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RESEARCH PAPER

The Influence of Anti-irritants on Captopril Hydrophilic Gel

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ABSTRACT

In this study, we compared the irritation inhibition of various types of anti-irritants such as antihistamines (cyprohetadine, diphenhydramine, and promethazine), alpha-hydroxy acids (gluconolactone and gluco-δ-lactone), corticosteroids (betamethasone and clobetasol), and ion channel modulating agents (amiloride, ethacrynic acid, nifedipine, and verapamil) on the adverse dermatological reaction caused by captopril gel using noninvasive bioengineering methods including measuring the trans-epidermal water loss (TEWL) and the color change of skin surface [such as change chroma (delta C) and difference in color (delta E) between the gel-treated site and the untreated site]. In addition, the influence of these anti-irritants on the penetration capacity of captopril through the rabbit skin was also investigated. The results showed that the TEWL, change chroma (delta C), and difference in color (delta E) of skin were significantly reduced via incorporating diphenhydramine and clobetasol, indicating that both substances had potent irritation inhibition activity. Moreover, these substances had no effect on the percutaneous absorption of captopril gel. However, flux of the captopril with anti-irritants was about 480 $\mu g/cm^2/h$ and the required minimum administration area to obtain the minimum effective concentration was about 15 cm^2, indicating that this formulation could possibly be developed for a transdermal drug delivery system.

Key Words: Captopril; Anti-irritant; Transepidermal water loss; Colorimetry; Percutaneous absorption.

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INTRODUCTION

Captopril, the first angiotensive converting enzyme (ACE) inhibitor, is used widely in treatment of hypertension and congestive heart failure. Captopril has a relatively short elimination half life 1.6 hr to 1.9 hr. Food may decrease oral absorption of captopril by up to 25–40%, and the oxidation rate of captopril in dermal homogenates is significantly lower than that in intestinal homogenates.[1–6] Moreover, in earlier study,[7] captopril showed a good skin penetration; the serum level (31 ng/mL) of therapeutic minimum effect is obtained by about 10 cm^2 of administered area of captopril gel. Hence, it is possible to develop the captopril delivery system.

However, one of the limitations regarding transdermal delivery system (TDS) development is that of contact dermatitis related to drugs, penetration enhancers, and excipients, which in some instances necessitate discontinuation of the TDS development. Captopril containing sulfhydryl groups in their chemical structure shows a variety of adverse effects such as skin rash and nephropathy caused by the induced drug. The incidence of adverse dermatological reactions to oral captopril has been reported to be 1.4–4.3%.[8] Further, contact allergy develops most often due to the active ingredient.[9,10] Therefore, as can be seen, topical application of captopril gel will be associated with a high dermatological reaction. Although skin rash is not a fatal effect, unlike systemic anaphylaxis represented by penicillin shock, preferably it should be avoided because a comparably high incidence of skin rash disturbs the long-term medication.

In general, the prevention of adverse dermatological reaction associated with the transdermal delivery of sensitizing drugs could be attainable via incorporating the anti-irritant. The ion channel modulating agents including amiloride (sodium/hydrogen ion), ethacrynic acid (potassium/sodium ion), nifedipine, and verapamil (calcium ion) could disrupt ion channels at the cellular level of the mast cell to inhibit the induction of skin immune response via dinitrochlorobenzene, a strong chemical sensitizer, in the Balb/c mouse model.[11] Alpha-hydroxy acid, gluconolactone, and glucono-δ-lactone are widely used in cosmetic products as anti-irritants to reduce skin irritation, which may be intrinsic skin irritation or irritation caused by hydroxyl acids or certain retinoids. The mechanism of gluconolactone is complicated. It may inhibit IL-1α-induced PGE2. IL-1α and PGE2 are well-known mediators of inflammation in the skin, diminishing or decreasing corneocyte cohesion and improving the skin surface cosmetically.[12–17] Topical hydrocortisone possesses inhibition of inflammatory, rash, and swelling activity. Hence, it has proved invaluable in the treatment of a wide range of dermatoses.[18,19] Antihistamines can block the actions of histamine and reduce the histamine-mediated response, with the result that topical antihistamine products are widely used to treat various dermatological reaction such as rash, erythema, and hypersensitivity reaction.[20]

