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Percutaneous Absorption-Enhancing Activity of Urea Derivatives

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Abstract □ The effect of urea and urea derivatives on the percutaneous absorption of salicylic acid and sodium salicylate through the skin of rabbit from petrolatum ointment was investigated. It was found that addition of urea or urea derivatives to the ointment base significantly increased the percutaneous absorption of the drugs in proportion to the concentratoin of the additive. The percutaneous absorptoin-enhancing activities of these compounds were that urea derivatives with the more and longer alkyl substituents showed the stronger activities. These activities of urea and urea derivatives were ascribed to the binding of these compounds with the lipids and proteins of the stratum corneum of the skin and the swelling of the tissues, which leads to the reduction of the barrier property of the layer. The preliminary skin irritation test showed that urea and urea derivatives were quite non-irritating to the skin. These results suggest that urea derivatives have a strong possibility to be developed as a percutaneous absorption enhancer.

Keywords □ Percutaneous absorption, salicylic acid, urea derivatives, skin ointment.

Recently, transdermal drug delivery to achieve systemic pharmacological effects has been developed as a viable means to administer therapeutic agents[1-3]. It has some advantages of bypassing hepatic first-pass elimination or GI degradation. It can also reduce side effects by optimizing the blood concentration-time profile, and avoid multiple-dosing inconveniences. Many drugs are candidates for transdermal delivery. Some drugs penetrate the skin at rates high enough to yield therapeutic levels in plasma. Such drugs are, however, the exception since most drugs do not penetrate intact skin in therapeutic amounts. This limitation is particularly evident with hydrophilic drugs. The excellent barrier properties of the stratum corneum of the skin have limited the drugs chosen for transdermal delivery to those whose daily requirements are on the order of mg/day. Other limiting factors include the irritation or allergic resposnses to the skin induced by the drugs or vehicles.

Since the skin acts as an excellent barrier, percutaneous absorption enhancers have ben developed, which would reversibly reduce the barrier resistance of the stratum corneum and thus allow the drug to penetrate to the viable tissues and enter the systemic circulation. The stratum corneum is compact and highly keratinized tissue, and lipids and proteins of the tissue provide a complex structure that is quite impermeable. Enhancers must alter the proteins and/or the lipids to make the skin easier for molecules to diffuse through the layer. An ideal percutaneous absorption enhancer in addition to being safe, non-toxic and non-irritating should also be pharmacologically inert.

Lipophilic solvents such as dimethylsulfoxide, dimethylformamide and 2-pyrrolidone have been well known as percutaneous absorption enhancers[4-7]. They can swell the stratum corneum may solubilize the lipids, and thus enhance the transdermal transport of drugs. Surface active agents have also been found to enhance the percutaneous absorption of drugs[8-14]. Surface active agents are, however, skin irritants; therefore a balance between absorption enhancement and irritation has to be considered. Polar lipids which have unsaturated fatty acids have been found to enhance the percutaneous ab-

sorption of drugs[15]. Recently, azone was found to have percutaneous absorption enhancing properties[1]. Unsaturated fatty acids and azone have a property in common; they have a bulky moiety in their chemical structures. When these compounds incorporate into the stratum corneum, they might induce less compact organization of the tissue and increase the fluidity of the tissue. This increment in fluidity might induce percutaneous absorption enhancement of drugs.

Urea is a protein denaturant and have been known to have a percutaneous absorption enhancing property[16]. In this laboratory, we have intensively studied effects of urea and its derivatives on the hydrodynamic properties of polyoxyethylated nonionic surfactants and some polymers. We found that alkyl derivatives of urea are far more effective than urea in affecting the solution properties of the surfactants and polymers[17,18]. We paid attention to this point and undertook this study to develop urea derivatives as percutaneous absorption enhancers.

EXPERIMENTAL

Materials

Tetramethylurea was purchased from Sigma Chemical Co., USA. Methylurea, 1,3-diemthylurea, ethylurea, and n-butylurea were obtained from Fluka Co., Switzerland. Dimethylsulfoxide (DMSO) was supplied from Kokusan Chemical Co., Japan. Urea, white vaseline, salicylic acid and sodium salicylate were supplied from Junsei Chemical Co., Japan and all other chemicals were of analytical grade.

Preparation of test ointments

Salicylic acid and sodium salicylate, previously reduced to fine powders in a ball mill, were passed through a No. 80 mesh sieve and dried at 50℃ in a heated vaccum dessicator for 48 hrs before use. The ointments prepared by a fusion method contained 10% (w/w) salicylic acid or 11.6% (w/w) sodium salicylate and a desired amount of urea, urea derivatives or DMSO. Each gredient was accurately weighed and incorporated into the ointment base. The base used was white vaseline.

