Enhancing Effect of Pyrroli done Derivatives on the Transdermal Penetration of Sulfaguanidine, Aminopyrine and Sudan III

Hitohoshi SASAKI,* Masaki KOJIMA, Junzo NAKAMURA, and Juichiro SHIBASAKI

Faculty of Pharmaceutical Sciences, Nagasaki University, Bunkyo-machi 1-14, Nagasaki 852, Japan

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The enhancing effect of pyrrolidone derivatives on the percutaneous penetration of sulfaguanidine, aminopyrine and sudan III was investigated using in vitro technique and excised rat skin. 1-Methyl (MP), 1-hexyl (HP) and 1-lauryl-2-pyrrolidone (LP) were used as penetration enhancers. Aminopyrine showed high penetration through skin although sulfaguanidine and sudan III showed little penetration. Pyrrolidone derivatives enhanced their penetrations. Especially HP and LP enhanced the penetration of sulfaguanidine to a high extent. Sudan III was not detected in the receptor phase regardless of the presence of enhancer. Pyrrolidone derivatives significantly increased the skin accumulation of sulfaguanidine, aminopyrine and sudan III. Penetration of pyrrolidone derivatives was also determined. MP and HP showed high penetrations. LP was not detectable in the receptor phase. MP, HP and LP showed skin accumulations. These results suggested the usefulness of pyrrolidone derivatives as percutaneous penetration enhancers.

Keywords — pyrrolidone derivative; transdermal drug delivery; penetration enhancer; skin accumulation; enhancing effect; rat skin; in vitro technique; sulfaguanidine; aminopyrine; sudan III

Introduction

Recently, percutaneous drug delivery has attracted a great interest not only in topical chemotherapy but also in systemic chemotherapy using controlled-release technology.1-4 However, efforts in this direction have been limited by the drawback that most drugs will not penetrate at rates high enough for therapeutic efficacy. One promising approach for enhancing the percutaneous absorption is use of a penetration enhancer. Many compounds have been reported to improve trans dermal delivery.5-11 Recently, 1-dodecylazacycloheptan-2-one (Azone®) was developed by Nelson Research, California, U.S.A. as a potential penetration enhancer without severe side effect.12-15 It was demonstrated that one possible mechanism for Azone® to enhance penetration of drugs is formation of a lipophilic ion pair with penetrant in the stratum corneum.16-18 Azone® may play a role of cationic form on anionic drug in the ion pair. In the previous papers, we reported that some pyrrolidone derivatives, which have similar structure to Azone®, could enhance the transdermal penetration of anionic drugs.19,20 In the present study, the enhancing effect of pyrrolidone derivatives on the penetration of sulfaguanidine, aminopyrine and sudan III, which are selected as model of unionized drugs in physiological pH, were investigated by using in vitro technique and excised rat skin.

Materials and Methods

Chemicals — Sulfaguanidine, aminopyrine, sudan III and 1-methyl-2-pyrrolidone (MP) were obtained from Nacalai Tesque, Inc., Kyoto, Japan. 1-Hexyl (HP) and 1-lauryl-2-pyrro lidone (LP) were prepared by a usual method.21 All other reagents were of reagent grade.

Determination of Rf Value in Thin Layer Chromatography (TLC) — TLC was carried out on TLC aluminum sheets pre-coated with a 0.2 mm layer of silica gel 60 F 254 (Merck, Darmstadt, West Germany) using the following solvent system; chloroform–ethyl acetate-methanol (90:7:3, v/v/v).

Solubility of Penetrant — The solubilities of drugs were determined at 32 °C by suspending excess amounts of them in isopropyl myristate with or without pyrrolidone derivatives (2 mmol/ml), followed by filtration and analysis.

* To whom correspondence should be addressed.
**In Vitro Penetration Experiment** — The in vitro diffusion cell was similar to the type used by Loftsson and Bodor.\(^{22}\) Rat full-thickness skin and isopropyl myristate were used as models of diffusion membrane and organic formulation, respectively. The membranes were full-thickness abdominal skins of male Wistar albino rats weighing 250–300 g. The hair of the rat was removed with an animal clipper and a shaver 24 h before the experiment. The animal was sacrificed with pentobarbital, given intraperitoneally. The skin was excised and mounted in the diffusion cell. The receptor phase was filled with isotonic sodium phosphate-buffered saline (pH 7.4, 49 ml) containing kanamycin sulfate (100 ppm). In the case of sudan III, a mixture of acetonitrile and saline (70:30, v/v) was used as receptor fluid because of its insolubility in water. Macroscopical change of the skin by organic solvent was not observed. Test formulations were prepared by suspending sulfaguanidine (200 mg), aminopyrine (200 mg) and sudan III (100 mg) in isopropyl myristate (1 ml) containing pyrrolidone derivatives (2 mmol) or none. The pyrrolidone derivatives were dissolved in isopropyl myristate. These test formulations were incubated at 32 °C for 5 h prior to the experiment. The formulation was gently applied on the donor side of the skin surface which had an available diffusion area of 6.8 cm\(^2\). The diffusion cell was placed in a chamber maintained at 32 °C and the receptor phase was stirred by a magnetic stirrer. At appropriate intervals, samples of the receptor fluid were withdrawn over a 10 h period.

