Percutaneous Delivery of Thalidomide and Its N-Alkyl Analogs

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Purpose. The purpose of this study was to determine the permeation parameters of thalidomide and three of its *N*-alkyl analogs and to establish a correlation between the physicochemical properties of these compounds and their percutaneous rates of absorption.

Methods. *In vitro* permeation studies were performed from buffer, *n*-alkanols and various mixed components using vertical Franz diffusion cells fitted with human epidermal membranes.

Results. Measured steady-state fluxes indicate that *N*-methyl thalidomide is a far better penetrant of human skin than the "parent molecule". However, fluxes through skin drop off markedly from that of the methylated compound when the chain length is extended to propyl and pentyl. However, they remain well above the flux of thalidomide, which is less than 0.025 $\mu g/cm^2/h$.

Conclusions. The best skin permeant of this series was the *N*-methyl analog, which also exhibited the highest water (buffer) solubility compared to thalidomide, and the *N*-propyl and *N*-pentyl analogs. The *N*-propyl and *N*-pentyl analogs were more lipid soluble and exhibited higher partition coefficient values than the *N*-methyl analog. From all the permeability data using buffer, a series of *n*-alkanols and various combinations of solvents and enhancers as vehicles, the more water-soluble compound and not the more lipid soluble one was the best skin permeant.

KEY WORDS: thalidomide; *N*-alkyl analogs; physicochemical properties; transdermal; percutaneous delivery.

INTRODUCTION

Thalidomide is a proven inhibitor of the biological synthesis of tumor necrosis factor alpha (TNF-α) and is believed to rely on this action for its suppression of the wasting of tissue that otherwise accompanies leprosy and other diffuse connective tissue disorders such as rheumatoid arthritis (1). There is good reason to believe that tissue wasting and other joint

damage done by arthritis can also be lessened or stopped upon use of this drug (2). However, the systemic levels, which have to be attained to gain this benefit from thalidomide in the instance of rheumatoid arthritis, are associated with serious untoward effects. The question we would like to answer, therefore, is whether thalidomide or a similarly acting compound can be delivered preferentially to local areas by applying it topically. The goal here is to assess the possibility of delivering a potent TNF-α inhibitor through skin as the first step in getting an effective agent into the synovial fluid of an infected rheumatoid arthritis joint. The ultimate intent would be to inhibit TNF-α production within the joints beneath and around the site of application without raising circulating levels of a drug to a point of concern. Among other possible benefits, percutaneous delivery would level out the peaks and valleys in blood levels seen with discrete oral dosages and this itself might lessen toxicity. Drug concentrations that can be achieved in deep tissues beneath the application (i.e. joints, masculature, etc.) are expected to be higher than can be achieved by oral administration of the same amount of drug (3).

Generally, molecules containing multiple hydrogen bonding centers and/or strong dipoles are high melting due to strong intermolecular crystalline self-association. Such molecules have little tendency to dissolve in organic phases and, consequently, their partitioning into the lipoidal barrier phases of the skin is minimal. Their permeation rates tend to be low even from their saturated solutions. Clearly, a low capacity to dissolve in the transport phases is a major obstacle to percutaneous delivery.

The easiest way to determine whether a drug can be delivered percutaneously is to assess the drugs *in vitro* permeability through skin. Thus, *in vitro* diffusion cell experiments were run to determine the skin absorption of thalidomide and closely structurally related analogs.

MATERIALS AND METHODS

Thalidomide and three of its odd chain *N*-alkyl analogs (methyl, propyl and pentyl), the structures of which appear in Fig. 1, were synthesized and identified as described previously (4). HPLC-grade acetonitrile and methanol were purchased from Fisher Scientific (Pittsburg, PA, USA) and used in the mobile phase of the HPLC method. For permeability studies, double-distilled deionized water and reagent grade organic solvents (Aldrich, Milwaukee, WI, USA) were used as received. Human female skin was obtained from the Anatomic Donation Program of the University of Michigan's Medical School.

Chromatographic Procedure

Thalidomide and its *N*-alkyl analogs were assayed by HPLC as described previously (4). Briefly, the HPLC consisted of a Beckman 114M solvent delivery system and a Spectraflow 783 variable wavelength UV detector set at 220 nm. A C_8 Spheri-5 micron cartridge (220 × 4.6 mm) with a guard column was used. The mobile phase consisted of 25% acetonitrile/water for thalidomide and *N*-methyl thalidomide and 35% and 45% acetonitrile for the *N*-propyl and *N*-pentyl analogs, respectively. The flow rate was set at 1.2 ml/min.

