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Synthesis and investigation of urea compounds as transdermal penetration enhancers

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Abstract

Twelve urea and thiourea compounds were examined for their transdermal penetration enhancing properties in vitro using hairless mouse skin and hydrocortisone as a model drug. Skins were pretreated for 1 h with the enhancer in propylene glycol before application of the drug also in propylene glycol. Enhancement ratios (*ER*) were calculated for permeability coefficient (*P*), 24 h receptor concentration (Q_{24}), and skin content of drug (*SC*) and compared to control values (no enhancer present). Control values for permeability coefficients were 0.71 ± 0.27 cm h^{-1}, 24 h receptor concentration: 11.9 ± 3.4 μM, and skin content of drug 44.6 ± 18.5 $\mu g\ g^{-1}$. The most effective enhancer of the series was 1-dodecyl-3-methylthiourea. This enhancer produced the highest values for all three penetration parameters with a permeability coefficient $4.7 \pm 1.8 \times 10^{-4}$ cm h^{-1} (ER_P 6.6), a 24 h receptor concentration of 62.7 ± 16.3 μM (ER_{Q24} 5.3), and a skin content of 90.1 ± 17.7 $\mu g\ g^{-1}$ (ER_{SC} 2.0). A structure-activity relationship was observed in that the thio-substituted analogs were more effective enhancers than the oxygen-containing compounds. Although these compounds show promise as penetration enhancers, further study is needed to determine their effectiveness with other drugs and their irritation potential.

Keywords: Transdermal drug delivery; Ureas; Hairless mouse; Percutaneous penetration; Hydrocortisone; Skin retention

1. Introduction

Due to its accessibility, skin has been considered as a potential route for systemic drug administration, and research into transdermal drug

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delivery has expanded greatly over the last two decades. Transdermal drug delivery possesses several advantages over more traditional methods. These include avoidance of first-pass intestinal and hepatic metabolism, avoidance of variable rates of absorption and metabolism inherent with oral treatment, continuous, non-invasive infusion of drugs which have short biological half-lives, avoidance of the risks and the inconvenience associated with parenteral treatment, and elimination of gastrointestinal irritation resulting from pharmaceutically active and inactive ingredients. Unfortunately, only a few drugs possess the physicochemical properties necessary for this route of delivery with most drugs being unable to cross the skin in quantities required for successful systemic therapy (Barry, 1983). The barrier to percutaneous drug penetration is presented by the top-most layer of the skin, the stratum corneum, which consists of protein filled cells surrounded by lipid lamellar sheets in a brick and mortar wall-like configuration (Barry and Bennett, 1987). Several methods have been employed to lessen the barrier function of the skin. One approach has been the use of transdermal penetration enhancers. These agents are chemical compounds which reversibly alter the barrier function of the skin and allow an increased rate of percutaneous permeation of coadministered drugs. Although the exact mechanism of action of enhancers has not been fully elucidated, it is known that they exert their effects partially by altering either the stratum corneum proteins, lipids, or both (Barry, 1987).

The selection of an enhancer for a transdermal product should be based on its efficacy, lack of toxicity, and compatibility with other components of the transdermal system (Skelly et al., 1987). An ideal enhancer should be pharmacologically inert, odorless, colorless, nontoxic, nonirritating, nonallergenic, and compatible with most drugs and excipients (Ranade, 1991). In addition, the onset and duration of the enhancer effect should be predictable, reproducible, and reversible (Skelly et al., 1987). In addition, enhancers should have solubility parameters similar to that of the skin (Barry, 1987).

The goal of this study is to identify potentially new urea-based penetration enhancers using an in vitro hairless mouse skin model and hydrocortisone as the model drug. Urea (compound **1**) is a colorless, odorless, slightly hygroscopic crystalline powder which is very soluble in water (1 g in 1.5 ml water) (Gunnar, 1995). It is used most commonly as a hydrating agent and is employed in the treatment of ichthyosis, psoriasis, and other hyperkeratotic skin conditions. It has been suggested that urea increases the water content of the skin and also acts as a mild keratolytic agent which could affect the stratum corneum corneocytes. These actions led researchers to believe that urea would increase the penetration of drugs through the skin (Williams and Barry, 1989).

2. Materials and methods

All chemicals and deuterated NMR solvents were purchased from Aldrich (Milwaukee, WS). Elemental analyses were conducted by Atlantic Microlabs (Atlanta, GA) and were within $\pm 0.4\%$ of theoretical for all compounds. Baxter Diagnostics (McGraw Park, IL) supplied reagent grade solvents, except for methanol and acetonitrile which were HPLC grade. All urea analogs were synthesized by the medicinal chemistry department at the University of South Carolina College of Pharmacy with the exception of **1**, **7**, and **8** which were purchased from Aldrich (Milwaukee, WS). The NMR spectra were determined on a Brucker WH-400 spectrometer.

