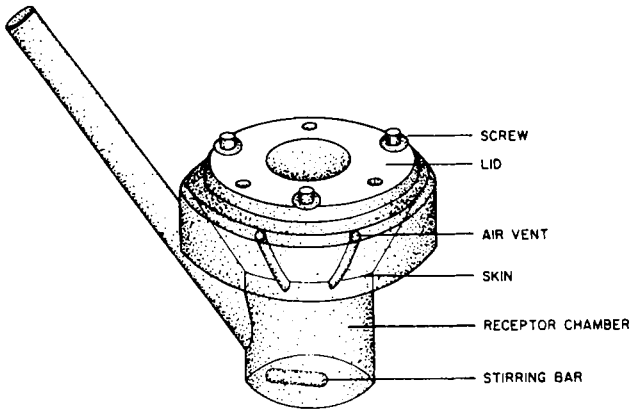

Figure 1—*Plexiglass diffusion cell with polytef lid.*

triethanolamine[11] and salicylic acid[12]. Idoxuridine[13], petrolatum USP, mineral oil[14], isopropyl myristate[1], *N,N*-diethyl-*m*-toluamide (I)[1], polyoxyethylene (2) stearyl ether[15], and polyoxyethylene 40 stearate[15] were additional compounds used. Other chemicals were reagent grade.

Procedure for Hairless Mouse Skin Diffusion Model—Transdermal drug delivery rates were determined using an *in vitro* diffusion cell procedure. Diffusion cells consisted of a plexiglass receptor chamber with a side arm to allow receptor phase sampling and a polytef lid[16] (Fig. 1). A polytef-coated stirring bar was used for receptor mixing. Female hairless mice[17] were sacrificed by cervical dislocation and the dorsal skin was removed in one piece. The skin was placed over the lower opening of the polytef lid and secured with a neoprene rubber gasket. The lid was then secured on the chamber. The exposed epidermal surface measured 8.0 cm^2. The receptor fluid was 45 ml of buffer solution consisting of 1.5×10^{-1} *M* NaCl, 5.0×10^{-4} *M* NaH_2PO_4, 2.0×10^{-4} *M* Na_2HPO_4, and 200 ppm gentamicin sulfate adjusted to pH 7.2 with sodium hydroxide or hydrochloric acid. In most cases, test formulations were applied in the amount of 0.1 ml (~100 mg/cell). The cell was placed in a thermostated chamber maintained at 32 ± 1°. The reservoir was stirred by a magnetic stirrer at 2.5 Hz. After 24 hr, a sample of the receptor fluid was withdrawn by a pipet through the side arm and emptied into a test tube. The test tube was then capped and frozen. The concentration of applied drug in the receptor fluid was measured using high-pressure liquid chromatography (HPLC). The results reported for each experiment were the average values from three replicate diffusion cells.

Procedures for Human Blanching Studies—Test formulations were applied to test areas (2 × 2 cm) on the lower backs of healthy human volunteers. Each formulation was applied to four or more test areas per subject and the results were averaged. All test areas were exposed to air (nonoccluded) for 3.5 hr after application. Any remaining formulation was then wiped off with ethanol. Blanching was evaluated at 5, 6, 7, and 8 hr postapplication. The blanching at each test area was visually appraised on a scale of 0–4, where 0 is no blanching, 1 is barely discernible blanching, and 4 is maximum blanching. Individual values for each formulation were averaged (n = 8–10). This value became the basis for comparisons.

Chromatographic Analysis—Samples of the *in vitro* receptor fluid were analyzed for the drug substance in each experiment by HPLC. The chromatographic components included pump[18], injector[19], detector[20], and recorder[21] coupled with a microparticulate column. The column type, mobile phase, and detection wavelength used for each drug substance are detailed in Table I.

Table I—HPLC Conditions for Analysis

Compound	Mobile Phase	Detection Wavelength, nm
Hydrocortisone[a]	35% acetonitrile–65% water	254
Hydrocortisone 21-acetate[b]	20% acetonitrile–20% tetrahydrofuran–60% water	254
Hydrocortisone 17-butyrate[b]	40% Tetrahydrofuran–60% water	254
Hydrocortisone 17-valerate[b]	30% acetonitrile–20% tetrahydrofuran–50% water	254
Dibucaine[c]	2 m*M* $NH_4H_2PO_4$ in 40% acetonitrile–60% water	254
Benzocaine[b]	15% acetonitrile–20% tetrahydrofuran–65% water	254
Indomethacin[c]	2 m*M* $NH_4H_2PO_4$ in 40% acetonitrile–60% water	254
Ibuprofen[c]	2 m*M* $NH_4H_2PO_4$ in 35% acetonitrile–65% water	205
Erythromycin[a]	2 m*M* $NH_4H_2PO_4$ in 30% acetonitrile–70% water	215
Tetracycline hydrochloride[a]	1 m*M* Na_2EDTA; 2 m*M* H_3PO_4 in 28% acetonitrile–72% water	254
Griseofulvin[c]	2 m*M* $NH_4H_2PO_4$ in 25% acetonitrile–75% water	295
Mycophenolic acid[c]	2 m*M* $NH_4H_2PO_4$ in 25% acetonitrile–75% water	295
Methyl salicylate[b]	4 m*M* H_3PO_4 in 40% tetrahydrofuran–60% water	254
Triethanolamine salicylate[b]	10 m*M* H_3PO_4 in 60% methanol–40% water	254