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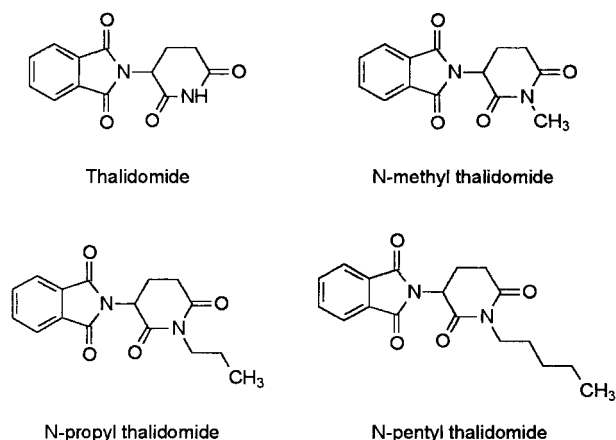
Fig. 1. Structural formula of thalidomide and its *N*-alkyl analogs.

Preparation of Donor Solutions

The donor solutions of thalidomide and its *N*-alkyl analogs were obtained by equilibrating excess amounts of each of the compounds with phosphate buffer (pH 6.4), a series of *n*-alcohols and mixed solvents and/or penetration enhancers after placing slurries of the test compounds individually into stoppered, water-jacketed, glass containers. The temperature was maintained at 32 °C by circulating water through the jackets from a constant temperature water bath. The slurries were vigorously and continuously mixed for 24 h using magnetic stirring bars. Preliminary work indicated that under the conditions used, a saturated state was achieved well within one day. Therefore, aliquots for assay were taken after 24 h of vigorous mixing. These samples were filtered through filters (PTFE filter media with Polypropylene housing, 0.45 μm pore size, Whatman Inc., Haverhill, MA, USA) preconditioned to the experimental temperature. The initial portion of the filtrate was discarded to discount possible absorption of the compounds on the film or filtering apparatus. A measured volume (300 μl) of clear filtrate was then drawn and added to the donor compartment.

Skin Preparation

Samples of split-thickness skin were removed from the thigh of female cadavers within 24 h postmortem, with the aid of a dermatome set at 250 μm. The epidermal layer was separated from a split-thickness skin section by immersing the skin section in 60 °C water for 1 min. The epidermal layer was gently teased away from the remaining tissue with forceps. The skin sections were cut into squares, wrapped in plastic film and stored in a freezer at −20 °C until they were needed. The frozen skin pieces were thawed and visually inspected for defects before mounting them within the diffusion apparatus. All frozen tissue was used within 2 months of its receipt.

Skin Permeation Method

Vertical Franz diffusion cells with a 4-ml capacity receptor compartment and a 0.8-cm^2 diffusion area were used in the permeation studies. The epidermal layer of the skin was mounted carefully onto the lower half of the cells of the diffusion apparatus with the stratum corneum facing up in the direction of the donor compartment. A donor compartment was fastened to its respective receptor compartment with a clamp, with the skin acting as a seal between the half-cells. The receptor compartments were then filled with isotonic phosphate buffer (pH 6.4). Care was taken to see that there were no consequential air bubbles left in the compartments. Skin surface temperature was maintained at 32 °C by circulating water from a constant temperature water bath (Lauda K-2/RD, Beckman Instruments, Inc., Fullerton, CA, USA) through the jacket of the lower compartment (5). The receptor cell compartments were filled and equilibrated with buffer 1 h before adding the drug-containing solution to the donor compartment. Stirring was maintained during the equilibration period and the remainder of the experiment. A small magnetic stirring bar was placed at the bottom of each receptor compartment to accomplish this. The donor compartment was filled with 300 μl of freshly prepared saturated solution of the drug and covered immediately with Parafilm to prevent evaporation of volatile components of the applied medium during the absorption experiment. At 5, 7, 9, 11, 13, and 15 h, the entire receptor volume was withdrawn and replaced with fresh buffer at 32 °C. This was done to insure that sink conditions existed throughout each experiment. Samples were directly assayed by HPLC to determine the drug concentrations in the receiver fluid. The permeation data were plotted as the cumulative amount of drug penetrated through skin as a function of time. The steady-state flux was determined from the slope of the linear portion of the cumulative amount-time plot. Permeation studies were performed using buffer and *n*-alkanols as vehicles. To enhance the skin flux and simultaneously determine which analog in the study penetrated the skin best, various combinations of solvents and penetration enhancers were combined and used as vehicles. The compositions of these formulations, A-C, were chosen based on the results of earlier experiments aimed at formulating thalidomide into a percutaneous application. Formulation A comprised of 70% isopropanol, 10% *n*-methyl pyrrolidone, 10% *n*-octanol, 5% citric acid and 5% isopropyl myristate ester (IPM). The composition of formulation B was 80% ethanol, 10% *n*-octanol, 5% citric acid and 5% IPM. Formulation C comprised of 57.5% water, 40% ethanol and 2.5% *n*-octanol.