At the end of a transfer period, the chamber mounting skin was soaked and shaken with 200 ml of water for 1 min five times. The skin was removed from the diffusion cell and homogenized in 50 ml of water with a Polytron Homogenizer® (Ikeemotorika Kogyo Co., Ltd., Tokyo, Japan). Sudan III in the homogenate was extracted with chloroform and assayed spectrophotometrically in the organic phase. The homogenate for sulfaguanidine and aminopyrine was diluted with an equal volume of methanol, shaken and filtered with a paper filter (Toyo Roshi Co., Ltd., Tokyo, Japan). The filtrate was used for high performance liquid chromatography (HPLC) assay.

**Analysis** — The pyrrolidone derivatives, sulfaguanidine and aminopyrine were determined by the use of HPLC system (LC-5A pump, SIL-1A injector, Shimadzu Co., Ltd., Kyoto, Japan) equipped with a variable wavelength ultraviolet (UV) absorbance detector (SPD-2A, Shimadzu Co., Ltd.) in a reverse phase mode. The stationary phase used was a Cosmosil 5C\(_{18}\) packed column (diameter 4.6 mm, length 150 mm, Nacalai Tesque, Inc.) and the peak was detected at 205 nm for pyrrolidone derivatives, 240 nm for sulfaguanidine and 260 nm for aminopyrine, respectively. The column was used at room temperature. Mixtures of methanol–water (MP, 5:95; HP, 55:45; LP, 85:15; sulfaguanidine, 5:95; aminopyrine, 55:45, v/v) were used as the mobile phase at a flow rate of 1.0 ml/min. The mobile phase was filtered by passing through a 0.45 \(\mu\)m pore size membrane filter (Toyo Roshi Co., Ltd.). The standard solutions were chromatographed and calibration curves were constructed on the basis of peak area measurements. This HPLC assay method was sensitive and reproducible.

Sudan III was assayed with a spectrophotometer (UV 110, Hitachi Co., Ltd., Tokyo, Japan) at 550 nm in chloroform.

![Fig. 1. Penetration-Time Profiles of Sulfaguanidine and Aminopyrine after Application Alone](image)

\(\bigcirc\), sulfaguanidine; \(\triangle\), aminopyrine. Vertical bars indicate S.E.M. and each point is the mean of at least 3 experiments.

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TABLE I. Physicochemical Properties and Penetration of Penetrants

<table>
<thead>
<tr>
<th>Penetrant</th>
<th>MW a)</th>
<th>mp (°C)</th>
<th>Rf b)</th>
<th>ȘIPM c) (mm)</th>
<th>n d)</th>
<th>Lag time e) (h)</th>
<th>Flux e) (nmol/cm²/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfaguanidine</td>
<td>214</td>
<td>192</td>
<td>0.01</td>
<td>0.056</td>
<td>4</td>
<td>4.30 ± 0.47</td>
<td>2.9 ± 0.6</td>
</tr>
<tr>
<td>Aminopyrine</td>
<td>231</td>
<td>108</td>
<td>0.24</td>
<td>88</td>
<td>5</td>
<td>0.28 ± 0.11</td>
<td>1142 ± 143</td>
</tr>
<tr>
<td>Sudan III</td>
<td>352</td>
<td>199</td>
<td>0.84</td>
<td>63</td>
<td>3</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

a) Molecular weight. b) Rf value in TLC. c) Solubility of penetrant in isopropyl myristate at 32 °C. d) Number of trials. e) Lag time and flux were calculated from in vitro experimental data graphically. Means ± standard error from the mean. f) Not detected.

Results and Discussion

The percutaneous penetration profiles of sulfaguanidine and aminopyrine applied alone in isopropyl myristate were shown in Fig. 1. Suspension was used as a formulation to determine the maximum penetration of penetrants. The profile showed a lag phase followed by the linear rise. The lag time and penetration flux of penetrants were determined graphically and summarized in Table I with their physicochemical properties. Sulfaguanidine and aminopyrine were reported to be almost completely undissociated at pH 7.4 and behave as cationic form in acidic condition. Aminopyrine showed higher Rf value, suggesting higher lipophilicity, than sulfaguanidine. Lipophilic aminopyrine showed high penetration although hydrophilic sulfaguanidine showed little penetration. Sudan III is a markedly lipophilic dye and there is no detectable sudan III in the receptor phase.