[a] Waters Associates, μBondapak, C18. [b] Brownlee Labs, RP-8. [c] Waters Associates, μBondapak, CN-RP.

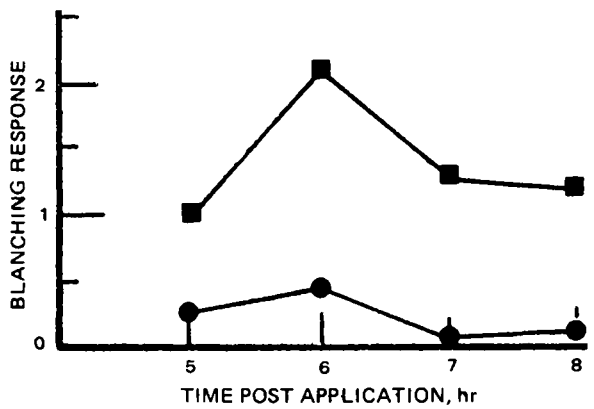

Figure 2—*Blanching results for hydrocortisone ointments in two subjects. Key: (■) 1% hydrocortisone in 5% I-petrolatum; (●) 1% hydrocortisone in petrolatum.*

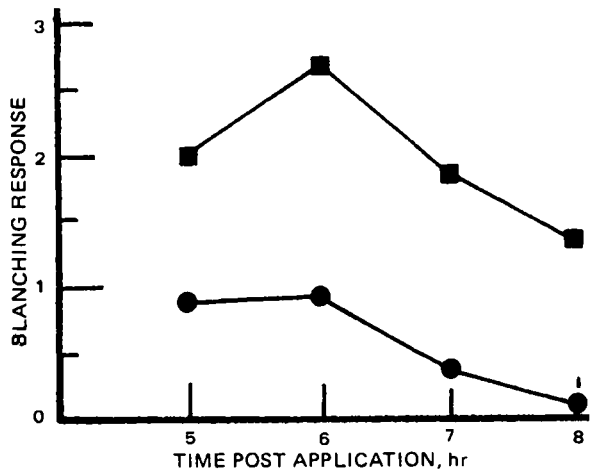

Figure 3—*Blanching results for hydrocortisone creams in two subjects. Key: (■) 1% hydrocortisone in 5% I-cream; (●) 1% hydrocortisone in cream.*

[11] Baker and Adamson Products.
[12] Fisher Scientific Co.
[13] Byron Chemical Co., Inc.
[14] Nujol, Plough, Inc.
[15] Brij 72 and Myrj 52, respectively, ICI United States, Inc.
[16] Kersco Engineering Consultants, Palo Alto, CA 94305.
[17] HRSJ, Jackson Labs, Bar Harbor, ME 04609.
[18] Model 6000A, Waters Associates.
[19] Model U6K, Waters Associates.
[20] Model 440 (at 254 nm) or Model 450 (variable), Waters Associates.
[21] Omniscribe model B5000.

Table II—*In Vitro* Diffusion of Various Drugs through Hairless Mouse Skin at 32° for 24 hr

Drug	Formulations	Applied Drug Delivered, %
Hydrocortisone	Hydrocortisone cream[a] 1% *versus* 1% drug in I	1.6 *versus* 35.0
Hydrocortisone acetate	Hydrocortisone acetate cream[b] 1% *versus* 1% drug in I	0.67 *versus* 27.6
Hydrocortisone 17-butyrate	Hydrocortisone 17-butyrate cream[c] 0.1% *versus* 0.1% drug in I	4.7 *versus* 63.1
	Hydrocortisone 17-butyrate ointment[b] 0.1% *versus* 0.1% drug in I	2.0 *versus* 63.1
Hydrocortisone 17-valerate	Hydrocortisone 17-valerate cream[d] 0.2% *versus* 0.2% drug in I	6.1 *versus* 40.8
Dibucaine	Dibucaine cream[e] 0.5% *versus* 0.5% drug in I	15.0 *versus* 82.0
Benzocaine	Benzocaine cream[f] 1% *versus* 1% drug in I	12.3 *versus* 35.7
Indomethacin	1% drug in petrolatum *versus* 1% drug in I	0.9 *versus* 37.6
Ibuprofen	1% drug in petrolatum *versus* 1% drug in I	63.5 *versus* ≃100
Erythromycin	1% drug in petrolatum *versus* 1% drug in I	not detectable *versus* 83.4
Tetracycline hydrochloride	Tetracycline lotion[g] *versus* 0.22% drug in I	not detectable *versus* ≃100
Griseofulvin	0.5% drug in petrolatum *versus* 0.5% drug in I	0.4 *versus* 29
Mycophenolic acid	1% drug in petrolatum *versus* 1% drug in I	not detectable *versus* 42
Methyl salicylate	5% drug in petrolatum *versus* 5% drug in I	not detectable *versus* 9
Triethanolamine salicylate	Triethanolamine salicylate lotion[h] 10% *versus* 1% drug in I	16 *versus* 89