RESULTS

The 32 °C aqueous (μg/ml) and octanol (mg/ml) solubilities, melting points and octanol/water partition coefficients (K_{oct}) of thalidomide and its *N*-alkyl analogs are summarized in Table I. Methylation of the thalidomide molecule enhanced the aqueous solubility 6-fold, but as the alkyl chain length is further extended from methyl to pentyl, the aqueous solubility decreased exponentially. The destabilization of the crystalline structure with increasing alkyl chain length led to an increase in lipophilicity of the analogs as evident in their higher octanol solubilities and octanol/water partition coefficients. By adding a methyl group to the thalidomide structure, the melting point drops by over 100 °C and upon increasing the alkyl chain length to five -CH$_2$- units, the melting points decrease linearly. When the experimental permeability coefficients from water are plotted against the partition coefficients (Fig. 2) a strong correlation is found between them. This correlation reflects the fact that skin partitioning is an element of the mass transport process, which share a common dependency with octanol/water partitioning. This alone indi-

Table I. Physicochemical Parameters and Solubilities of Thalidomide and Its *N*-Alkyl Analogs[a]

Compound	Melting point (°C)	Log K_{oct}	32°C Aqueous (pH 6.4) solubility (μg/ml)	32°C Octanol solubility (mg/ml)
Thalidomide	275	0.49	61.4	0.07
N-methyl thal.	159	1.15	370.4	2.64
N-propyl thal.	136	2.11	59.4	6.21
N-pentyl thal.	105	3.01	9.0	20.19

[a] Source: Data from Ref. 4.

cates that the skin is acting to a first good approximation as a lipophilic barrier.

The predicted permeability coefficients (K_p) of thalidomide and its N-alkyl analogs from their saturated aqueous solutions (pH 6.4) are summarized in Table II. The maximal flux of a given solute from a particular vehicle can be estimated from the product of its permeability coefficient across the membrane and its solubility in the vehicle. The maximal predicted and experimental fluxes for thalidomide and its *N*-alkyl analogs from an aqueous vehicle (buffer) are given in Table II. These fluxes were found to increase when the thalidomide molecule (log K_{oct} = 0.49) was methylated (log K_{oct} = 1.15) and decreased when the alkyl chain length was extended to propyl and pentyl (log K_{oct} = 2.11 and 3.01, respectively). Similar results were observed for NSAIDs (8). The experimental determined flux values are the mean ± standard deviation (SD) of six diffusion experiments. The flux of thalidomide was so low that it could not be detected by the HPLC method. The limit of detection of the HPLC method was 0.01 μg/ml. Thus, the flux of thalidomide was less than 0.025 μg/cm²/h. The fluxes of thalidomide and its *N*-alkyl analogs were all statistically different from one another at p < 0.01 with one exception. The fluxes of *N*-methyl thalidomide and *N*-propyl thalidomide were statistically different only at a 90% confidence level (p < 0.1).

The fluxes of thalidomide and its *N*-alkyl analogs through human skin at 32 °C using a series of *n*-alkanols as solvents are summarized in Table III. These data are plotted in Fig. 3 as the steady-state flux (μg/cm²/h) against the number of carbons in the *n*-alkanols. Declining fluxes with increased alkanol chain length can be seen with every one of the four test compounds. Thalidomide is the poorest penetrant from every one of the alkanols and *N*-methyl thalidomide turns out to be the most facile penetrant of the series in study.