Test animals

Rabbits weighing between 2.5 to 3.0 kg were commercially supplied. The rabbits were maintained on rabbit chow and water ad libitum and housed individually in an animal room at room temperature. Twenty-four hours prior to the application of the ointment to the rabbit, hair was carefully removed with an animal clipper from the skin of the dorsal area on both sides of the spine.

Application of ointments

Accurately weighed 6.9g of the ointment was uniformly spread over the shaved skin of the animal ($8\times10\,cm^2$) and covered with a linear-low-density polyethylene wrap film. To minimize contamination and to bring the ointment into adequate contact with the wrap film, the applied site was wrapped with a adhesive bandage. The rabbits were fixed during experiment. For 6 hours, the ointment was left on the skin, and food and water were not given. On completion of a single test, the application was removed and the applied area was thoroughly washed with warm water. The ointment left on the application site and the washing were collected. A 7-day rest period ensued before reapplication of ointment.

Procedure

One-half m*l* of blood was withdrawn from the marginal ear vein of the rabbit at thirty minutes after the ointment application and then hourly intervals for 6 hours. Then, 0.1 m*l* of heparin sodium was added to the blood sample. This mixture was centrifuged at 2,000 rpm for 10 minutes, and 0.2 m*l* of the resulting plasma was taken into a test tube containing 2 m*l* of distilled water and 2 m*l* of Trinder's reagent[19]. Colorimetric analysis for salicylate was performed by reading the absorbance of the developed color at 540 nm. The concentration of the salicylate was read from the calibration curve. The residual ointment at the site of application of the skin was recovered as well as possible. The residue was diluted with methanol to 100 m*l* and shaken well. From the clear part of the solution, a desired amount of the solution was taken, and diluted with water. A certain amout of aqueous ferric chloride solution was added to this solution. Colorimetric analysis of salicylate was performed as formerly described. The experiments were repeated four times. The recovery of the drug in the residual ointment was investigated in a separate control experiment.

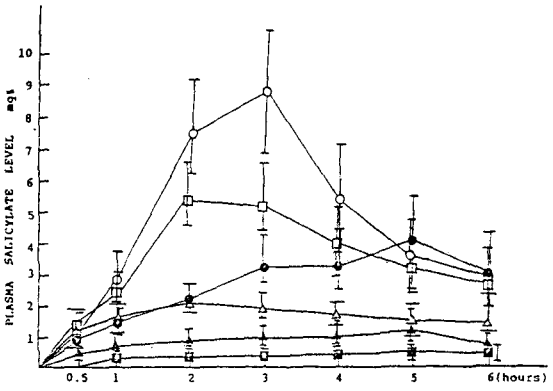

Fig. 1. Effect of urea derivatives and DMSO on percutaneous absorption of salicylic acid from petrolatum ointment containing 10% salicylic acid (n=4).
Key; 10% salicylic acid plus 10% tetramethylurea (○), 10% salicylic acid puls 10% butylurea (●), 10% salicylic acid plus 10% methylurea (△), 10% salicylic acid plus 10% urea (▲), 10% salicylic acid plus 10% DMSO (□), and control (■).

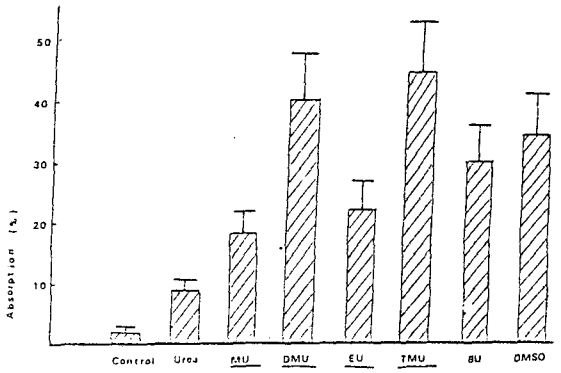

Fig. 2. Effect of urea derivatives and DMSO on percutaneous absorption of salicylic acid from petrolatum ointment containing 10% salicylic acid. The concentrations of urea derivatives and DMSO were 5%, and the absorption (%) was calculated from the amount of the residual drug in ointment after the application period.
Key; MU; methylurea, DMU; 1,3-dimethylurea, EU; ethylurea, TMU; tetramethylurea, and BU; *n*-butylurea.

The average recovery of the five control experiments was 96% (±2%).

Preliminary skin irritation test

The general preliminary skin irritation test of ointment vehicles was modified for simplicity in this study. Two grams of the test ointment containing 5% or 10% urea derivative was applied to rabbit ($4\times4\,cm^2$) with or without occlusive dressing. In order to avoid differences in stratum corneum thickness at different body areas, all of these test agents were applied to the dorsal area where the experiment for percutaneous absorption was carried out. For 10 days, the test ointment was applied to the rabbit and the doses were exchanged every 12 hours. Each time the applied skin was carefully searched for adverse reactions that usually show erythema and scaling. Adverse reactions were graded on scale of none, mild, moderate and severe.