[a] Hytone Cream, Dermik Laboratories, Inc., Fort Washington, PA 19034. [b] Carmol Cream and Locoid Ointment, respectively, Ingram Pharmaceutical Co., San Francisco, CA 94111. [c] Locoid Cream, Torii Pharmaceutical Co., Tokyo, Japan. [d] Westcort Cream, Westwood Pharmaceuticals, Inc., Buffalo, NY 14213. [e] Nupercainal Cream, Ciba Pharmaceuticals, Summit, NJ 07901. [f] Solarcaine Cream, Plough, Inc., Memphis, TN 38151. [g] Topicycline Lotion, Proctor and Gamble, Cincinnati, OH 45202. [h] Asper Lotion, Thompson Medical Co., Inc., New York, NY 10022.

Table III—*In Vitro* Diffusion of Hydrocortisone through Hairless Mouse Skin at 32°

Hydrocortisone in	Percent of Applied Drug[a] Delivered in 24 hr
Petrolatum	0.3
5% I-petrolatum	0.8
Cream	0.5
5% I cream	1.5

[a] One hundred milligrams (~0.1 ml, containing 1 mg of hydrocortisone) of the formulation was applied.

RESULTS AND DISCUSSION

In the *in vitro* diffusion experiments, hairless mouse skin was used as the barrier membrane. The data were generally reproducible allowing for comparisons between the relative rates of penetration of a wide variety of drugs. These diffusion cell results are not necessarily applicable to skin *in vivo*, but they do provide an indication of the relative penetration enhancement. Literature references suggest that a correlation between human and hairless mouse skin can be expected for some drugs (16, 17). Table II contains diffusion results for various drugs dissolved in pure Compound I and allowed to diffuse through the hairless mouse skin at 32° for 24 hr. All the drugs in I were solutions; however, griseofulvin required warming to obtain a solution rapidly. The I formulation was compared to a commercial product whenever possible. Where no comparable product was available, the drug was dispersed in petrolatum USP. It should be noted, however, that both ibuprofen and methyl salicylate dissolve in petrolatum, while the others are suspensions. From the results presented in Table II, it is apparent that enhancement of drug delivery is dramatic when I is present in the formulation.

The amount of pure I which diffused through the barrier membrane was also measured. Over 90% of the sample of I diffused across the hairless mouse skin in 24 hr. In an investigation (18) studying the evaporation of I from human skin *in vivo*, it was found that ~50% of the applied amount of I (25 $\mu g/cm^2$) could be recovered by evaporation, skin wiping, and skin stripping after 30 min. The remaining 50% of I, therefore, appeared to have been absorbed or associated with nonstripped skin.

Although solution preparations dissolved in I were used in the *in vitro* studies, they are less commonly used for topical application *in vivo*. Creams and ointments are the more conventional mode. To investigate enhanced drug delivery with I *in vivo*, hydrocortisone cream and ointment formulations were prepared. Hydrocortisone was chosen as the model drug due to the blanching effect it causes on human skin. The amount of skin blanching due to local vasoconstriction occurring after corticosteroid application was used as a relative measure of drug penetration.

As a preliminary experiment to examine hydrocortisone delivery, the following formulations were prepared: 1, 1% hydrocortisone in petrolatum; 2, 1% hydrocortisone in petrolatum plus 5% I; 3, 1% hydrocortisone cream; and 4, 1% hydrocortisone cream plus 5% I. These formulations differed only in that 2 and 4 contained 5% I. These preparations were examined in the hairless mouse skin diffusion test, the results of which are shown in Table III.

The hydrocortisone preparations were then applied to humans as described in the *Experimental* section. Figures 2 and 3 show the results obtained from two subjects. A large increase in the blanching response was observed for Formulations 2 and 4 relative to 1 and 3. Control experiments demonstrated that I alone does not cause skin blanching. Therefore, the increase in the blanching response clearly indicates enhanced dermal delivery of hydrocortisone effected by the presence of I in the hydrocortisone formulations. It is interesting to note that hydrocortisone delivery is improved with as little as 5% I, whereas studies with other dermal penetration enhancers such as dimethyl sulfoxide appear to require higher concentrations.

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