The steady-state fluxes of thalidomide and its *N*-alkyl analogs, from formulations A-C can be seen in Table III and Fig. 4. To make comparisons between the four test compounds, the same skin specimen was used for the compounds applied within each individual formulation and therefore normalization of fluxes was not necessary (9). For comparisons between formulations, normalization of fluxes would be appropriate (10,11). The flux of *N*-methyl thalidomide is statistically higher (p < 0.05) than that of thalidomide and the other analogs in all three formulations.

DISCUSSION

We are working on the hypothesis that thalidomide or an analog thereof can be delivered into tissues beneath the skin from topical applications in sufficient quantity to arrest the degenerative changes associated with rheumatoid arthritis.

The purpose of this study was to determine the permeation parameters of thalidomide and several *N*-alkyl analogs from water to obtain baseline permeation data that allow comparisons with other compounds. We also set about testing a series of *n*-alkanols and various other vehicles to take measures of the intrinsic percutaneous permeabilities of these drugs as a step towards predicting their potentials as topical therapeutic systems. Our intent was also to establish a correlation between the physicochemical properties of these compounds and their percutaneous rates of absorption.

Compounds that are absorbed through skin *in vivo* are mainly taken up and cleared systemically by blood vessels directly beneath the epidermis. Thus, compounds do not have to penetrate the full thickness of the skin (epidermis and dermis) before entering the vasculature system. Accordingly, for *in vitro* permeation studies, the epidermis (including the stratum corneum) is often if not usually separated from the underlying dermis using a heat separation technique (12). This technique cannot confidently be applied to hairy skin because the hair shafts are anchored firmly in and remain in the dermis, creating holes in the epidermal membrane as the dermis is pulled away. To obviate all concern here, only skin from female cadavers was used in this study. Harrison and co-workers (13) examined the integrity of the barrier layer following storage after various time periods. No differences
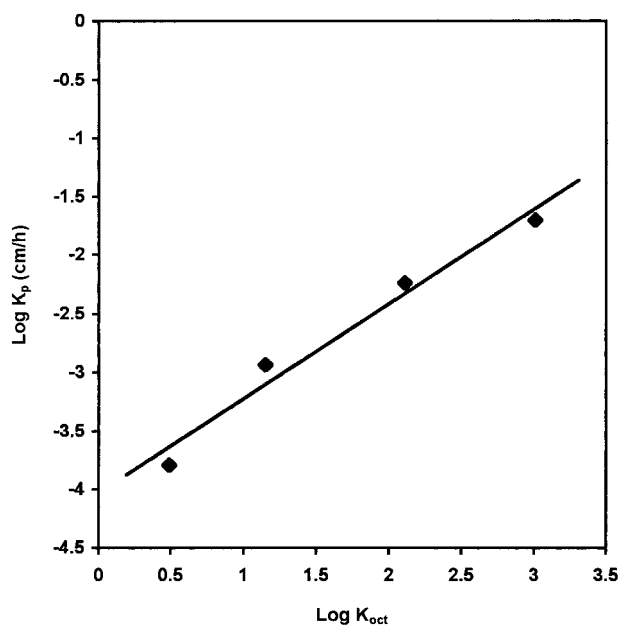
Fig. 2. Logarithmic plot of experimentally derived aqueous permeability coefficient (Log K_p) vs. partition coefficient (Log K_{oct}) for thalidomide and its *N*-alkyl analogs.

Table II. Predicted and Experimental Permeation Parameters of Thalidomide and its *N*-Alkyl Analogs through Human Skin from Saturated Buffer Solutions

Compound	Predicted permeability coefficient[a] (cm/h)	Predicted flux[b] (μg/cm^2/h)	Experimental flux $\pm$ SD (μg/cm^2/h)
Thalidomide	0.119×10^{-3}	0.007	—[c]
N-methyl thal.	0.287×10^{-3}	0.105	0.43 ± 0.08
N-propyl thal.	0.929×10^{-3}	0.055	0.34 ± 0.13
N-pentyl thal.	2.730×10^{-3}	0.025	0.18 ± 0.06

[a] Calculating according to Ref. 6.
[b] Calculating according to Ref. 7.
[c] Flux was too low to be detected by HPLC method.

were found between measurements of *in vitro* percutaneous penetration of tritiated water in skin stored at –20 °C for up to 466 days vs. fresh skin stored at 10 °C and used within 2–3 days of autopsy, indicating the barrier properties inherent in skin remain intact under the conditions of storage used in this work.