The penetration profiles of penetrants and enhancers after coapplication of sulfaguanidine and aminopyrine with pyrrolidone derivatives were shown in Figs. 2 and 3, respectively. The penetration of sulfaguanidine was largely en-

![Fig. 2. Penetration-Time Profiles of Sulfaguanidine (A) and Pyrrolidone Derivatives (B) after Their Coapplication.](image)

○, MP; △, HP; □, LP. Vertical bars indicate S.E.M. and each point is the mean of at least 3 experiments.

![Fig. 3. Penetration-Time Profiles of Aminopyrine (A) and Pyrrolidone Derivatives (B) after Their Coapplication.](image)

○, MP; △, HP; □, LP. Vertical bars indicate S.E.M. and each point is the mean of at least 3 experiments.
TABLE II. Flux and Lag Time for Penetration of Penetrants Applied with Pyrrolidone Derivatives

<table>
<thead>
<tr>
<th>Penetrant</th>
<th>Enhancer</th>
<th>n a)</th>
<th>Lag time b) (h)</th>
<th>Flux b) (nmol/cm²/h)</th>
<th>E_{pen} c)</th>
<th>S_{enh} d) (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfaguanidine</td>
<td>MP</td>
<td>4</td>
<td>0.91 ± 0.27</td>
<td>67 ± 21</td>
<td>23</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>HP</td>
<td>4</td>
<td>0.92 ± 0.03</td>
<td>698 ± 72</td>
<td>237</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>LP</td>
<td>5</td>
<td>2.78 ± 0.32</td>
<td>810 ± 78</td>
<td>275</td>
<td>4.9</td>
</tr>
<tr>
<td>Aminopyrine</td>
<td>MP</td>
<td>3</td>
<td>0.01 ± 0.01</td>
<td>4040 ± 274</td>
<td>3.5</td>
<td>383</td>
</tr>
<tr>
<td></td>
<td>HP</td>
<td>3</td>
<td>0.90 ± 0.29</td>
<td>26880 ± 3098</td>
<td>23.5</td>
<td>351</td>
</tr>
<tr>
<td></td>
<td>LP</td>
<td>4</td>
<td>2.16 ± 0.13</td>
<td>14689 ± 1627</td>
<td>12.9</td>
<td>282</td>
</tr>
</tbody>
</table>

a) Number of trials. b) Lag time and flux were calculated from in vitro experimental data graphically. Means ± standard error from the mean. c) The factor of penetration enhancement (= (flux of penetrant applied with enhancer)/(flux of penetrant applied alone)). d) Solubility of penetrant in isopropyl myristate with enhancer at 32 °C.

enhanced by HP and LP. All pyrrolidone derivatives enhanced the penetration of aminopyrine to a high extent. The saturation tendency of the flux of aminopyrine applied with LP may reflect the equilibrium state because of its rapid penetration. Sudan III was hardly detected in the receptor phase regardless of the presence of enhancer (data not shown).

Penetration of enhancer was also determined. High penetration of MP to reach the equilibrium state was observed. HP penetrated slightly. LP was not detectable in the receptor phase.

The lag time and penetration flux of penetrants after coapplication with enhancer were determined graphically and summarized in Table II with the factor (E_{pen}) of penetration enhancement by pyrrolidone derivatives. The solubility of penetrants in isopropyl myristate containing enhancer (2 mmol/ml) were added in the table. Pyrrolidone derivatives enhanced the penetration of sulfaguanidine by 23–275 times and aminopyrine by 4–24 times. Pyrrolidone derivatives also increased the solubility of sulfaguanidine and aminopyrine in isopropyl myristate, suggesting an interaction between enhancer and penetrant. This interaction in the stratum corneum seems to be one possible mechanism of penetration enhancer.

Hadgraft and coworkers investigated the ability of Azone® to facilitate the transport of salicylate anion across an inert membrane impregnated with isopropyl myristate using a rotating diffusion cell. They demonstrated that penetration enhancer may be capable of forming an ion pair with penetrant in stratum corneum and enhanced the transport of penetrant using a pH gradient. 16–18) However, the pyrrolidone derivatives, which have similar structure to Azone®, enhanced the penetration of not only anionic drugs 19,20) but also sulfaguanidine and aminopyrine. They are almost completely undissociated at pH 7.4 and behave as cationic form in acidic condition. 23) The interaction between enhancer and penetrant might be explained by hydrogen bonding rather than ion pair. It was reported that the pyrrolidone moiety of the polyvinylpyrrolidone could form hydrogen bonds with a number of drugs. 24,25)

Lag time for penetration was prolonged with an increase of lipophilicity of pyrrolidone deriva-