Therefore, the objective of this present study was to evaluate the effect of prevention and improvement of various types of anti-irritants such as antihistamines, alpha-hydroxy acids, corticosteroids, and ion channel modulating agents on the adverse dermatological reaction caused by captopril gel. Two noninvasive bioengineering methods, including measuring the transepidermal water loss (TEWL)[21,22] and the color change of skin surface[23–25] between the gel treated site and the untreated site, were used to quantify the adverse dermatological reaction and assess the effect of anti-irritants in this study. In addition, another important obstacle that must be overcome for a transdermal delivery system is the penetration capacity of the drug. Therefore, the influence of these anti-irritants on the penetration flux of captopril through the rabbit skin was also investigated.

MATERIALS AND METHODS

Materials

The following reagents were used: captopril, ethacrynic acid, amiloride hydrochloride, nifedipine, gluconolactone, glucono-δ-lactone (Sigma Chemical Company, St. Louis, Mo), diphenhydramine hydrochloride, clobetasol propionate, betamethasone propionate, hydroxypropyl cellulose, carboxymethyl cellulose sodium (CMC), ethylenediaminetetraacetic acid trisodium salt (EDTA-3Na) (TCI, Japan). All other chemicals and solvents were of analytical reagent grade.

Animals

Male New Zealand White rabbits (10–12 weeks old, 2.0–2.5 Kg) were used in this study. The hair of the abdominal region was removed with electric hair clippers. In the skin irritation evaluation, 0.8 g of captopril gel was spread uniformly over a sheet of cotton cloth (2×2 cm^2) for a 4-hr administration period by the occlusive dressing technique.

Measurement the Transepidermal Water Loss

In order to avoid experimental error, the rabbits were kept in controlled laboratory (temperature and relative

humidity were maintained at 22–24° C and 55–60%) during the study. Disturbances in the laboratory during measurement were kept to a minimum. The TEWL was measured quantitatively using an evaporimeter (Tewameter TM210, Koln, Germany). The probe was protected using a protection cover with the screen and grid. Measurement was taken at days 1, 2, 3, and 4 after gel removal and readings at a stable level were preformed 40 sec after application of the probe to the skin. The test sites were dabbed dry and air convection in the laboratory was avoided. The TEWL was calculated automatically and displayed digitally in $g/m^2/h$.

Colorimetry

A colorimeter (Chroma Meter-CR 221, Minolta, Japan) was used to measure erythema color. The instrument records three-dimensional color reflectance including ''L,'' ''a,'' and ''b,'' as recommended by (CIE). The luminance ''L'' gives the relative brightness ranging from total black (0) to total white (100). The ''a'' is the balance between red (100) and green (−100) and ''b'' represents the balance between yellow (100) and blue (−100) (Pierard and Pierard-Franchimont, 1993). The change chroma (delta C) and difference in color (delta E) between the gel treated site and the untreated site as the basis are described as follows:[26–29]

$$\text{delta C} = [(\text{delta a})^2 + (\text{delta b})^2]^{1/2}$$

$$\text{delta E} = [(\text{delta L})^2 + (\text{delta a})^2 + (\text{delta b})^2]^{1/2}$$

The colorimetric results were expressed as difference from the control, value obtained from one adjacent untreated side at days 1, 2, 3, and 4 after gel removal. Thereafter, changes in color on the treated sites were determined against the subject's own baseline standard.

In Vitro Skin Penetration Experiments

The extent and rate of skin permeation of captopril from gel were determined using a Keshary–Chien glass diffusion cell fitted with excised rabbit abdominal skin.[30] The detailed procedure and analysis were described in our earlier study.[31] Each data point represents the average of three determinations.

Data Analysis

The irritation inhibition ratio of anti-irritants was calculated using the equation:

$$\text{Irritation inhibition ratio} = \text{Cr} - \text{Sr}/\text{Cr} \times 100$$

where Cr and Sr are the value of TEWL or color change of skin after treatment with the captopril gel with and without anti-irritants.