Ethanol is one of the most commonly used skin permeation enhancers and its use as part of a co-solvent system with water has been observed to increase permeation of a wide range of drugs through human and animal skin both *in vivo* or *in vitro*. Several compounds are commercially delivered from transdermal systems having alcohol-containing reservoirs (14). Estradiol and fentanyl are two transdermal drugs, which appears in formulations containing ethanol as a penetration enhancer. It was hypothesized that this solvent or a closely related compound might prove to be of benefit with respect to delivering thalidomide. Systematic studies were begun using a range of homologous *n*-alkanols to explore the hypothesis. It is also known that the degree of flux enhancement may increase upon blending a nonpolar, long-chain enhancer into ethanol or isopropanol (15). It has been reasoned that, while the polar enhancers traverse the skin, the nonpolar enhancers are largely retained in the stratum corneum, aspects that appear to make such combinations superior enhancer systems

(16). In terms of toxicology, ethanol and isopropanol are the most acceptable of the short-chain alcohols. However, to better understand the structural requirements for enhancement, and to determine which compound in study penetrates the skin best, flux studies were performed using all alkanols that are liquid at room temperature, e.g. alkanols up to a chain length of twelve, irrespective of toxicity. Truly extraordinary results were obtained upon doing so.

Steady-state fluxes of thalidomide and its three *N*-alkyl analogs through human skin when each of the compounds was administered as a saturated solution in each of the alkanols are presented in Fig. 3. The first pattern that emerges upon viewing the data is that the alkanols become increasingly less effective vehicles as their alkyl chain length is extended. Although there is variability in the data and some minor reversals in the general pattern, a trend to declining permeability as the alkanol chain is lengthened is seen with every one of the four test compounds. The experimental fluxes of thalidomide and its *N*-alkyl analogs from buffer, a series of *n*-alkanols and different formulations (Tables II and III) showed clearly that thalidomide is the poorest penetrant from every one of these donor vehicles. These data also indicate that *N*-methyl thalidomide is the most facile penetrant from saturated solutions throughout the alkanol series and
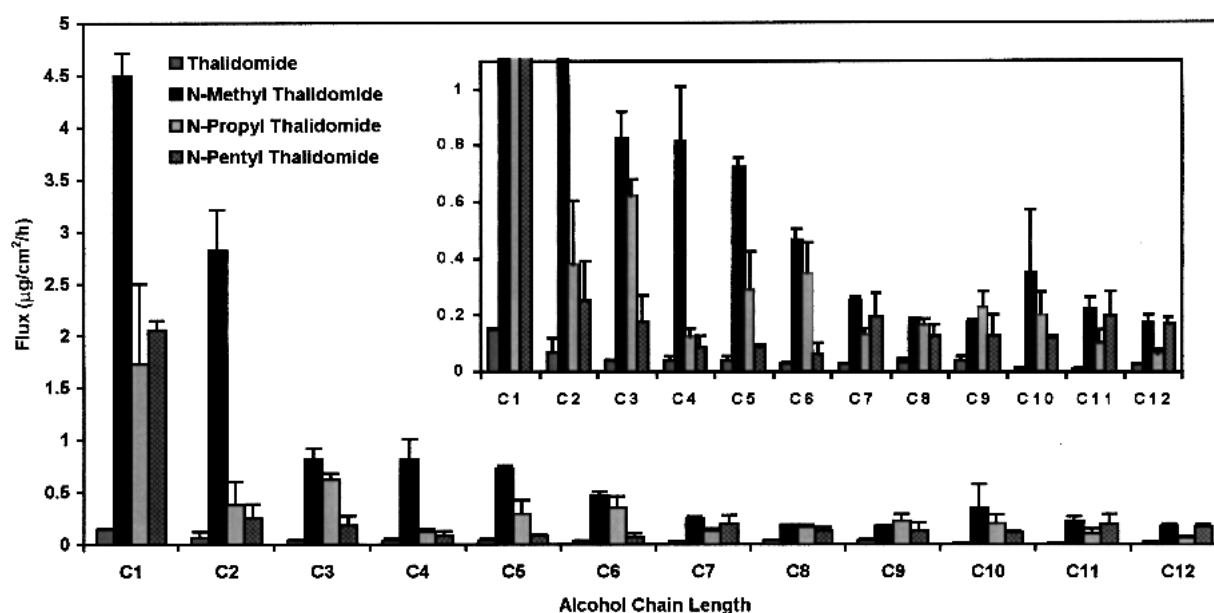
Fig. 3. Steady-state fluxes of thalidomide and its *N*-alkyl analogs from saturated *n*-alcohol solutions plotted against the alcohol chain length. These data are the mean $\pm$ standard deviation (SD) of 3 diffusion experiments.