2.1. Enhancer synthesis

*2.1.1. 1-Dodecylurea (**2**)*

Urea (7.18 g, 39 mmol) and dodecylamine (2.32 g, 39 mmol) were dissolved in dry pyridine (60 ml). The solution was refluxed for 4 h and the insoluble product was removed by filtration. The crystals were washed with water, then recrystallized from chloroform (4.62 g, 52%). Further recrystallization from methanol yielded a product with a melting point of 106–107°C which was in agreement with the melting point reported in the literature (106.8–107.5°C) for this compound (Williams and Barry, 1989).

^{1}H NMR(DMSO − d_6)δ0.86(t, 3H, CH_3)

×, 1.24(m, 20H, $(C\underline{H}_2)_{10}CH_3$)

×, 2.91(m, 2H, $C\underline{H}_2N(C{=}O)NH_2$)

×, 5.33(s, 2H, NH_2), 5.87(bs, 1H, NH).

*2.1.2. 1-Dodecyl-3-methylurea (**3**)*

Employing established synthetic technology (Vishnyakova et al., 1985) dodecylamine (1.87 g, 10 mmol) was dissolved in 100 ml methylene chloride and cooled to 0°C. Methyl isocyanate (0.57 g, 10 mmol) was added to the solution in one portion with immediate formation of a precipitate. The mixture was then refluxed for 2 h, the solution was cooled, then allowed to stir at room temperature for 12 h. The resulting crude product was removed by filtration and recrystallized from benzene to yield white crystals (1.82 g, 75%). mp 82–83°C.

Anal ($C_{14}H_{30}N_2O$) Calc.: C, 69.37; H, 12.47; N, 11.56; O, 6.60. Found: C, 69.17; H, 12.33; N, 11.65.

^{1}H NMR($CDCl_3$)δ 0.86(t, 3H, CH_3)

×, 1.23(m, 18H, $(C\underline{H}_2)_9CH_3$)

×, 1.48(m, 2H $C\underline{H}_2CH_2N$), 2.77(s, 3H, NCH_3)

×, 3.13(m, 2H, CH_2N).

*2.1.3. 1-Dodecyl-3-methythiourea (**4**)*

Dodecylamine (4.63 g, 25 mmol) was dissolved in 100 ml methylene chloride and cooled to 0°C. Methyl isothiocyanate (1.83 g, 25 mmol) was added to the solution in one portion with immediate formation of a precipitate. The mixture was then refluxed for 2 h, the solution was cooled, then allowed to stir at room temperature for 12 h. The resulting crude product was removed by filtration and recrystallized from benzene to yield white crystals (5.44 g, 84%). A melting point of 68–69°C was in agreement with the melting point (68–69°C) reported in the literature (Erickson, 1956).

^{1}H NMR($CDCl_3$)δ 0.86(t, 3H, CH_3)

×, 1.24(m, 18H, $(C\underline{H}_2)_9CH_3$)

×, 1.58(broad m, 2H, $C\underline{H}_2CH_2N$)

×, 3.02(s, 3H, NCH_3), 3.36(broad m, 2H, CH_2N).

*2.1.4. 1,3-Didodecylurea (**5**)*

Urea (2.0 g, 35 mmol) and dodecylamine (13.0 g, 70 mmol) were dissolved in butan-1-ol (20 ml) and refluxed for 18 h. The solution was cooled and the precipitated product removed with vacuum filtration and then recrystallized from methanol (9.6 g, 71%). A melting point of 102–103°C was in agreement with the melting point reported (103.3–105.5°C) in the literature (Erickson, 1954).

^{1}H NMR($CDCl_3$)δ 0.86(t, 6H, CH_3)

×, 1.23(m, 36H, $(C\underline{H}_2)_9CH_3$)

×, 1.57(m, 4H $C\underline{H}_2CH_2N$), 3.13(t, 4H, CH_2N).

*2.1.5. 1,3-Didodecylthiourea (**6**)*

Carbon disulfide (0.76 g, 10 mmol) and dodecylamine (3.76 g, 20 mmol) were dissolved in toluene (100 ml). The resulting solution was refluxed for 12 h, cooled, and filtered to yield white crystals (3.12 g, 82%). A melting point of 77–78°C was in agreement with the melting point reported (69–70°C) in the literature (Hoskins et al., 1940).

