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Effect of antioxidants and anti-irritants on the stability, skin irritation and penetration capacity of captopril gel

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Abstract

The effects of antioxidants and chelating agent on the stability of captopril in aqueous and semisolid were determined in this study. Then the influence of the combination of additives including antioxidants, anti-irritants and penetration enhancer on stability, skin irritation and penetration capacity of captopril in semisolid dosage form was investigated. In the stability study, the degradation of captopril followed the first-order kinetic. The chelating agent EDTA showed a potent stability effect and obviously increased the shelf-life up to 14-fold that of control gel. The anti-irritants such as clobetasol and diphenhydramine had potent inhibition irritation activity and the effect was not retarded by the addition of EDTA. Moreover, the captopril gel containing penetration enhancer, anti-irritants and chelating agent had a higher penetration capacity and the minimum therapeutic concentration could be obtained by applying about 13.24 cm^2 of administered area, indicating that the formulation can possibly be developed for a transdermal drug delivery system.

Keywords: Captopril; Antioxidant; Stability; Anti-irritant; Percutaneous absorption

1. Introduction

Transdermal pharmaceutical products, whether ointments, matrix formulations or reservoir systems, provide the considerable advantages of a noninvasive parental route for drug therapy, avoidance of first-pass gut and hepatic metabolism, potentially decreased side effects and relative ease of drug input termination in problematic cases (Chien, 1992). Therefore, the transdermal therapeutic system has become increasingly popular for administration—e.g. clonidine, fentanyl, glyceryl trinitrate and estradiol, etc. (Good, 1983; Karim, 1983; Burris and Mroczek, 1986; Sclar et al., 1991; Seki et al., 1990). Captopril is an orally effective angiotensin

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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 to 1.9 h (Jarrott et al., 1982; Raia et al., 1990; Levy et al., 1991). Food may decrease oral absorption of captopril by up to 25–40% (Ohman et al., 1985; McEvoy, 1993). The oxidation rate of captopril in dermal homogenates is significantly lower than that in intestinal homogenates (Zhou and Li Wan Po, 1994). Moreover, in earlier studies (Wu et al., 2000), captopril has good penetration effect; the serum level (31 ng/ml) of therapeutic minimum effect is obtained by using about 10 cm^2 of administered area of captopril gel. Consequently, the transdermal drug delivery system (TDDS) may be suitable for captopril as a successful dosage form.

According to earlier studies (Lee and Notari, 1987; Timmins et al., 1982; Nahata et al., 1994; Lye et al., 1997) captopril is unstable and undergoes oxidation to form captopril disulfide in aqueous solution and the oxidation reaction can be delayed by lowering the pH of the solution, adding chelating agents, increasing the captopril concentration, using a nitrogen or low-oxygen headspace, or incorporating antioxidants and the anti-irritants such as clobetasol and diphenhydramine can alleviate the adverse dermatologic reaction. In addition, captopril containing sulfhydryl groups in their chemical structure show a variety of adverse side effects such as skin rash and nephropathy caused by drug induced. Although skin rash is not a fatal adverse 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.

Therefore, in order to develop the captopril delivery system, the effect of antioxidants and anti-irritants on the stability of captopril formulations and skin irritation after topical application were evaluated in this study. Furthermore, the influence of the combination of additives including antioxidants, anti-irritants and penetration enhancer on stability, skin irritation and penetration capacity of captopril in semisolid dosage form was investigated.

2. Materials and methods

2.1. Materials

The following reagents were used: captopril, ascorbic acid, α-tocopherol, γ-tocopherol, sodium sulfite, sodium bisulfite (Sigma Chemical Company, USA), Diphenhydramine, clobetasol, betamethasone, hydroxypropyl cellulose (HPC), carboxymethyl cellulose sodium (CMC), ethylenediaminetetraacetic acid trisodium salt (EDTA-3Na) (TCI, Japan), Alpha-tocopheryl polyethylene glycol succinate (TPGS) (Eastman, TN). All other chemicals and solvents were of analytical reagent grade.