Statistical comparisons between data were made using analysis of variance (ANOVA) analysis. Subgroup comparisons were made using the Newman–Keuls multiple comparisons.

RESULTS AND DISCUSSION

Adverse Dermatological Reaction Evaluation

The adverse dermatological reaction including TEWL and color change of skin surface (delta E and delta C) after a 4-hr application of captopril gel is shown in Fig. 1. The quantity of adverse dermatological reactions gradually became significant on the second day of captopril administration, and then gradually remitted on the third day of administration. The skin irritation response—time profiles of the captopril gel formulations prepared with CMC and high performance chromatography (HPC)—were similar and showed less skin irritation than that prepared with chitosan. Thereafter, captopril gel was prepared with CMC. In order to clearly distinguish the anti-irritant effect between formulations with or without the anti-irritant agent, the maximum measured value of adverse dermatological reaction at the third day was used in comparison (Table 1). As shown in Table 1, the values of TEWL, delta E, and delta C of formulation F1 (with captopril) were significantly ($p<0.05$) greater than that of the vehicle without captopril (F00), indicating that the active substance (captopril) was a potential irritant. In order to circumvent the adverse dermatological reaction associated with the topical use of captopril gel, some substances such as antihistamines, alpha-hydroxy acids, corticosteroids, and ion channel modulating agents, which possess anti-irritant properties,[11–19] were incorporated into the captopril gel formulations. As shown in Table 1, the values of TEWL, delta E, and delta C were significantly ($p<0.05$) reduced by incorporating diphenhydramine and clobetasol. This indicated that, compared to the captopril gel without anti-irritant, both diphenhydramine and clobetasol had significant inhibitory effects on skin irritation, including the barrier function integrity (reduction in TEWL) and erythema (reduction in delta E and delta C) improvement. According to a previous study,[32] the skin rash caused by captopril may be the result of the direct pharmacological action of captopril on the inhibition of kinase-II, the same

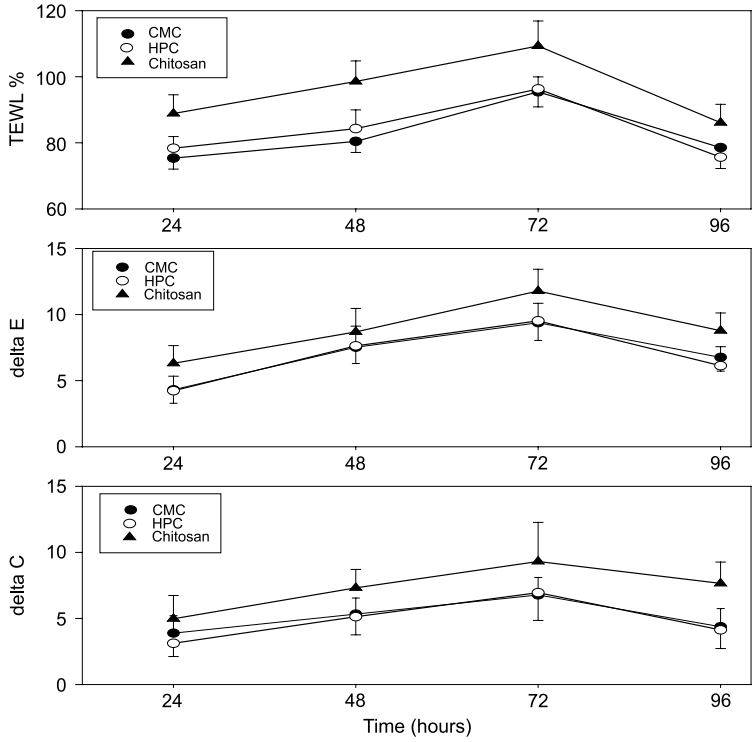

Figure 1. The skin irritation quantification measured by TEWL and chromametry measurement after 4-hr topical applications of various captopril gels. All data represent the means of three experiments ± SD.

enzyme as ACE. Kinin activity would then be potentiated in the skin, thereby leading to the histamine-mediated inflammatory reaction. Therefore, diphenhydramine, a potent antihistamine, could antagonize the action of histamine, leading to a marked decrease in adverse dermatological reactions. Furthermore, the irritation inhibition ratio of diphenhydramine increased with increasing the concentration (Fig. 2).