^{1}H NMR($CDCl_3$)δ 0.86(t, 6H, CH_3)

×, 1.24(m, 40H, $(C\underline{H}_2)_{10}CH_3$)

×, 3.50(m, 4H, CH_2N).

*2.1.6. 1-Dodecyl-3-phenylurea (**9**)*

Dodecylamine (4.63 g, 25 mmol) was dissolved in 100 ml methylene chloride and cooled to 0°C. Phenyl isocyanate (2.98 g, 25 mmol) was added to the solution in one portion with immediate formation of a precipitate. The mixture was refluxed for 2 h, the solution was cooled, then allowed to stir at room temperature for 12 h. The resulting crude product was removed by filtration and recrystallized from benzene to yield white crystals (5.44 g, 84%). mp 83–83.5°C.

Anal ($C_{19}H_{32}N_2O$) Calc.: C, 74.95; H, 10.59; N, 9.20; O, 5.25 Found: C, 74.78; H, 10.46; N, 9.18.

^{1}H NMR(DMSO-d_6)δ 0.86(t, 3H, CH_3)

×, 1.24(m, 18H, $(C\underline{H}_2)_9CH_3$)

×, 1.41(broad m, 2H $C\underline{H}_2CH_2N$)

×, 3.04(t, 2H, CH_2N), 6.90–7.40(m, 5H, C_6H_5).

*2.1.7. 1-Dodecyl-3-phenylthiourea (**10**)*

Dodecylamine (4.63 g, 25 mmol) was dissolved in 100 ml methylene chloride and cooled to 0°C. Phenyl isothiocyanate (3.38 g, 25 mmol) was added to the solution in one portion with immediate formation of a precipitate. The mixture was refluxed for 2 h, the solution was cooled, then allowed to stir at room temperature for 12 h. The resulting crude product was removed by filtration and recrystallized from benzene to yield white crystals (5.45 g, 68%). A melting point of 70.5–72°C was in agreement with the melting point (70–72°C) reported in the literature (Williams and Barry, 1989).

^{1}H NMR(DMSO-d_6)δ 0.86(t, 3H, CH_3)

×, 1.25(m, 18H, $(C\underline{H}_2)_9CH_3$)

×, 1.52(m, 2H $C\underline{H}_2CH_2N$), 3.43(m, 2H, NCH_2)

×, 7.09(t, 1H, NH), 7.27

–7.41(m, 5H, C_6H_5), 7.72(broad m, 1H, NH).

2.2. Solubility determinations of ureas in propylene glycol

A small amount of solid urea enhancer was dissolved in 500 μl of propylene glycol in a small test tube and covered with Parafilm®. The test tube was vortexed and placed in a heat block maintained at 32 ± 0.2°C, which is the temperature of the surface of the skin (Sheard et al., 1939). The tubes were then vortexed every 24 h and additional enhancer was added until visual saturation was achieved.

2.3. In vitro permeability studies

Permeability studies were performed using methods previously described (Michniak et al., 1993a). Male hairless mice strain SKH1 (hour–hour) 8 weeks old, were obtained from Charles Rivers Laboratories (Wilmington, MA). Animals were sacrificed by CO_2 asphyxiation and full-thickness abdominal and dorsal skin was excised. Any extraneous subcutaneous fat was removed from the dorsal surface. The skins were stored at −80°C (Revco Scientific, Asheville, NC) until utilized. Skins were then slowly thawed, cut into small pieces and mounted on modified Franz diffusion cells (Vangard International, Neptune, NJ).

Each diffusion cell (donor surface area 3.14 cm^2; receptor volume 12 ml) contained isotonic phosphate buffer solution (pH 7.2), 0.1% v/v 36% aqueous formaldehyde as a preservative (Sloan et al., 1991), and 0.5% w/v polyoxyethylene 20 cetyl ether as a solubilizer (Chien, 1982). The receptors were maintained at 37 ± 0.5°C through the use of a circulating water bath. The receptors were continuously stirred at 600 rpm using magnetic stirring bars. Skins were allowed to hydrate for 1 h prior to experimentation.

Control experiments consisted of no pretreatment of the skin with enhancer solution, while propylene glycol (PG) control consisted of pretreating the skin with 8 μl of PG. All enhancers were tested at their maximal solubility in propylene glycol (Table 1) or 0.4 M for compound **1**. Following hydration each skin was covered with 8 μl of enhancer solution. Suspensions of enhancers in PG were prepared by adding excess enhancer to

Table 1
Approximate saturation solubilities of urea transdermal penetration enhancers in propylene glycol at 32 ± 0.5°C (Phillips, 1995)

Compound #	Approximate saturation solubility (M)
1	>0.4
2	0.026
3	0.038
4	0.053
5	0.001
6	0.002
7	0.028
8	0.057
9	0.007
10	0.026

propylene glycol, centrifuging the excess solid to the bottom of the tube, and using the supernatant as the enhancer solution. The enhancer solution was left on the skin for 1 h prior to drug application and was not washed off at the end of this period.