Table III. Steady-State Flux Values of Thalidomide and its *N*-Alkyl Analogs from a Series of n-Alcohols and Three Different Formulations

Vehicle	Steady-state flux (μg/cm^2/h) $\pm$ standard deviation			
	Thalidomide	*N*-methyl thalidomide	*N*-propyl thalidomide	*N*-pentyl thalidomide
Methanol (C1)	0.147 ± 0.005	4.498 ± 0.220	1.730 ± 0.768	2.059 ± 0.086
Ethanol (C2)	0.066 ± 0.053	2.819 ± 0.391	0.378 ± 0.226	0.250 ± 0.139
Propanol (C3)	0.039 ± 0.002	0.822 ± 0.096	0.621 ± 0.058	0.178 ± 0.096
Butanol (C4)	0.037 ± 0.016	0.813 ± 0.196	0.123 ± 0.03	0.083 ± 0.044
Pentanol (C5)	0.039 ± 0.014	0.722 ± 0.034	0.291 ± 0.134	0.086 ± 0.01
Hexanol (C6)	0.028 ± 0.007	0.468 ± 0.037	0.347 ± 0.111	0.065 ± 0.036
Heptanol (C7)	0.029 ± 0.002	0.250 ± 0.016	0.129 ± 0.023	0.192 ± 0.084
Octanol (C8)	0.035 ± 0.010	0.185 ± 0.003	0.164 ± 0.022	0.124 ± 0.038
Nonanol (C9)	0.036 ± 0.018	0.174 ± 0.008	0.225 ± 0.062	0.126 ± 0.077
Decanol (C10)	0.018 ± 0.001	0.351 ± 0.220	0.201 ± 0.081	0.117 ± 0.007
Undecanol (C11)	0.010 ± 0.003	0.223 ± 0.040	0.101 ± 0.047	0.196 ± 0.091
Dodecanol (C12)	0.025 ± 0.004	0.172 ± 0.030	0.063 ± 0.016	0.169 ± 0.023
Formulation A	0.156 ± 0.021	2.070 ± 0.282	0.666 ± 0.141	0.096 ± 0.048
Formulation B	0.323 ± 0.193	2.710 ± 0.538	0.563 ± 0.172	0.244 ± 0.096
Formulation C	0.831 ± 0.218	6.540 ± 0.448	2.125 ± 0.293	2.142 ± 0.178

from buffer. This is not a phenomenon strictly associated with alkanols and buffer, the same happened when the three vehicles identified as A, B, and C was employed. Formulations A–C were selected based on earlier experiences we had for mulating thalidomide for percutaneous application. The bar plots in Fig. 4 indicate that, relative to thalidomide, there is initially an extraordinary increase in steady-state flux when the compound is methylated. However, fluxes through skin fall back markedly when the chain length is extended to propyl and pentyl. *N*-Methyl thalidomide has the highest maximum flux, the smallest log K_{oct} and octanol solubility, but the highest water solubility of the *N*-alkyl analogs. The maximum flux decreased in the order *N*-methyl, *N*-propyl, *N*-pentyl, and thalidomide. This rank order in skin permeability correspond well with the results from a study done by Le and Lippold

Fig. 4. Bar plot showing a mean (n = 3) steady-state flux ± standard deviation (SD) of thalidomide and its *N*-alkyl analogs from different formulations.