In the study, Clobetasol also showed a potent anti-inflammatory effect at low concentration, but the

Table 1. The values of TEWL and erythema measured at third day after removal of the captopril gel with and without anti-irritants in rabbit by bioengineering methods.

No.	Anti-irritant	%	TEWL	Delta E	Delta C
F00	– (No captopril)	0.0	20.43 ± 1.59	3.46 ± 0.38	2.25 ± 0.56
F01	–	0.0	95.03 ± 5.39[a]	8.10 ± 1.11[a]	6.85 ± 1.93[a]
F02	ethacrynic acid	0.1	98.93 ± 6.98	9.88 ± 1.23	7.69 ± 1.86
F03	amiloride	0.1	99.97 ± 8.37	10.92 ± 1.53	7.54 ± 1.39
F04	nifedipine	0.1	98.73 ± 7.77	10.31 ± 1.39	8.37 ± 1.35
F05	gluconolactone	5.0	94.90 ± 6.57	8.65 ± 2.31	7.10 ± 1.64
F06	glucono-δ-lactone	5.0	97.67 ± 7.38	8.94 ± 1.32	6.93 ± 1.86
F07	cyprohetadine	0.1	99.13 ± 7.32	9.78 ± 1.21	6.57 ± 0.39
F08	diphenhydramine	0.2	74.47 ± 6.92[b]	5.09 ± 1.01[b]	3.51 ± 0.34[b]
F09	promethazine	1.0	99.60 ± 6.57	9.98 ± 2.11	6.71 ± 0.68
F10	betanethasone	0.05	94.03 ± 4.21	10.88 ± 2.31	6.31 ± 1.35
F11	clobetasol	0.05	68.10 ± 4.11[b]	5.14 ± 1.13[b]	3.76 ± 0.39[b]
F12	diphenhydramine	0.20	45.62 ± 2.32[b,c]	4.38 ± 2.11[b]	3.42 ± 1.21[b]
	clobetasol	0.05			

Note: Captopril gel containing 5% sod. CMC, 5% menthol, 20% ethanol, and 5% captopril.
[a]Significant difference compared with F0 (negative control, gel without captopril) (ANOVA, $p<0.05$).
[b]Significant difference compared with F01 (positive control, gel with captopril) (ANOVA, $p<0.05$).
[c]Significant difference compared with F08, F11, F12, and F6 (ANOVA, $p<0.05$).

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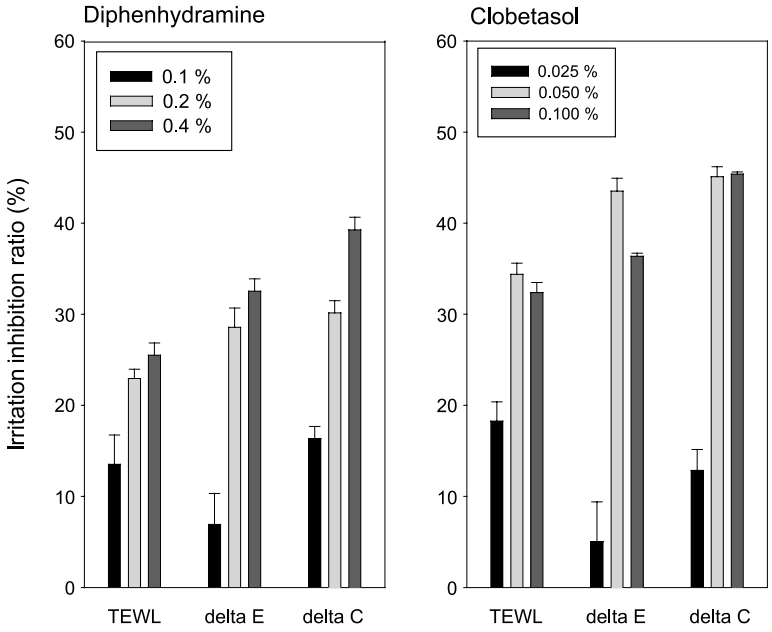