At this time 80 μl of a suspension of hydrocortisone in propylene glycol was placed on each skin (Michniak et al., 1993b). The hydrocortisone suspensions were prepared in a manner similar to the enhancer solution with the exception of the centrifugation. There was solid hydrocortisone suspended in the propylene glycol to provide a high enough dose of drug to maintain a hydrocortisone concentration gradient across the skin for the entire 24 h experiment. The solubility of hydrocortisone in propylene glycol at 32 ± 0.5°C was 0.03 M (Phillips, 1995). The donor compartment was then occluded with Parafilm®. Samples (300 μl) of receptor phase were withdrawn at specified time points over 24 h with the samples being immediately replaced with fresh buffer. Analysis of samples was corrected for all previous samples removed.

2.4. Skin homogenization

At 24 h, the skins were removed from the receptor cells and washed three times in 100 ml of methanol for a total of 15 s. Following room temperature drying, each skin was weighed, cut up, placed in 4 ml of methanol, and homogenized using a tissue homogenizer (Kinematica, Switzerland). The homogenate was then gravity filtered, passed through a Sep–Pak C_{18} cartridge (Waters, Milford, MA), and all samples were stored at -80°C until analysis (Michniak et al., 1993a).

2.5. Sample analysis

Analysis of samples for drug content was performed using high performance liquid chromatography. All solvents were HPLC grade. HPLC analysis were performed using a Perkin–Elmer system which consisted of an ISS 100 Automatic Sampling System, an SEC-4 Solvent Environment Control, a Series 410 LC pump, and a LC-235 diode array detector. The HPLC was controlled by an Epson III + computer with Omega peak integration software (Perkin–Elmer, CT).

All drug samples were analyzed using a reversed-phase C_{18} column (Rainin Microsorb MV, 4.6 mm I.D. $\times$ 25 cm, 5 μm) kept at room temperature. Hydrocortisone was detected at 242 nm with a retention time of approximately 4.5 min using a mobile phase of 40:60 acetonitrile:water at a flow rate of 1.0 ml min^{-1}. An external standard of hydrocortisone (5 mg/100 ml) was used. Testing the linearity of the validation plot from 1.0 to 100 μg ml^{-1} revealed a correlation coefficient of 0.9960. Intraday and interday variabilities were determined to be 10.1 and 14.0% respectively.

2.6. Data analysis

Cumulative amounts of drug (μM) corrected for sample removal were plotted against time (hours). Permeation profiles were calculated from permeability coefficients (cm h^{-1}), receptor concentrations at 24 h ($Q_{24,}$ μM), and skin content of drug (SC, μg g^{-1}). Enhancement ratios (ER) were calculated from permeability coefficients (P, flux–solubility), 24 h receptor concentration (Q_{24}), and skin content of drug (SC) after enhance treatment of skin divided by the same parameter after no enhancer treatment (control).

$$\mathrm{ER} = \frac{\text{permeation parameter after enhancer treatment}}{\text{permeation parameter from control}}$$

Statistical treatment of the data involved the use of analysis of variance (ANOVA) and two-tailed Student's t-test. The α-value was set at 0.05 and the null hypothesis assumed the variances between enhancer and control to be equal. Therefore if $p < 0.05$, there was a significant difference between the enhancer and control.

3. Results and discussion

A series of urea and thiourea compounds (Fig. 1) were tested for their ability to enhance the

