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In vitro percutaneouus absorption of captopril

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Abstract

Four available skin membranes (mouse, rat, rabbit, pig) and human skin were utilized to evaluate the in vitro penetration of captopril. The flux of captopril increased in the order of human ≒ pig < rabbit < rat < mouse. The penetration rate of captopril through rabbit skin was optimal to evaluate the variation of formulations. Hence, the rabbit skin was picked out as a model membrane for in vitro penetration experiments of captopril. The flux of captopril was increased linearly when the concentration of captopril increased from 1% up to 10% ($r = 0.9777$). The enhancing effect of penetration enhancers including fatty alcohols (2C–14C), aliphatic esters (2C–10C) and other compounds on captopril penetration through excised rabbit skin were also evaluated. The enhancement of fatty alcohols and aliphatic esters were related to the chain length of enhancers. The C_6–C_{10} of fatty alcohols, butyl acetate and *N*-dodecyl-γ-lactame showed the most enhancing effect on captopril penetration. © 1997 Elsevier Science B.V.

Keywords: Captopril; Human skin; Pig skin; Concentration; Penetration enhancer; Fatty alcohol; Aliphatic esters; *N*-dodecyl-γ-lactame; Penetration index

1. Introduction

The potential advantages associated with transdermal drug delivery were well documented and included avoidance of first-pass gut and hepatic metabolism, potentially decreased side-effects and the relative ease of drug input termination in problematic cases (Chien, 1992).

Captopril is an orally effective angiotensin I-converting enzyme inhibitor and is used in treatment of hypertension and congestive heart failure. Captopril has a relatively short elimination half life in plasma with estimates in man ranging from 1.6 h to 1.9 h (Jarrott et al., 1982; Raia et al., 1990; Levy et al., 1991). The oxidation rate of captopril in dermal homogenate is significantly lower than that in intestinal homogenates (Zhou and Li Wan Po, 1994). Consequently transdermal drug delivery system (TDDS) may be suitable for captopril as a successful dosage form.

The objective of the present study were to investigate the difference of penetration rate of

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captopril through various types of animal skins including mouse, rat, rabbit, pig and human skin and to pick through an advisable and easy model membrane for the in vitro penetration experiments of captopril. Furthermore, the effect of concentration of captopril were evaluated. In order to increase the permeability of captopril, various types of penetration enhancer including fatty alcohols, aliphatic esters and other compounds which have been successfully used as penetration enhancers for some drug, such as thiamine disulfide and sodium nonivamide acetate etc., were used in this study (Aungst et al., 1986; Goodman and Barry, 1988; Komata et al., 1992a,b; Fang et al., 1996).

2. Materials and methods

2.1. Materials

The following reagents were used: captopril (Sigma, USA), Urea (Merck, Germany), fatty alcohols (C_2–C_{18}), ethyl acetate (C_2), butyl acetate (C_4), hexyl acetate (C_6), octyl acetate (C_8), undecanoic acid methyl ester (C_{10}) and tocopherol (TCI, Japan), *N*-dodecyl-γ-lactame (NDL) was a gift from the Schools of Chemistry of Kaohsiung Medical College (Kaohsiung, Taiwan). All other chemicals and solvents were of analytical reagent grade.

2.2. Preparation of skin membranes

Samples of whole adult human skin (24–50 years old) were obtained from breast reduction operations. Subcutaneous fat was carefully trimmed and the skin of breast was rinsed with normal saline. Then the skin was then sealed in aluminum foil and a plastic bag and stored at −20°C. The mean thickness of human skin was about 2.68 mm.

The male Wistar rates (6–8 weeks old; 150–200 g), the male Balb/c mice (4–6 weeks old; 25–30 g), the male New Zealand rabbits (12–14 weeks old, 2.5–3.0 kg) and the male Yorkshire pig (1–2 weeks old; 5–6 kg) were used. The hair of the abdominal region was removed with electric hair clippers and skin was excised after careful shaving. The excised full thickness skin samples were stored at −20°C prior to use.

