# Acute Toxicity and Skin Irritation of Pyrrolidone Derivatives as Transdermal Penetration Enhancer

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**We investigated preliminary acute toxicity and primary skin irritation of nine pyrrolidone derivatives which had been previously developed as transdermal penetration enhancers. The acute toxicity was observed at a dose of 500 mg/kg after intraperitoneal administration in mice. Their primary skin irritations were examined with rabbit dorsal skin. 1-Lauryl-2-pyrrolidone induced the most severe irritation among the derivatives. Pyrrolidone derivatives having methyl group and methyloxycarbonyl group caused little irritation. The primary irritation indices of pyrrolidone derivatives were not relative to their accumulations in the skin but to their enhancing effects. In conclusion, 1-hexyl-4-methyloxycarbonyl- and 1-lauryl-4-methyloxycarbonyl-2-pyrrolidone are suggested to be adequate enhancers, judging from the balance of their enhancing activity and irritation.**

**Keywords** percutaneous absorption; transdermal drug delivery; pyrrolidone derivative; penetration enhancer; acute toxicity; primary skin irritation; Draize's method; mouse; rabbit; enhancing effect

The search for transdermal penetration enhancers has attracted special interest because of the desire to extend the range of pharmacologically active substances which can be delivered *via* the skin.[1] Several organic solvents and surfactants are known to enhance the transdermal penetration of a drug.[2,3] Recently, new amphiphilic molecules such as 1-dodecylazacycloheptan-2-one (Azone®),[4] *N,N*-diethyl-*m*-toluamide,[5] amides of cyclic amines,[6] 1-alkyl- or 1-alkenylazacycloalkanone derivatives,[7] unsaturated cyclic ureas[8] and alkyl *N,N*-dialkyl-substituted amino acetates[9] have been developed as potential enhancers without side effect. In a previous report,[10] we also demonstrated that some pyrrolidone derivatives had high enhancing effect on the penetration of hydrophilic dye. If these compounds are to be used for transdermal drug delivery, there are various criteria which a penetration enhancer should fulfill. These ideal properties such as specificity and reversibility of action, absence of toxicity and pharmacological response, and suitability for formulations have been reviewed.[2] Important criteria for an enhancer are that it should be non-toxic and non-irritating. In the present study, preliminary acute toxicity in mice and primary skin irritation in rabbits of pyrrolidone derivatives were investigated.

## Experimental

**Materials** 1-Methyl-2-pyrrolidone (I) was obtained from Nacalai Tesque, Inc., Kyoto, Japan. 1-Alkyl-2-pyrrolidones (hexyl, II; lauryl, III), 1-alkyl-4-carboxy-2-pyrrolidones (methyl, IV; hexyl, V; lauryl, VI) and 1-alkyl-4-methyloxycarbonyl-2-pyrrolidones (methyl, VII; hexyl, VIII; lauryl, IX) were prepared by the usual method.[11] Azone® was synthesized in this laboratory. All other reagents were of reagent grade.

**Acute Toxicity Study of Enhancer** Male albino ddY mice weighing 25—30 g were used in this study. Pyrrolidone derivatives (1000 mg/ml) were dispersed in 1% carboxymethylcellulose sodium salt (CMC) dissolved in deionized and distilled water. Three mice were injected intraperitoneally with the enhancer formulation at doses of 250, 500 and 1000 mg/kg. Three control mice were injected with an equivalent volume of 1% CMC solution. The mice were observed for 2 d post-injection and those that died during this period were recorded.

**Primary Skin Irritation Test** Male albino rabbits weighing 2.0—2.5 kg were used to examine the dermal irritations of pyrrolidone derivatives. The dorsal hair of four rabbits was removed with electric clippers 24 h before the application of a test compound. Each compound was dissolved or dispersed in isopropyl myristate at 0.2 mmol/ml. The test solutions (0.1 ml) were applied to circular gauze pads (1 mm thickness and 20 mm in diameter) of adhesive plaster for patch test (Torii & Co., Ltd., Tokyo, Japan) and affixed to the animals' dorsal surface. A supporter around the trunk of the rabbit secured the contact of the test compound and the skin. The occlusive dressings were removed after 24 h, and the dorsal site was assessed for redness and edema on 0, 1 and 7 d after removal of the test compound. Erythematous and edematous responses were totally evaluated for each site according to a modification of Draize's method and graded on a scale of 0—8 (8 being the maximum response).[12] The grader had no information about the application of the test compounds. Primary skin irritation indices were calculated by averaging the irritation scores of rabbits on 0 and 1 d after removal of test compounds.

An *in vitro* transfer experiment through rat skin and its analysis are described in a previous report.[10]

## Results and Discussion

Table I shows the acute toxicity of pyrrolidone derivatives after intraperitoneal administration to mice. CMC (1%) was used to disperse the enhancer. The mice administered II, III, V, VI, VIII and IX survived at a dose of 250 mg/kg but died at a dose of 500 or 1000 mg/kg. Mice administered I, IV and VII, which have methyl group, survived at a dose of 1000 mg/kg. A median lethal dose ($LD_{50}$) of Azone® was reported to be 232 mg/kg in mice.[13] These results suggested that the pyrrolidone derivatives in the present study would be safe regardless of transdermal penetration.