![Fig. 4. Relationship between Factor (E_{pen}) of Penetration Enhancement by Enhancer and Rf Value of Penetrant](image-url)

Fig. 4. Relationship between Factor (E_{pen}) of Penetration Enhancement by Enhancer and Rf Value of Penetrant

O, MP; △, HP; □, LP. 1, phenol red; 2, sulfaguanidine; 3, 5-fluorouracil; 4, trimcinolone acetone; 5, aminopyrine; 6, indomethacin; 7, flurbiprofen. The lines through the data were obtained from a linear regression fit. MP, Y = -0.122X + 0.622 r = 0.266; HP, Y = -0.728X + 0.683 r = 0.760; LP, Y = -0.849X + 0.389 r = 0.816.
Table III. Skin Accumulation of Penetrants and Pyrrolidone Derivatives at 10 h after Their Coapplication

<table>
<thead>
<tr>
<th>Penetrant</th>
<th>Enhancer</th>
<th>n</th>
<th>Skin accumulation (µmol) b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Penetrant</td>
</tr>
<tr>
<td>Sulfaguanidine</td>
<td>Control</td>
<td>4</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>MP</td>
<td>4</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>HP</td>
<td>4</td>
<td>3.9 ± 0.4 e)</td>
</tr>
<tr>
<td></td>
<td>LP</td>
<td>5</td>
<td>5.2 ± 1.4 d)</td>
</tr>
<tr>
<td>Aminopyrine</td>
<td>Control</td>
<td>5</td>
<td>4.2 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>MP</td>
<td>3</td>
<td>8.5 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>HP</td>
<td>3</td>
<td>37.8 ± 16.7</td>
</tr>
<tr>
<td></td>
<td>LP</td>
<td>4</td>
<td>31.7 ± 10.4 f</td>
</tr>
<tr>
<td>Sudan III</td>
<td>Control</td>
<td>3</td>
<td>126.8 ± 12.7</td>
</tr>
<tr>
<td></td>
<td>MP</td>
<td>4</td>
<td>281.5 ± 40.5 c)</td>
</tr>
<tr>
<td></td>
<td>HP</td>
<td>4</td>
<td>358.1 ± 39.2 d)</td>
</tr>
<tr>
<td></td>
<td>LP</td>
<td>4</td>
<td>208.7 ± 36.8</td>
</tr>
</tbody>
</table>

a) Number of trials. b) Skin accumulation of penetrant and enhancer at 10 h after their applications. Means ± standard error from the mean. Statistical significance (Student's t-test) from control: c) p < 0.05, d) p < 0.01, e) p < 0.001.

tives. Lipophilic compound, LP, might need a long time to penetrate into skin and show the constant enhancing effect because of its slow penetration.

The relationship between the factor \( E_{pen} \) of penetration enhancement by enhancers and lipophilicity \( (Rf) \) of penetrants were shown in Fig. 4. The \( E_{pen} \) values of phenol red, 5-fluorouracil, triamcinolone acetonide, indomethacin, and flurbiprofen, reported previously, were added in the figure. Enhancing effect of HP and LP is maximal when the drug is hydrophilic, like sulfaguanidine and 5-fluorouracil, and not so effective for lipophilic compounds. Lipophilic compounds which were not enhanced by enhancer seem to have two types. One compound had a low melting point and showed high penetration regardless of the presence of enhancer, like flurbiprofen. The other compound had a high melting point or marked lipophilicity and showed little penetration regardless of the presence of enhancer, like triamcinolone acetonide. The enhancing effect of MP was not related to the \( Rf \) value of penetrant.

The skin accumulation of penetrants and enhancers at 10 h after their coapplication were described in Table III. The amount of penetrant in the skin increased with an increase of lipophilicity of penetrants. Pyrrolidone derivatives enhanced not only penetration but also skin accumulation of sulfaguanidine and aminopyrine. The skin accumulation of sulfaguanidine and aminopyrine were relative to their flux (correlation coefficient: 0.990 and 0.958, data not shown). The intercept of skin accumulation (0.7 µmol for sulfaguanidine and 4.6 µmol for aminopyrine) on flux was observed. It might be explained by the adsorption of drugs on skin. The skin accumulation of sudan III, which showed no appearance in the receptor phase, was significantly enhanced by MP and HP. The high skin accumulation of pyrrolidone derivatives was also observed. LP showed high accumulation in spite of no appearance in receptor phase. This behavior of a markedly lipophilic compound may be explained by its high affinity for stratum corneum.

Thus, the pyrrolidone derivatives are useful for transdermal delivery of various drugs. Especially, the lipophilic enhancers, HP and LP, could effectively enhance the hydrophilic penetrant. The optimal selection of drug, enhancer and vehicle is important for developing the useful transdermal drug delivery system.

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Penetration Enhancement by Pyrrolidone

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References