2.3. In vitro permeation studies

Skin permeation of captopril was measured using diffusion cell which was similar to the Franz horizontal diffusion assembly (Franz, 1975). Animal skins and human skin were used as the barrier membrane. The receptor compartment contained 15 ml of deoxygenated distilled water. The donor compartment contained 15 ml of 1% captopril solution incorporated with or without enhancers. The temperature of the cell was maintained at 37 ± 0.5°C by thermostatically controlled water which was circulated through a jacket surrounding the cell body. Samples (0.5 ml) were removed from the receptor compartment at regular intervals and an equal volume of fresh deoxygenated water was added. Samples were assayed using HPLC after subsequent dilution (Wu et al., 1996).

3. Results and discussion

In these studies, four available skin membranes (mouse, rat, rabbit and pig) and human breast skin were utilized to evaluate the in vitro penetration of captopril. The flux of captopril through various types of skin are shown in Fig. 1. The flux of captopril increased in the order of human ≒ pig < rabbit < rat < mouse. The mouse skin

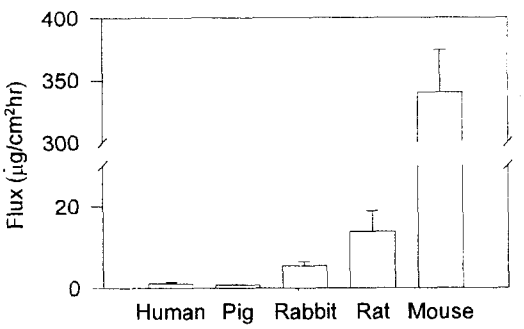

Fig. 1. Comparison of flux through various types of skins ($n = 3$).

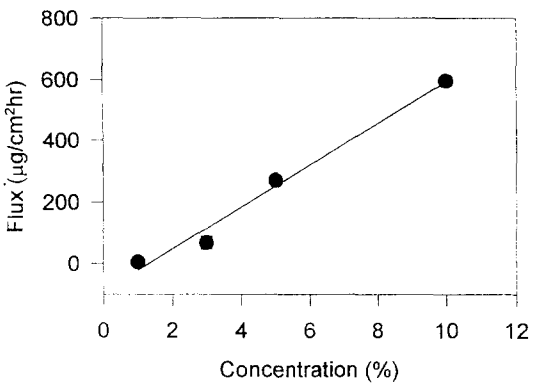

Fig. 2. The effect of captopril concentration on percutaneous absorption through excised rabbit abdominal skin ($n = 3$).

showed the greatest permeability about 300 times than that of human breast skin since the thickness of whole skin is lower than that of other animals (Bronaugh and Stewart, 1986). The excised human breast skin was the least permeable of all the skin type and no significant difference compared with pig skin. According to the previous reports (Harada et al., 1993), the human breast skin showed lower penetration rate than the other anatomical sites, so as the higher permeability of captopril through other sites of human skin were presumed. In this present study, the penetration rate of human breast skin and pig skin were too slow to distinguish the difference of formulations. As shown in Fig. 1, the flux of captopril through rabbit skin was advisable (about 4.8 times of flux of human breast skin) and easy to evaluate the difference of formulations. Comparison of the results exhibited that the rabbit skin could be used as a model membrane for in vitro penetration experiments of captopril.

Fig. 2 shows the flux of captopril with different concentrations through rabbit skin. The flux of captopril was increased linearly when the concentration of captopril increased from 1% up to 10% ($r = 0.9777$). Since the thiol group within the structure of captopril, higher penetration and irritation to skin were produced simultaneously. Therefore, the lower concentration of captopril (1%) was selected to decrease irritation and the penetration enhancers were used to increase the percutaneous absorption of captopril from solution formulations. Various carbon numbers of fatty alcohols (2C–14C), aliphatic esters (2C–10C) and other penetration enhancers were used in this present study. These penetration enhancers have been used as potent enhancers for many drugs such as naloxone, thiamine disulfide and 5-fluorouracil etc. (Aungst et al., 1986; Goodman and Barry, 1988; Komata et al., 1992a,b).