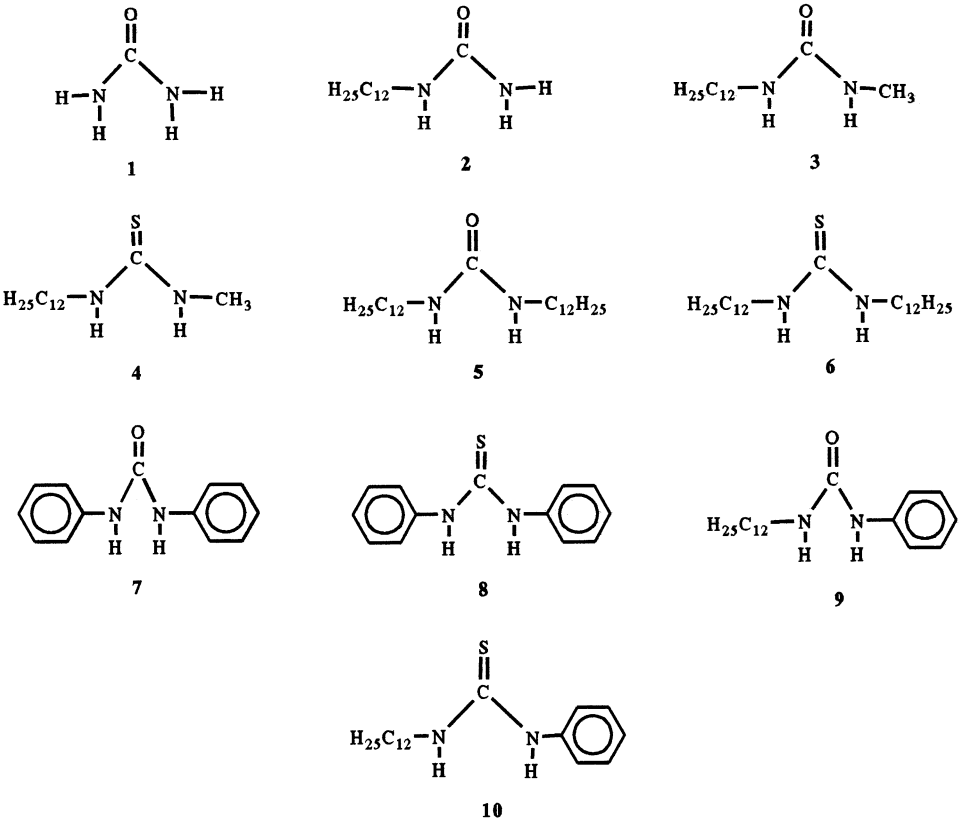

Fig. 1. Structures of urea enhancers: (**1**) urea; (**2**) 1-dodecylurea; (**3**) 1-dodecyl-3-methylurea; (**4**) 1-dodecyl-3-methylthiourea; (**5**) 1,3-didodecylurea; (**6**) 1,3-didodecylthiourea; (**7**) 1,3-diphenylurea; (**8**) 1,3-diphenylthiourea; (**9**) 1-dodecyl-3-phenylurea; (**10**) 1-dodecyl-3-phenylthiourea.

penetration of hydrocortisone across hairless mouse skin. All urea compounds, except for **1**, had approximate saturation solubilities which were below 0.4 M (Table 1) thus these enhancers were applied to the skin at maximum thermodynamic activity (Phillips, 1995). All results are expressed as mean $\pm$ standard deviation. Control experiments are run with only propylene glycol and hydrocortisone present with no enhancer. Control values for permeability coefficients are 0.71 ± 0.27 cm h^{-1}, Q_{24}: 11.9 ± 3.4 μM, and skin content of drug 44.6 ± 18.5 μg g^{-1}.

All urea compounds give significantly higher ($p < 0.05$) Q_{24} data with the exception of compound **9** (Table 2; Figs. 2 and 3). The most effective enhancer of the urea series was compound **4** This enhancer had with a 24 h receptor concentration of 62.7 ± 16.3 μM (ER_{Q24} 5.3). Compound **8** had the next highest receptor concentration with a Q_{24} of 44.4 ± 7.7 μM (ER_{Q24} 3.7). Compound **10** also had significantly increased Q_{24} when compared to control with a value of 40.0 ± 14.3 μM (ER_{Q24} 3.4).

The urea compounds do not show as high a degree of enhancement for permeability coefficients as compared to Q_{24}. Only compounds **4**, **5**, **7**, **8**, and **10** show significant increases in permeability coefficients, while nine of the ten enhancers had significant increases in receptor concentrations (Table 2). The most effective enhancer was compound **4** with a permeability coefficient of 4.7 ± 1.8 cm h^{-1} (ER_P 6.6). The next most active enhancer was compound **8** with a value of 1.9 ± 0.34 cm h^{-1} (ER_P 2.6). These data suggest that

Table 2
Percutaneous absorption parameters for hydrocortisone with urea enhancers