RESULTS

The effect of urea and various urea derivatives on percutaneous absorption of salicylic acid and sodium salicylate was investigated. There was only little percutaneous absorption of salicylic acid when petrolatum ointment containing 10% salicylic acid without any enhancer was applied to the skin of rabbit. Fig. 1 shows that percutaneous absorption of salicylic acid was significantly increased with adding urea or urea derivative to the vehicle. All urea derivatives tested in this experiment, methylurea, 1,3-dimethylurea, ethylurea, *n*-butylurea and tetramethylurea showed the better effects than urea. Of them, tetramethylurea showed a remarkably high absorption enhancing effect. When the potency of tetramethylurea was compared with that of DMSO which is well known as an excellent percutaneous absorption enhancer, the effect of tetramethylurea was beffer than that of DMSO under the experimental condition.

The percutaneous absorption experiments were evaluated with two different ways; determination of plasma concentration of the drug and measurement of the residual amount of the drug on the skin. The residual amount of salicylic acid after 6 hours application of the ointment containing 10% salicylic acid and 10% urea derivative or DMSO was determined and the results were shown in Fig. 2. The results also showed that urea and urea derivatives significantly increased the percutaneous absorption of salicylic acid, and alkyl substitution in urea enhances the percutaneous absorption enhancing

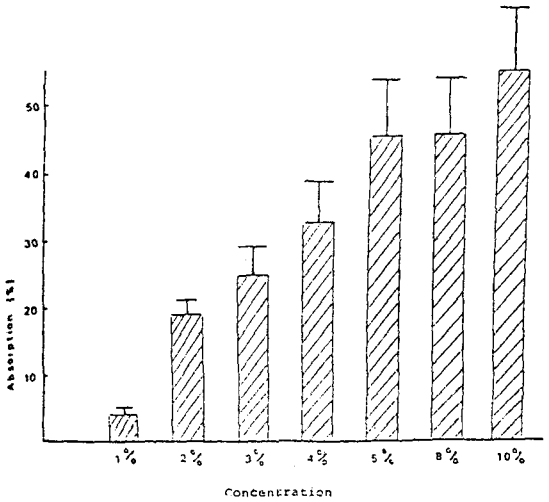

Fig. 3. Effect of various concentration of tetratmethylurea on percutaneous absorption of salicylic acid from petrolatum ointment containing 10% salicylic acid. The absorption (%) was calculated from the amount of the residual drug in ointment after the application period.

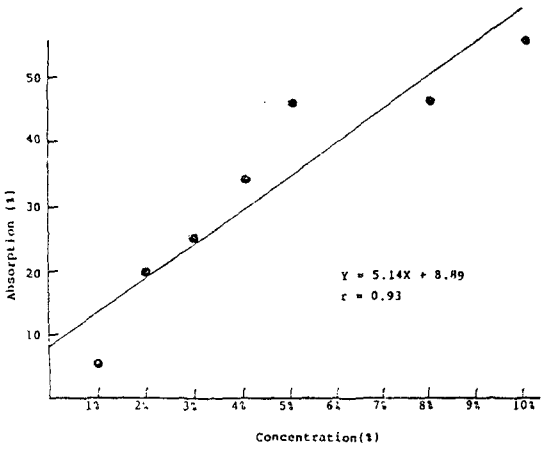

Fig. 4. Dependence of the absorption rate of salicylic acid on the concentration of TMU in the petrolatum ointment.

activitity of urea. Of the urea derivatives, tetramethylurea, *n*-butylurea and 1,3-dimethylurea were remarkably effective and these effects were comparable to the activity of DMSO. The effect of the concentration of tetramethylurea in ointment vehicle on the percutaneous absorption enhancing activity was examined and the results was shown in Fig. 3. The result revealed that the percutaeous absorption enhancing activity of tetramethylurea was concentration dependent and increased in proportion to the concentration of the urea derivative as shown in Fig. 4. The maximum increment observed with tetramethylurea was about 27-fold higher relative to the control at the concentration level of 10% tetramethylurea in ointment.

The effect of urea and urea derivatives on the percutaneous absorption of sodium salicylate, an ionic form was investigated. The results were illustrated in Fig. 4. Urea and urea derivatives also increased the percutaneous absorption of sodium salicylate. However, the activities were consistently less potent than the effects of these compounds on the percutaneous absorption of salicylic acid.