(17) where methyl nicotinate has the highest maximum flux, the smallest log K_{oct}, but the highest water solubility. Roy and co-workers (18) also showed that the skin flux of alkyl *p*-aminobenzoates was in the order methyl > ethyl > butyl. Again, the methyl ester has the lowest silicone/water partition coefficient but the highest solubility in water. The rank order in skin permeability for thalidomide and its *N*-alkyl analogs (*N*-methyl > *N*-propyl > *N*-pentyl > thalidomide) from all the different donor vehicles also correlates well with the maximum predicted flux (Table II) when the buffer solubility is taken into account. No correlation was observed between lipid solubility and skin permeability. Waranis and Sloan (19) also noted that there is no correlation between lipid solubility of a prodrug and relative ability to deliver a drug through the skin. They also stated that the best prodrugs of the series in terms of delivering 6-mercaptopurine (6-MP) through the skin, regardless of the vehicle, has been attributed mainly to the increased water solubility of these two prodrugs compared with that of 6-MP and the other prodrugs.

CONCLUSION

Since the stratum corneum is basically a lipophilic barrier, drug lipophilicity is regarded as one of the key parameters which controls drug skin permeation and thus it is expected that more lipophilic drug derivatives could show better partitioning and solubility into the stratum corneum which could result in enhanced skin permeation. However, due to the biphasic nature of the skin, a balance should exist between the lipid and water solubilities of drugs needed for enhanced transdermal drug delivery. Based on experimental data, authors have pointed out that water solubility plays a role as important as lipophilicity in the skin permeation process (19–21). From all the permeability data of thalidomide and its *N*-alkyl analogs, using water, a series of *n*-alkanols and various combinations of solvents and enhancers as vehicles, it is clear that *N*-methyl thalidomide is the best permeant of this series. The *N*-propyl and *N*-pentyl analogs exhibit significantly higher lipid solubility and lower aqueous solubility than *N*-methyl thalidomide. The results in this study show that in each donor vehicle the more water-soluble compound
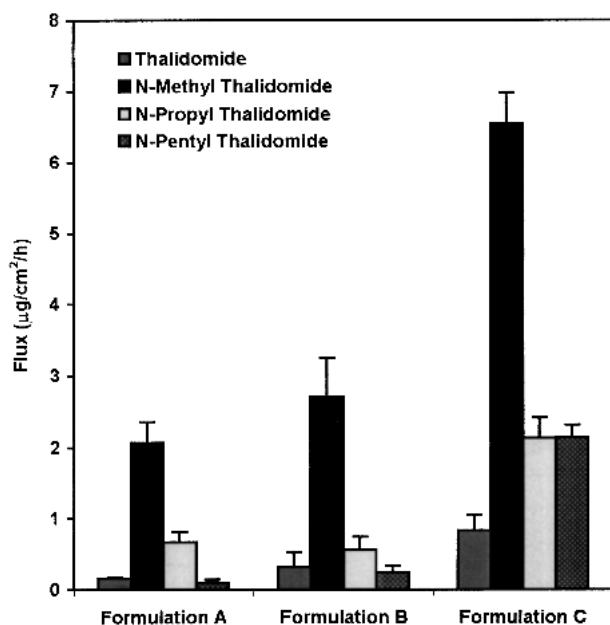
and not the more lipid soluble one was more efficient in penetrating the skin. The high transdermal flux of *N*-methyl thalidomide is associated with its high aqueous solubility, low molecular weight, low melting point and moderately high lipophilicity. In conclusion, this study showed that increased lipophilicity or partition coefficient values did not result in a higher skin flux, but the highest flux through skin is achieved by the more lipophilic analog that showed the highest water solubility.

ACKNOWLEDGMENTS

REFERENCES

1. E. P. Sampaio, E. N. Sarno, R. Galilly, Z. A. Cohn, and G. Kaplan. Thalidomide selectively inhibits tumor necrosis factor alpha production by stimulated human monocytes. *J. Exp. Med.* **173**:699–703 (1991).

2. O. Gutiérrez-Rodríguez. Thalidomide: a promising new treatment for rheumatoid arthritis. *Arthritis Rheum.* **27**:1118–1121 (1984).

3. P. Singh and M. S. Roberts. Skin permeability and local tissue concentrations of nonsteroidal anti-inflammatory drugs after topical application. *J. Pharmacol. Exp. Ther.* **268**:144–151 (1994).