The cumulative amount of captopril with 5% of various carbon numbers of fatty alcohols permeated through excised rabbit skin at intervals are shown in Fig. 3. The penetration profile of captopril exhibited a zero-order permeation at a constant penetration rate. The permeation parameters calculated using the profiles are presented in Table 1. The flux value of captopril incorporated with 5% fatty alcohols except ethanol showed a significant effect (Newman-Keuls test) as compared with that of the control. The fluxes were increased approximately 5.24–65.24-fold. According to the previous reports (Cooper et al., 1985; Aungst et al., 1986; Komata et al., 1992a,b), the enhancement of fatty alcohols were related to the carbon numbers. The enhancing capacity of fatty alcohols was increased following the increase of carbon numbers, then it levelled off after the carbon numbers which was higher than C_8. So it was predicted that the fatty alcohols showed the highest enhancing effect when possessing the moderate carbon numbers. However, the shorter carbon numbers of alcohol showed a poor perme-

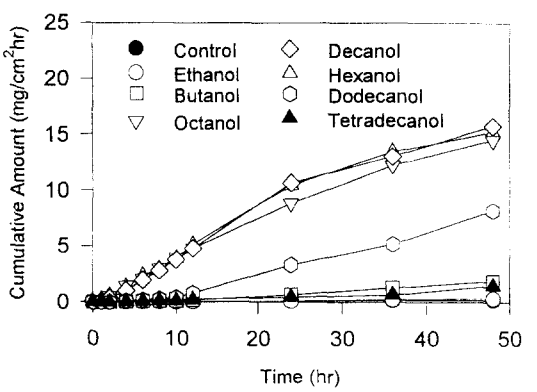

Fig. 3. The effect of 5% of various fatty alcohols on captopril percutaneous absorption through excised rabbit abdominal skin ($n = 3$).

Table 1
Permeation parameters of 1% captopril solution with fatty alcohols (5%) through rabbit skin ($n = 3$)

Enhancer (5%)	Flux ($\mu g/cm^2/h$)	48-h Accumulative amount ($\mu g/cm^2$)	Lag time (h)	PI
Control	5.40+1.03	242.61 ± 29.53	2.17 ± 0.12	1.00
Ethanol	7.43 ± 1.03	351.86 ± 214.41	3.24 ± 0.60	1.38
Butanol	41.56 ± 13.83	1892.58 ± 655.22	4.36 ± 0.48	7.70
Hexanol	349.17 ± 43.04	15 240.50 ± 1714.56	—	64.66
Octanol	315.13 ± 16.58	14 550.01 ± 1189.46	—	58.36
Decanol	352.27 ± 37.23	15 773.00 ± 1961.89	—	65.24
Dodecanol	176.19 ± 81.05	8203.73 ± 3697.82	4.77 ± 0.52	32.63
Tetradecanol	28.27 ± 19.06	1494.65 ± 1092.90	2.51 ± 1.46	5.24

$PI = J_{\text{with enhancer}}/J_{\text{control}}$.

ability which was due to its physicochemical inherent hydrophilicity. The higher carbon numbers of alcohol also show a similar phenomenon since the lipophilic characteristic limited its solubility in the medium resulted in the low penetration capacity through excised rabbit skin. In this present study, hexanol, octanol and decanol showed higher enhancing effect than the other fatty alcohols did. Fatty alcohols were reported to show their percutaneous enhancing effect because of disrupting the lipid of stratum corneum and reducing the resistance of stratum corneum to permeation compounds (Cooper et al., 1985; Komata et al., 1992a,b).