2.2. Accelerated stability evaluation

The stability study was conducted by incubating captopril solution and semisolid formulations at 60 and 30 °C, respectively, with a relative humidity of 70%. The samples were withdrawn at appropriate time intervals for a period of 1 and 8 weeks, respectively. After that, an accurately weighted 0.5 g of gel sample was mixed with 8 ml methanol in a glass-stopper centrifuge tube, followed by mechanical shaking at 200 rpm for 12 h. After centrifugation for 10 min at 3000 rpm, the 100 μl of supernatant was diluted with mobile phase solution and filtrated through a 0.45 μm PVDF syringe filter. The filtrate was analyzed by HPLC (Wu et al., 2002).

2.3. Adverse dermatologic reaction evaluation

The adverse dermatologic reactions, including the skin irritation and barrier damage caused by captopril gel were evaluated by an evaporimeter (Tewameter TM210, Koln, Germany) for transepidermal water loss (TEWL) and a colorimeter (Chroma Meter-CR 221, Minolta, Japan) for chrommetry measurement. The detailed process was described in our previous study (Wu et al., 2002). In brief, a sheet of cotton cloth (2×2 cm^2) containing 0.8 g gel was applied to the abdominal skin of rabbit for 4 h administration period by the occlusive dressing technique, and then the administration site was measured at days 1, 2, 3 and 4 after gel removal.

2.4. In vitro skin penetration experiments

The extent and rate of skin permeation of captopril from gel formulations were determined using a modified glass diffusion cell fitted with excised rabbit skin (Hsu et al., 1994). The donor cell was filled with 2 g of 5% captopril gel with or without various additives. The receptor compartment was filled with 20 ml of deionized water and its temperature was maintained at 37 ± 0.5 °C by thermostatic water pump during the experiment. Approximately, 0.5 ml of the receptor medium was withdrawn at determined intervals and replaced immediately with an equal volume of receptor solution to maintain a constant volume. The sample withdrawn from the receptor compartment was then analyzed by HPLC system using a Lichrospher® 100, RP-18e 250×4 mm, 5 μm column (Merk). The column was eluted with methanol/water containing 0.016% phosphoric acid (1/1, v/v) at a flow rate 0.5 ml/min. The concentration of captopril was determined by UV absorbance at 210 nm.

3. Results and discussion

3.1. The stability evaluation

Fig. 1 presents the percentage of remaining as a function of time in various quantities of water at 60 °C. The remaining captopril at various time intervals would be fitted to an apparent first-order plot. The regression lines were calculated and the rate constants obtained. The degradation rate increased in the order of deionized water with or without degas < distilled water < tap water. The more rapid oxidation in tap water and distilled water might be explained by the presence of an amount of metal ions. The degas time had no effect on captopril stability, suggesting the absence of oxygen in deionized water. Therefore, the deionized water was suitable to prepare captopril solution. The influence of pH on the rate of degradation of captopril in phosphate-citrate buffer is shown in Fig. 2. The reaction rate increased with pH and sharply so above pH 4.0. The rate constant at pH 4.0 with ionic strength 0.18, 0.9 and 1.8 were 0.028, 0.030 and 0.033 per day, respectively. This revealed that the stabilizing effect was inversely related to ion strength. Form the above data, the pH below 4 and deionized water was the appropriate vehicle for preparing captopril dosage form.

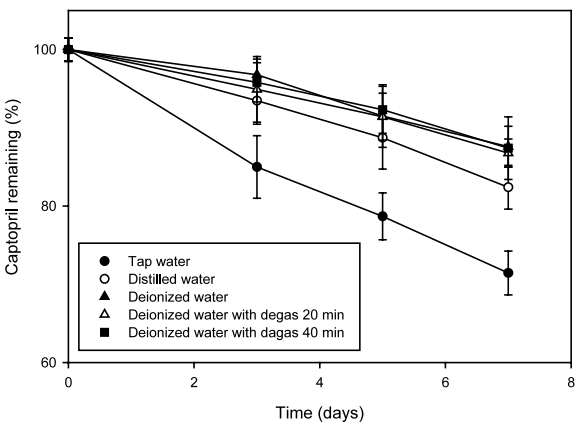

Fig. 1. Percentage of captopril remaining as a function of time in various quality of water at 60 °C.