Enhancer in PG[a]	$P \times 10^{-4}$[b] (cm h^{-1})	ER_P[c]	Q_{24}[d] (μM)	ER_{Q24}[c]	SC[e] ($\mu g\ g^{-1}$)	ER_{SC}[c]
Control ($n=9$)	0.71 ± 0.3	1.0	11.9 ± 3.4	1.0	44.6 ± 18.4	1.0
PG control ($n=14$)	0.79 ± 0.22	1.1	11.7 ± 3.9	0.99	32.8 ± 20.2	0.72
1 ($n=5$)	0.86 ± 0.16	1.2	18.2 ± 3.0	1.5	33.9 ± 7.5	0.76
2 ($n=4$)	0.78 ± 0.62	1.1	32.9 ± 10.5	2.8	50.3 ± 14.3	1.1
3 ($n=5$)	0.83 ± 0.46	1.1	21.5 ± 4.3	1.8	16.4 ± 5.4	0.37
4 ($n=5$)	4.7 ± 1.8	6.6	62.7 ± 16.3	5.3	90.1 ± 17.7	2.0
5 ($n=5$)	1.7 ± 0.5	2.4	22.3 ± 5.2	1.9	35.3 ± 4.8	0.79
6 ($n=4$)	0.98 ± 0.30	1.4	18.5 ± 5.1	1.6	36.5 ± 13.4	0.82
7 ($n=5$)	1.8 ± 0.24	2.5	23.7 ± 3.44	2.0	62.9 ± 39.8	1.4
8 ($n=5$)	1.9 ± 0.34	2.6	44.4 ± 7.7	3.7	79.3 ± 16.1	1.7
9 ($n=5$)	1.2 ± 0.67	1.7	13.2 ± 3.4	1.1	48.3 ± 12.6	1.1
10 ($n=5$)	1.7 ± 0.5	2.4	40.0 ± 14.3	3.4	71.2 ± 26.8	1.6

[a] Propylene glycol
[b] Permeability coefficient.
[c] *ER*, enhancement ratio.
[d] Q_{24}, receptor concentration after 24 h.
[e] *SC*, skin content of hydrocortisone).

ureas slowly increase the permeability of hairless mouse skin. Permeability coefficients are calculated from the initial (1–4 h) portion of the permeability curve, thus the initial enhancement of drug delivery may not be as great as the enhancement over the full 24 h of experimental time. This time may be required for the ureas to breakdown the keratinocytes of the stratum corneum to increase permeability. In order to increase the initial rate of penetration of drug, an increase in pretreatment time may be required.

The urea compounds tested do not show a high degree of enhancement in skin contents of drug. Only three compounds have significant increases in skin content of hydrocortisone. Compound **4** had an ER_{SC} of 2.0, compound **8** had an ER_{SC} of 1.7, and compound **10** had an ER_{SC} of 1.6. All other compounds showed insignificant enhancement or skin content less than control.

The most effective urea enhancer was compound **4** which had the highest enhancement ratios for all three transdermal categories with ER_P of 6.6, ER_{Q24} of 5.3, and ER_{SC} of 2.0. The second most active urea analog was compound **8**. This analog had the second highest enhancement ratios for each of the permeability parameters with ER_P 2.6, ER_{Q24} 3.7, and ER_{SC} 1.7. The third most effectual enhancer was compound **10** with ER_P 2.4, ER_{Q24} 3.4, and an ER_{SC} of 1.6. It may be noted that in each case the thio analog of each urea compound was more effective than the oxygen-containing structure.

Urea has long been known to increase the penetration of drugs into the skin through several mechanisms of action (Raab, 1989). Its keratolytic properties can lead to the breakdown of corneocytes over time (Williams and Barry, 1989). The hygroscopic nature of urea can also be the cause of penetration enhancement since the increased water content of stratum corneum will increase the amount of drug penetrating through the skin. This has also been suggested as being the main mechanism of action of urea (Barry, 1991). However, it has been proposed that urea may alter the integrity of the lipids of the epidermal barrier. Beastall suggested that urea may lower the phase transition temperature of the lipids of the stratum corneum so that they become fluidized at the ambient temperature of the skin (Beastall, 1986). Finally, due to the fact that urea decreases the lag time required to reach steady-state diffusion of drugs such as 5-fluorouracil, one study has suggested that urea may interact with the proteins of the stratum corneum thereby decreasing drug binding (Williams and Barry, 1989).