Fig. 4 shows the percutaneous profiles of captopril with or without 5% aliphatic esters through excised rabbit skin. The penetration profile of captopril exhibited a zero-order permeation at a constant penetration rate. Moreover, Table 2 depicts the permeation parameters, the flux value of captopril incorporated with 5% aliphatic esters were significantly higher (ANOVA test, $P < 0.05$) than that of control group. The flux of captopril with aliphatic esters increased approximately 5.64–57.79-folds. The enhancing effect of aliphatic esters on captopril penetration increased in the order of ethyl acetate (C_2) < octyl acetate (C_8) ≒ undecanoic acid methyl ester (C_{10}) < butyl acetate (C_4) ≒ hexyl acetate (C_6). These results also indicated that the aliphatic esters showed the highest enhancing effect when possessing the moderate carbon numbers (C_4–C_6).

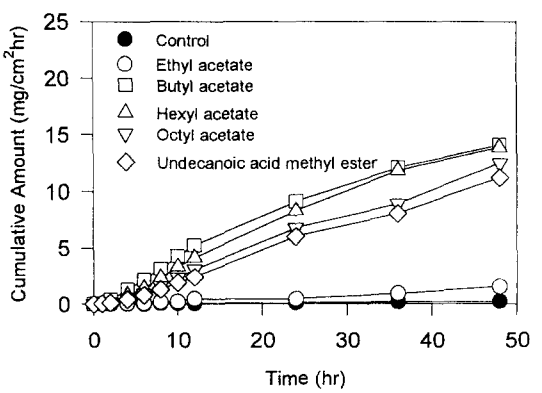

Fig. 4. The effect of 5% of various aliphatic esters on captopril percutaneous absorption through excised rabbit abdominal skin ($n = 3$).

The enhancing effect of fatty alcohols and aliphatic esters to the percutaneous absorption of captopril at the same carbon numbers are compared as shown in Fig. 5, there was no significant difference between the enhancing effect of fatty alchols and aliphatic ester as the carbon numbers range from C_6 to C_8. However, the enhancement of aliphatic ester was significantly higher than that of fatty alcohol at lower carbon numbers (C_2 and C_4) which was due to the physicochemical inherent hydrophilicity of fatty alcohol resulting in the poor permeability of captopril. The opposite results was observed at higher carbon numbers (C_{10}) since the lipophility of aliphatic ester was higher than that of fatty alcohol, so its solubility was limited in the medium resulting in the low penetration capacity of captopril (Cooper et al., 1985; Aungst et al., 1986; Komata et al., 1992a; Santoyo et al., 1995).

Table 2
Permeation parameters of 1% captopril solution with various aliphatic esters (5%) through rabbit skin ($n = 3$)

Enhancer (5%)	Flux ($\mu g/cm^2/h$)	48-h Accumulative amount ($\mu g/cm^2$)	Lag time (h)	PI
Control	5.40 ± 1.03	242.61 ± 29.53	2.17 ± 0.12	1.00
Ethyl acetate	30.47 ± 0.56	1576.98 ± 65.14	1.83 ± 1.64	5.64
Butyl acetate	308.03 ± 9.93	14 098.13 ± 341.29	—	57.04
Hexyl acetate	312.68 ± 20.91	13 889.82 ± 848.65	0.23 ± 0.79	57.79
Octyl acetate	268.59 ± 54.17	12 479.73 ± 2214.33	1.58 ± 0.98	49.74
Undecanoic acid methyl ester	243.44 ± 6.06	11 225.13 ± 229.17	1.82 ± 0.21	45.08

$PI = J_{\text{with enhancer}}/J_{\text{control}}$.