Primary skin irritation of pyrrolidone derivatives on rabbit skin is listed in Table II. In most cases little erythema and edema were observed. Lowered irritation scores were observed on 7 d than on 0 and 1 d after removal of a test formulation. Compound III caused the highest primary irritation index among derivatives. Pyrrolidone derivatives

TABLE I. Acute Toxicity of Pyrrolidone Derivatives in Mice

| Compound[a] | Toxicity (dead mice/total mice)[b] | | |
|---|---|---|---|
| Dose (mg/kg) | 250 | 500 | 1000 |
| I | | | 0/3 |
| II | 0/3 | 2/3 | 3/3 |
| III | 0/3 | 0/3 | 3/3 |
| IV | | | 0/3 |
| V | 0/3 | 0/3 | 3/3 |
| VI | 0/3 | 2/3 | 2/3 |
| VII | | | 0/3 |
| VIII | 0/3 | 0/3 | 2/3 |
| IX | 0/3 | 1/3 | 3/3 |

*a*) Pyrrolidone derivatives (1000 mg/ml) were dispersed in 1% CMC solution. *b*) Three mice were injected intraperitoneally with pyrrolidone derivatives. They were observed for 2 d post-injection and those dying during this period were recorded.

having methyl group and methyloxycarbonyl group caused little irritation. Azone® stimulated a high irritation index of 3.25±0.31 in the same system; this may be due to the lengthy occlusion of skin in this system. Rabbit skin is also known to be more sensitive than human skin, therefore it is often used in chemical irritation testing.

Penetration properties of pyrrolidone derivatives and their enhancing effects on phenol red penetration are summarized in Table III. Penetration properties are given from an *in vitro* diffusion experiment reported previously.[10] Derivatives with methyl or hexyl group showed good penetration although little of the lipophilic compound with lauryl group was transferred through the skin. Wiechers and coworkers demonstrated that Azone® is safe for clinical use in human because of its poor skin absorption and rapid clearance from the body.[13] Pyrrolidone derivatives showed high accumulation in the skin at 10 h after application. These penetrations and skin amounts were not related to their enhancement of phenol red penetration.

TABLE II. Primary Skin Irritation of Pyrrolidone Derivatives in Rabbits

| Compound[a] | Irritation score[b] Day after removal (d) 0 | 1 | 7 | PII[c] |
|---|---|---|---|---|
| Control | 0 | 0 | 0 | 0 |
| I | 0.25±0.25 | 0 | 0 | 0.13±0.13 |
| II | 2.25±0.63 | 1.50±0.29 | 0.75±0.48 | 1.88±0.35 |
| III | 4.00±0.41 | 3.25±0.25 | 2.50±0.29 | 3.63±0.26 |
| IV | 1.00 | 0 | 0 | 0.50±0.19 |
| V | 1.25±0.48 | 0.50±0.29 | 0 | 0.88±0.30 |
| VI | 2.25±0.95 | 1.25±0.25 | 0.50±0.29 | 1.75±0.49 |
| VII | 0.75±0.25 | 0.25±0.25 | 0 | 0.50±0.19 |
| VIII | 0.75±0.48 | 0.25±0.25 | 0.25±0.25 | 0.50±0.27 |
| IX | 0.75±0.48 | 0.50±0.29 | 0.25±0.25 | 0.63±0.26 |

*a*) Test compounds were dissolved or dispersed in isopropyl myristate at 0.2 mmol/ml. *b*) Test solutions (0.1 ml) were applied to skin for 24 h. Erythematous and edematous responses were evaluated and graded on a scale of 0—8 on 0, 1 and 7 d after removal of test compounds. *c*) Primary irritation indices (PII) were calculated by averaging the irritation scores on 0 and 1 d after removal of test compounds. Means±standard error of four rabbits.

The relationships between primary irritation indices of pyrrolidone derivatives and their accumulations in the skin or enhancing effects were evaluated and are shown in Fig. 1 (A, B and C). Primary irritation indices of the derivatives were not relative to their accumulations in the skin (Fig. 1A). On the other hand, there was roughly a linear relationship between irritations and enhancing effects of pyrrolidone derivatives (Fig. 1B and C). The same tendency was reported by Okamoto *et al.* using 1-alkyl- or 1-alkenylazacycloalkanone derivatives as an enhancer and 6-mercaptopurine as a penetrant.[7]