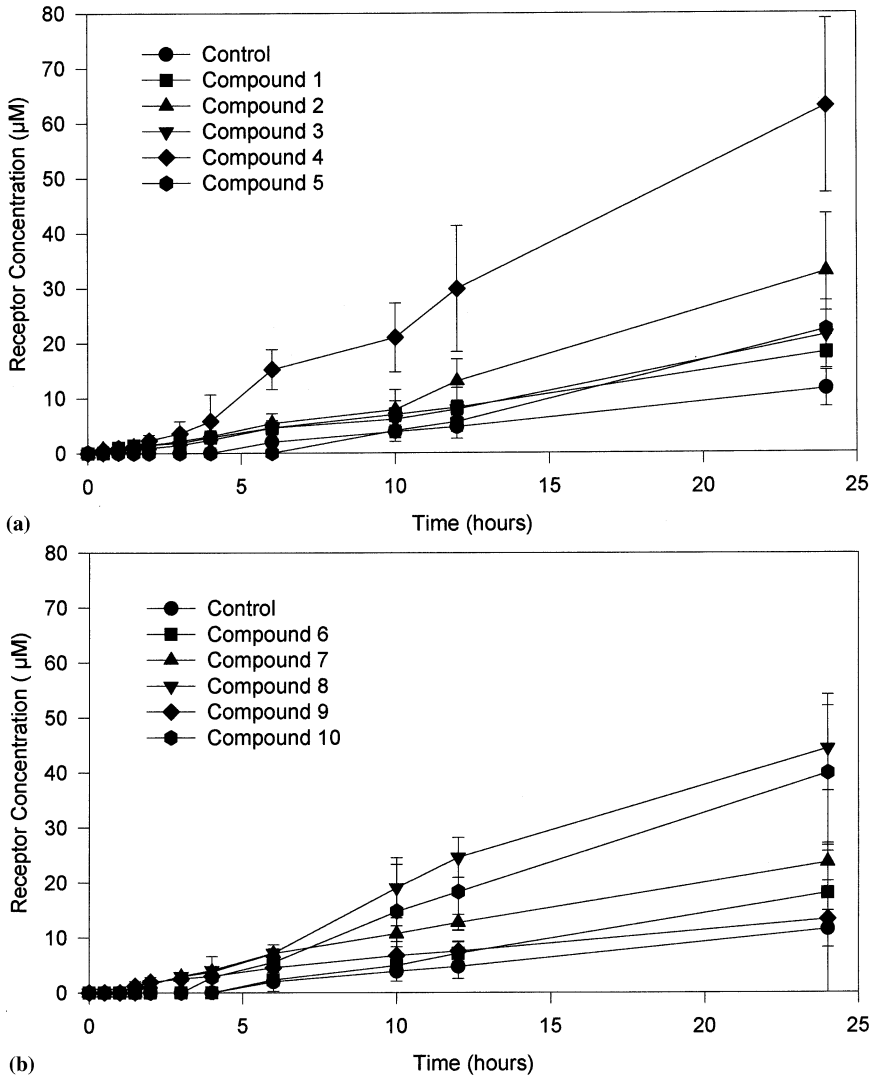

Fig. 2. Effect of urea enhancers on diffusion of hydrocortisone through hairless mouse skin. (a) Compounds **1**–**5** and (b) compounds **6**–**10**.

Urea is also a component of the natural moisturizing factor (NMF) present in the skin. The major components of NMF are free fatty acids (40%), pyrrolidonecarboxylic acid (12%), and urea (7%) (Ritschel and Sprockel, 1988; Lippold and Hackemuller, 1990). Some of the earliest studies of transdermal penetration enhancement used urea to increase the penetration of hydrocortisone. One study using human volunteers found that a cream containing hydrocortisone and 10% urea increased the steroid permeation 2-fold (Feldman and Maibach, 1974). Another study, which examined six hydrocortisone creams, found that the urea containing creams significantly increased the activity and bioavailabiltiy of the steroid when compared to the non-urea containing creams (Barry and Woodford, 1976). Another study revealed that the addition of urea to a 1% hydrocortisone cream elevated the penetration of hydrocortisone 2–3-fold within all three layers of