Preliminary skin irritation test of urea and urea derivatives on the skin of rabbit was carried out. The results were that urea and urea derivatives except tetramethylurea did not show any significant skin irritation on test animals within 10-day period. However, the ointment containing 10% tetramethylurea induced mild skin irritation on one of five test animals on the ninth day of the experiment when the test agent was applied without occlusive dressing. The ointment containing 10% tetramethylurea induced mild skin irritation on one of five test animals on the seventh day, on three of five test animals on the ninth day, when the agent was applied under occlusive dressing. The ointment containing 5% tetramethylurea did not show any positive reactions when applied without occlusive dressing and a mild reaction on one of five test animals on the tenth day when applied under occlusive dressing.

DISCUSSION

It has been known that urea and urea derivatives act as a protein denaturant and enhance the solubility of hydrophobic compounds in water[20,21]. These properties of urea and urea derivatives have been ascribed to the direct binding of these compounds with proteins or hydrophobic compounds[22-26].

Some suggest that urea and urea derivatives reduce hydrophobic bonding via breaking the iceberg water structure around the hydrophobic moieties[27,28]. The results of this study showed that urea and urea derivatives have significant percutaneous absorption

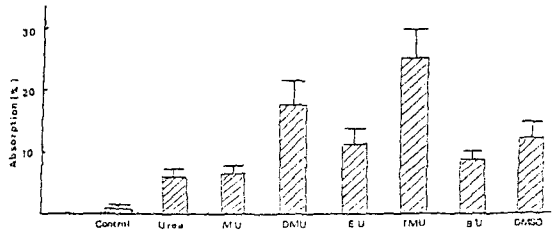

Fig. 5. Effect of urea derivatives and DMSO on percutaneous absorption of sodium salicylate from petrolatum ointment containing 11.6% sodium salicylate. The concentrations of urea derivatives and DMSO were 5%, and the absorption (%) was calculated from the amount of the residual drug in ointment after the application period.
Key; MU; methylurea, DMU; 1,3-dimethylurea, EU; ethylurea, TMU; tetramethylurea, and BU; *n*-butylurea.

enhancing activities for salicylic acid and sodium salicylate from petrolatum ointment base. The general trend of this activity was that the more and longer alkyl substitution in urea provides the stronger activities. Especially, tetramethylurea was remarkable in increasing the percutaneous absorption of both of these drugs. This trend is similar to that of the effects of these compounds on denaturing proteins[29]. However, *n*-butylurea shows some deviation from this trend. Although *n*-butylurea is very effective protein denaturant, this compound showed less percutaneous enhancing activity than tetramethylurea. This might be ascribed to the low solubility of *n*-butylurea in water. It is worthwhile to note that tetramethylurea is in a liquid state while other urea derivatives are solids at room temperature and it mixes well with organic solvents as well as with water. These properties of tetramethylurea might have some relation with the precutaneous absorption enhancing activity. Marongiu *et al.*[30] measured the heats of solution of urea derivatives. They interpreted the results in terms of intracomponent molecular interaction and stabilization of the water structure by the solute molecules. They suggested that the geometry, the length and number of alkyl groups have an important action on these two effects. The same trend was observed for the percutaneous absorption enhancing activity of urea derivatives. The geometry of the alkyl substitution might have an important bearing and further study along this line would be interesting.

The results of this research suggest that urea and urea derivatives could be used as a percutaneous absorption enhancer for hydrophobic compounds as well as for hydrophilic ones. These properties might be ascribed to the binding of these compounds with lipids or proteins of the stratum corneum[25-27]. This binding would modify the nonpolar pathway as well as the polar pathway in the skin. This naturally leads to the modification of the heterogeneous pathway.

This study was performed under the condition that salicylic acid was saturated in a vehicle which was composed of petrolatum and urea derivative. So comparisons of the fluxes across the skin of the permeant under the condition of the equal thermodynamic activity were performed. If none of kinetic factors act, all of the fluxes across the skin of the permeant would be same. This study showed that the flux was increased with increasing concentrations of tetramethylurea. This indicates that the percutaneous absorption enhancing activity of tetramethylurea would be rather kinetically controlled. Probably urea and urea derivatives modify the stratum corneum and reduce the resistance to the flow of the solute across the skin

The mechanism of the percutaneous absorption enhancing activity of urea and urea derivatives might not be fully defined by the results of this study. However, one possible explanation is that urea and urea derivatives bind with proteins or lipids in the stratum corneum and this might induce swelling of the tissue as proposed for DMSO[31]. This leads to less compact packing of the layer and easier fluxes of drugs.

The preliminary skin irritation tests show that urea and urea derivatives are quite non-irritating to the skin if the concentration of the enhancers is not too high. Though this is not to guarantee safe use of these agents on human skin, it might be expected that the urea derivatives are relatively safe to human skin. This is a very attractive property for the compounds to be developed as a percutaneous absorption enhancer, because skin irritation is the most annoying problem in dermal formulations.

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