4. C. Goosen, T. J. Laing, J. du Plessis, T. C. Goosen, and G. L. Flynn. Physicochemical characterization and solubility analysis of thalidomide and its *N*-alkyl analogs. *Pharm. Res.* **19**:13–19 (2002).

5. J. A. Cordero, L. Alarcon, E. Escribano, R. Obach, and J. Domenech. A comparative study of the transdermal penetration of a series of nonsteroidal antiinflammatory drugs. *J. Pharm. Sci.* **86**:503–508 (1997).

6. R. O. Potts and R. H. Guy. Predicting skin permeability. *Pharm. Res.* **9**:663–669 (1992).

7. J. Hadgraft, J. du Plessis, and C. Goosen. The selection of nonsteroidal anti-inflammatory agents for dermal delivery. *Int. J. Pharm.* **207**:31–37 (2000).

8. T. Yano, A. Nakagawa, M. Tsuji, and K. Noda. Skin permeability of various non-steroidal anti-inflammatory drugs in man. *Life Sci.* **39**:1043–1050 (1986).

9. S. D. Roy and E. Manoukian. Permeability of ketorolac acid and its ester analogs (prodrug) through human cadaver skin. *J. Pharm. Sci.* **83**:1548–1553 (1994).

10. U. Hagedorn-Leweke and B. C. Lippold. Absorption of sunscreens and other compounds through human skin in vivo: derivation of a method to predict maximum fluxes. *Pharm. Res.* **12**:1354–1360 (1995).

11. B. P. Wenkers and B. C. Lippold. Skin penetration of nonsteroidal antiinflammatory drugs out of a lipophilic vehicle: influence of the viable epidermis. *J. Pharm. Sci.* **88**:1326–1331 (1999).

12. O. Díez-Sales, A. C. Watkinson, M. Herráez-Domínguez, C. Javaloyes, and J. Hadgraft. A mechanistic investigation of the *in vitro* human skin permeation enhancing effect of Azone®. *Int. J. Pharm.* **129**:33–40 (1996).

13. S. M. Harrison, B. W. Barry, and P. H. Dugard. Effects of freezing on human skin permeability. *J. Pharm. Pharmacol.* **36**:261–264 (1984).

14. N. A. Megrab, A. C. Williams, and B. W. Barry. Estradiol permeation across human skin, silastic and snake skin membranes: the effects of ethanol/water co-solvent systems. *Int. J. Pharm.* **116**:101–112 (1995).

15. D-D Kim, J.L. Kim, and Y.W. Chien. Mutual hairless rat skin permeation-enhancing effect of ethanol/water system and oleic acid. *J. Pharm. Sci.* **85**:1191–1195 (1996).

16. R. Kadir, D. Stempler, Z. Liron, and S. Cohen. Delivery of theophylline into excised human skin from alkanoic acid solutions: A "Push-Pull" mechanism. *J. Pharm. Sci.* **76**:774–779 (1987).

17. V. H. Le and B. C. Lippold. Influence of physicochemical properties of homologous esters of nicotinic acid on skin permeability and maximum flux. *Int. J. Pharm.* **124**:285–292 (1995).

18. S. D. Roy, J. Fujiki, and J. S. Fleitman. Permeabilities of alkyl *p*-aminobenzoates through living skin equivalent and cadaver skin. *J. Pharm. Sci.* **82**:1266–1268 (1993).

19. R. P. Waranis and K. B. Sloan. Effects of vehicles and prodrug properties and their interactions on the delivery of 6-mercaptopurine through skin: bisacyloxymethyl-6-mercaptopurine prodrugs. *J. Pharm. Sci.* **76**:587–595 (1987).

20. F. P. Bonina, L. Montenegro, P. De Caprariis, F. Palagiano, G. Trapani, and G. Liso. *In vitro* and in vivo evaluation of polyoxyethylene indomethacin esters as dermal prodrugs. *J. Control. Release* **34**:223–232 (1995).

21. J. Rautio, T. Nevalainen, H. Taipale, J. Vepsäläinen, J. Gynther, T. Pedersen, and T. Järvinen. Synthesis and *in vitro* evaluation of aminoacyloxyalkyl esters of 2-(6-methoxy-2-naphthyl)propionic acid as novel naproxen prodrugs for dermal drug delivery. *Pharm. Res.* **16**:1172–1178 (1999).