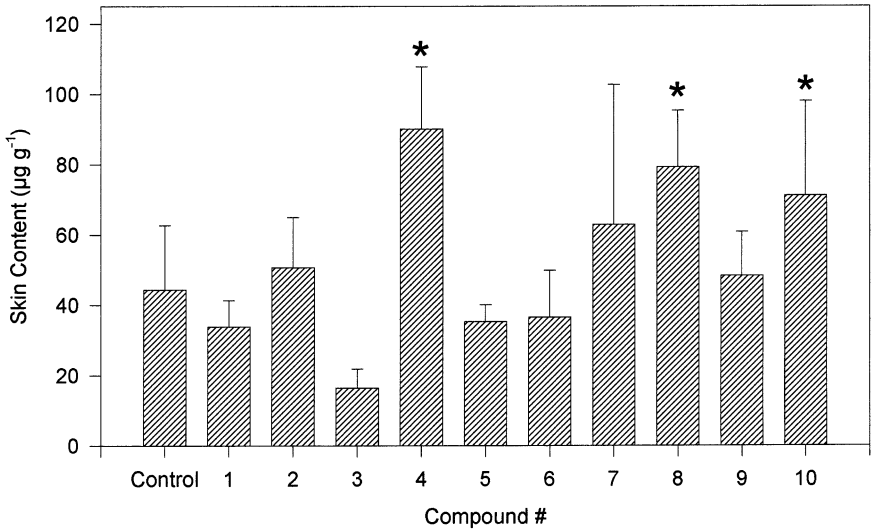

Fig. 3. Effect of urea enhancers on skin content of hydrocortisone in hairless mouse. * Denotes statistically significant difference from control ($p < 0.05$).

the skin (stratum corneum, epidermis, and dermis) (Wohlrab, 1984). This data is in agreement with the current study which showed that urea increased the penetration of hydrocortisone 1.5-fold through hairless mouse skin.

In addition, a study examining the effect of urea on the penetration of ketoprofen through rat skin showed that urea elevated permeation 8–50-fold depending on the vehicle used. The increased degree of enhancement over hydrocortisone can be explained by the fact that ketoprofen has a lower molecular weight than hydrocortisone. The study also revealed that urea formed large, hydrophilic channels through the excised skin (Kim et al., 1993). The small ketoprofen molecules will more readily pass through these channels than hydrocortisone. This size dependence on enhancement is further demonstrated by a study which revealed that a formulation of dihydroergotamine in propylene glycol and 6% urea showed no significant enhancement from the control (Niazy, 1991). Dihydroergotamine has a molecular weight of 679.8 as compared to 362.5 for hydrocortisone, and 254.3 for ketoprofen. There seems to exist an inverse relationship between permeation enhancement by urea and molecular weight of permeant.

A study examining the effect of urea enhanced transport of indomethacin (mol.wt. 357.8) revealed that urea alone did not increase the penetration of the model drug. However, in combination with various length alkanols, urea did increase the penetration 1–2-fold. There exists a direct relationship between alkanol chain length and degree of enhancement. The researchers theorized that the alkanols accelerated the transport of urea into the stratum corneum, and urea, after uptake, disrupts the barrier function of the skin (Nishihata et al., 1990).

In addition to urea, there have been a few studies done on analogs of urea. Williams and Barry (1989) studied urea (compound **1**) and its analogs 1-dodecylurea (compound **2**), 1,3-didodecylurea (compound **5**), 1,3-diphenylurea (compound **7**) for their permeation enhancing abilities with 5-fluorouracil as a model drug. They reported that 1-dodecylurea was the most effective compound with an ER_P of 4.3. 1,3-diphenylurea had the next highest enhancement ratio with an ER_P of 3.5. 1,3-didodecylurea and urea had permeability coefficient ratios of 2.8

and 1.1, respectively (1992). The results from the current study are in agreement with this data.

Results from the current studies display trends which have been demonstrated by past studies involving enhancement of transdermal drug delivery. The most effective compound (**4**) contains a dodecyl side chain and a methyl group. The dodecyl chain has been shown to be important in enhancing the penetration of drugs through the stratum corneum. It is known that the dodecyl chain disrupts the stratum corneum structure and increase penetration through this less ordered environment (Michniak et al., 1993c, 1994a,b, 1995; Sasaki et al., 1990, 1991). In addition to compound **4**, other compounds within this series also contain dodecyl side chains (compounds **5**, **6**, **9**, and **10**). However, in addition to the single dodecyl chain, these compounds also contain additional functional groups: compounds **5** and **6** contain two dodecyl chains, while compounds **9** and **10** contain phenyl groups. It is likely these relatively large groups decreased the penetration of the urea analog into the stratum corneum, thereby decreasing their penetration enhancing abilities.

The data from this study of urea enhancers also suggests a structure-activity relationship concerning the substitution of a sulfur for the oxygen of the urea analogs. In three of four cases, the substitution of a sulfur for an oxygen increased the enhancing ability of the compound. Compounds **4**, **8**, and **10** (the thio analogs of compounds **3**, **7**, and **9**) had higher ER_P and ER_{Q24} than the oxygen containing compound (Figs. 2 and 3; Table 2). Only compound **5** was a better enhancer than its thio analog compound **6** and that difference was not statistically significant.

In conclusion, this study has shown urea and thiourea analogs to be effective penetration enhancers for hydrocortisone. Further studies of these compounds will be necessary to determine their enhancing properties for more hydrophilic model drugs. In addition, irritation studies should be performed to determine the urea analogs potential as transdermal penetration enhancers.

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