Figure 2. The irritation inhibition ratio on TEWL, delta E, and delta C after 4-hr topical applications of captopril gel incorporating various concentrations of diphenhydramine and clobetasol.

irritation inhibition ratio did not conspicuously increase clobetasol concentration, as shown in Fig. 2. Clobetasol is widely considered to be the most potent of the currently available corticosteroids. The mechanism of action of clobetasol is suggested to stimulate the synthesis of enzymes needed to decrease inflammation and suppress metabolic activity.[19] When clobetasol (0.05%) and diphenhydramine (0.2%) were used simultaneously (F12), the values of delta E and delta C showed no significant difference ($p>0.05$), but the TEWL was conspicuously reduced ($p<0.05$) when clobetasol or diphenhydramine was used alone (F08 and F11). The results indicted that the integrity of the stratum corneum was remarkably improved by adding a combination of clobetasol and diphenhydramine into captopril gel. It is suggested that both anti-irritants

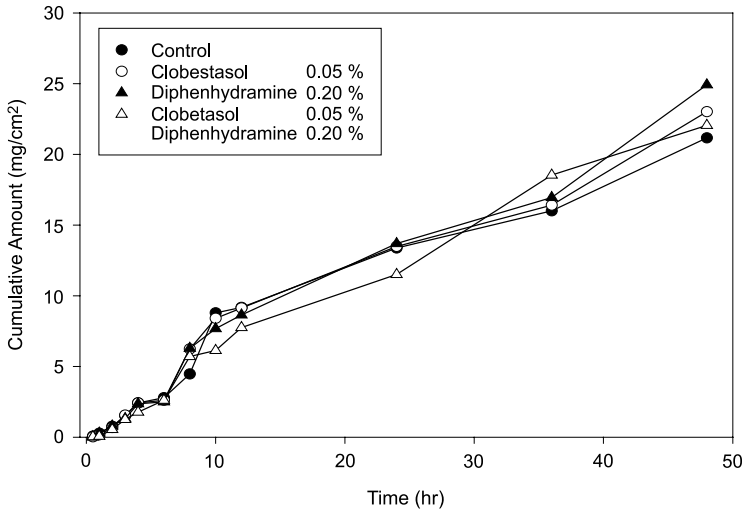

Figure 3. Permeation-time profile of captopril gel with and without anti-irritants including clobetasol and/or diphenhydramine through excised rabbit skin (n=3).

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(clobetasol and diphenhydramine) could provide different mechanisms in the anti-inflammatory reaction to obtain a synergistic effect.

In Vitro Skin Penetration Experiments

The cumulative amounts of drug penetration through the skin were plotted against time as shown in Fig. 3. A linear relationship was obtained for each formulation (R^2>0.9397), showing that the penetration of captopril from the gel was well described by the zero-order kinetics. The fluxes of captopril with and without anti-irritants (clobetasol or diphenhydramine alone and combined clobetasol and diphenhydramine) were 444±31, 473±8, 506±30, and 480±40 μg/hr/cm^2, respectively. There was no significant difference (p>0.05) in the flux between the captopril gel with and without anti-irritants, which indicated that the anti-irritants did not influence the percutaneous absorption of captopril gel. The results might be due to such low content levels (0.2% and below), since the thermodynamic activity of captopril would be largely unaffected.

According to previous studies,[7] the flux required to maintain the minimum effective concentration in the captopril transdermal delivery system was 1488 μg/cm^2/h through human skin and about 4.8-fold higher through rabbit skin. Therefore, the required flux through rabbit skin was about 7142 μg/cm^2/h. In this study, the flux of experimental captopril was 480 μg/cm^2/h. Therefore, the required minimum administration area to obtain the minimum effective concentration was about 15 cm^2, indicating that the captopril gel formulation can possibly be developed for a transdermal drug delivery system.

CONCLUSIONS

The addition of diphenhydramine and clobetasol to captopril gel was shown to reduce formulation induced irritation without inhibiting the transdermal penetration of captopril. The required minimum administration area to obtain the minimum effective concentration of experimental formulations was about 15 cm^2, indicating that these formulations could possibly be developed for a transdermal drug delivery system.

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