TABLE III. Enhancing Effect and Penetration Property of Pyrrolidone Derivatives

| Compd. | Penetration property Receptor[a] (mM) | Skin[a] (μmol) | Enhancing effect Flux[b] (μmol/h) | Skin[b] (μmol) |
|---|---|---|---|---|
| I | 18.8±1.2 | 9.5±1.3 | 0.11±0.04 | 0.58±0.05 |
| II | 16.4±0.6 | 126.4±16.7 | 9.15±1.24 | 20.61±0.85 |
| III | 0.0012±0.0006 | 64.6±20.9 | 9.13±0.99 | 27.83±19.73 |
| IV | 1.0±0.2 | 35.8±6.5 | 0.06±0.01 | 0.65±0.08 |
| V | 5.1±1.8 | 29.7±7.4 | 0.94±0.17 | 2.49±0.38 |
| VI | 0.16±0.03 | 28.5±3.7 | 4.92±1.65 | 8.69±1.69 |
| VII | 10.7±1.3 | 63.5±8.8 | 0.04±0.003 | 0.48±0.05 |
| VIII | 9.3±1.6 | 81.9±19.4 | 3.51±0.67 | 12.55±3.38 |
| IX | 0.048±0.018 | 36.9±13.0 | 6.37±1.37 | 15.12±2.83 |

*a*) Concentration of pyrrolidone derivatives in the receptor phase and its amount in the skin 10 h after application of phenol red with pyrrolidone derivatives on rat skin. *b*) Percutaneous penetration flux of phenol red and its amount in the skin 10 h after its application with pyrrolidone derivatives on rat skin. Mean±standard error of at least six experiments.

TABLE IV. Ratio of Phenol Red Flux on Primary Irritation Index after Application of Pyrrolidone Derivatives

| Compound | Flux/PII ratio | Compound | Flux/PII ratio |
|---|---|---|---|
| I | 0.84 | VI | 2.81 |
| II | 4.87 | VII | 0.08 |
| III | 2.51 | VIII | 7.02 |
| IV | 0.12 | IX | 10.11 |
| V | 1.07 | | |

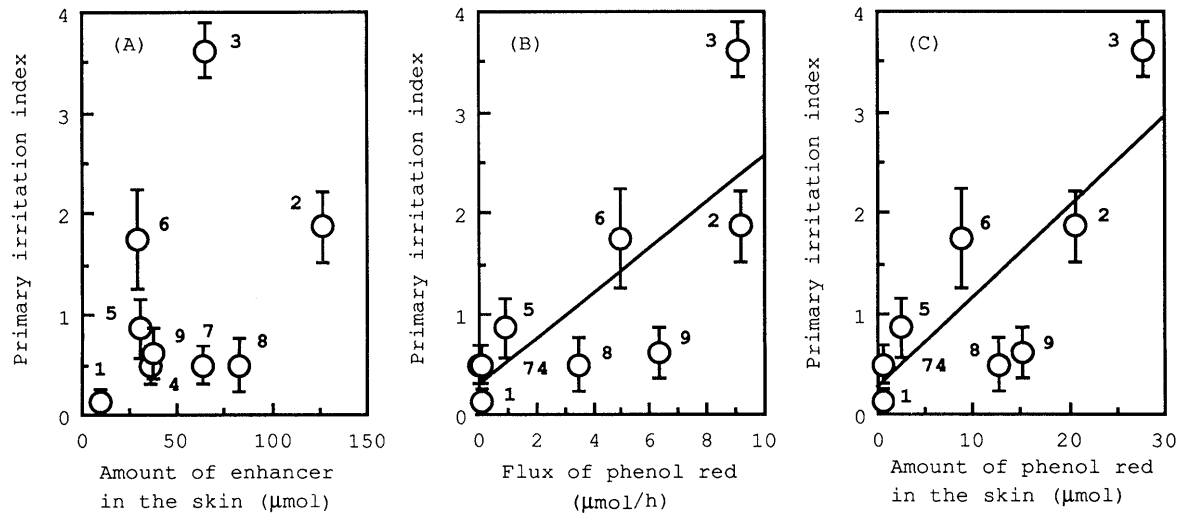


Fig. 1. Relationships between Primary Irritation Indices of Pyrrolidone Derivatives and Their Amounts in the Skin (A), Fluxes of Phenol Red (B) or Amounts of Phenol Red in the Skin (C) after Application of Phenol Red with Pyrrolidone Derivatives

**1**, I; **2**, II; **3**, III; **4**, IV; **5**, V; **6**, VI; **7**, VII; **8**, VIII; **9**, IX. The lines through the data in (B) and (C) were obtained from a linear regression fit. (B) $Y=0.227X+0.291$, $r=0.780$; (C) $Y=0.090X+0.268$, $r=0.809$. Vertical bars indicate standard errors and each point is the mean of four rabbits.

The ratios of phenol red flux on the primary irritation index are compared in Table IV. Compounds VIII and IX showed higher value, suggesting them to be the optimal enhancers with high enhancing activity and low irritation among the derivatives in this study. In fact, Fig. 1(B, C) without VIII and IX showed higher correlation coefficients (B, $Y=0.256X+0.429$, $r=0.898$; C, $Y=0.103X+0.421$, $r=0.947$). A part of VIII (60%) and IX (39%) was detected as V and VI in the skin homogenate sample. VIII and IX in a stratum corneum might contribute to a high enhancing effect. V and VI in an epidermal layer might decrease irritation slightly. Additional work on the retention of enhancer, its long-term chronic toxicity and the toxicity of practical formulation are necessary before clinical use is advisable.

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