On the other hand, the enhancing effect of other compounds including propylene glycol, span 80, urea, *N*-dodecyl-γ-lactame and vitamin E (α-tocopherol) are also shown in Fig. 6 and the permeation parameters are depicted in Table 3. From the result, propylene glycol, span 80, PEG 400 and urea showed non-significant enhancement. In 1995, Trivedi et al. (1995) reported that vitamin E was generally thought to be non-irritating, and possess anti-oxidation and emollient properties. It acts as a penetration enhancer by intercalating within the lipid bilayer region of the stratum corneum, thus altering the characteristics of the membrane affecting permeability. In this study, vitamin E showed moderate enhancing effect on captopril penetration. The PI was increased about 13.5-fold. The *N*-dodecyl-γ-lactame was a synthetic analogous of azone which was reported to possess a potent enhancing effect, induced by disrupting the lipid of the stratum corneum, for many drugs (Lambert et al., 1989; Goodman and Barry, 1989; Rigg and Barry, 1990; Wu et al., 1995). In this study, the *N*-dodecyl-γ-lactame remarkably increased the permeation of captopril through excised rabbit skin and showed the most enhancing effect in comparison with others. The PI was increased about 56.7-fold. It was deduced from their structure that the *N*-dodecyl-γ-lactame could attain effective enhancement because of the similar mechanism to azone.

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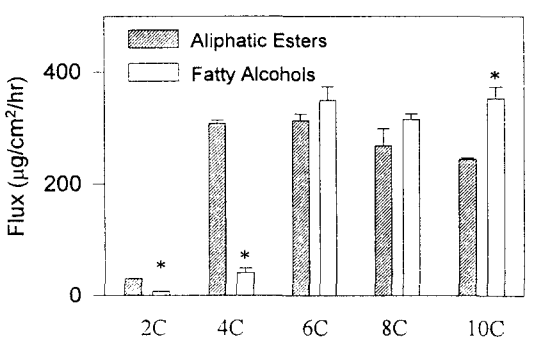

Fig. 5. Comparison of fatty alcohols and aliphatic esters in various carbon numbers on flux through excised rabbit abdominal skin ($n = 3$). * Significant; Student's *t*-test.

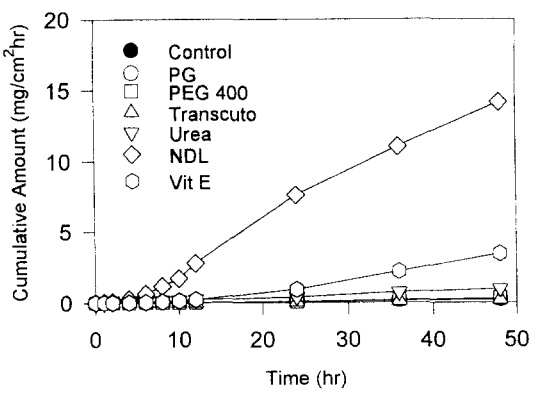

Fig. 6. The effect of 5% of various types of penetration enhancers on captopril percutaneous absorption through excised rabbit abdominal skin ($n = 3$).

Table 3
Permeation parameters of 1% captopril solution with various types of enhancers (5%) through rabbit skin ($n = 3$)

Enhancer (5%)	Flux ($\mu g/cm^2/h$)	48-h Accumulative amount ($\mu g/cm^2$)	Lag time (h)	PI
Control	5.40 ± 1.03	242.61 ± 29.53	2.17 ± 0.12	1.00
N-Dodecyl-γ-lactame	322.16 ± 16.77	$14\,181.05 \pm 592.61$	2.78 ± 1.05	56.66
Urea	20.83 ± 6.49	975.07 ± 261.27	1.47 ± 0.30	3.86
Vitamin E	72.90 ± 16.05	3455.52 ± 867.82	5.15 ± 0.30	13.50
Propylene glycol	6.13 ± 0.90	289.89 ± 39.03	3.27 ± 0.57	1.14
PEG 400	6.73 ± 2.39	321.48 ± 113.74	3.76 ± 0.15	1.25
Span 80	29.67 ± 17.02	1460.22 ± 901.22	2.90 ± 0.15	5.49

$PI = J_{\text{with enhancer}}/J_{\text{control}}$.

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