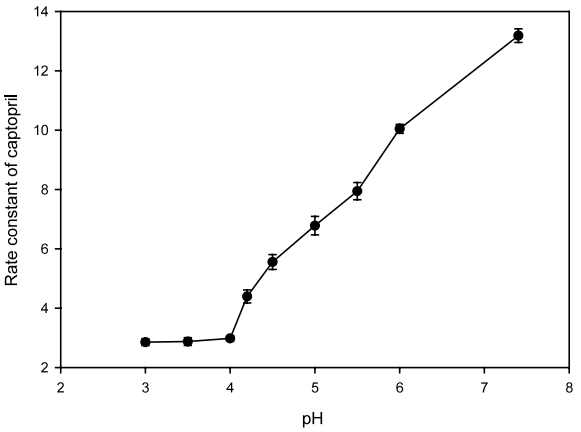

Fig. 2. The degradation rate constant of captopril in various pH value of phosphate-citrate buffer with 1.8 μ ionic strength at 60 °C.

In general, various actions of additives such as anti-irritants, antioxidants and enhancers were added into the formulation forming an acceptable preparation. Some of additives are insoluble, so semi polar vehicle such as ethanol, Propylene glycol and PEG 400 were incorporated into the formulation to increase the solubility of insoluble

additives and drug. As shown in Table 1, the degradation rates in ethanol solution were much lower than those of propylene glycol and PEG solution. The rate increased with increase in concentration of propylene glycol and PEG. It was proposed that the propylene glycol and PEG were used without further purification, which contained some metal ion leading to catalyze the oxidation reaction. Another possibility was that captopril easily undergoes oxidation in lower polar solvent to form captopril disulfide, a lower polarity substance. In addition, Kadin (1982) reported that the oxidation of captopril seems to occur less readily in methanol. Hence, alcohols seem to be the better choice of cosolvent for the insoluble additives in captopril preparations.

Table 1
Stability studies of 1% captopril solution without and with various additives at 60 °C and 70% RH

Additives	%	K (per day)
DI-water		0.019
Ethanol	5	0.020
	10	0.020
	20	0.020
PG	5	0.040
	10	0.050
	20	0.058
PEG 400	5	0.030
	10	0.035
	20	0.038
TPGS	1	0.019
	3	0.021
	5	0.020
EDTA	1	0.005
	3	0.005
	5	0.005
Ascorbic acid	1	0.020
	3	0.021
	5	0.021
Sodium ascorbate	1	0.040
	3	0.046
	5	0.052
Sodium bisulfite	1	0.021
	3	0.020
	5	0.020
Sodium sulfite	1	0.020
	3	0.021
	5	0.020
Enhancer-5	5	0.019
Diphenhydramine	0.2	0.020
Clobetasol	0.05	0.020

TPGS, alpha-tocopheryl polyethylene glycol succinate (water-soluble vitamin E); DI-water, deionized water.

The stability of captopril in aqueous liquids is limited by its oxidation to captopril disulfide (Pereira and Tam, 1992; Nahata et al., 1994). Therefore, the antioxidants and chelating agent were added into captopril solution to improve the stability in this study. As shown in Table 1, the additional ingredients, ascorbic acid, sodium ascorbate, sodium sulfite, sodium bisulfite and tocopherol did not improve captopril stability. The mechanism of EDTA, a chelating agent, is well known to chelate the catalytic metal ions reducing the oxidation reaction (Pereira and Tam, 1992; Lye et al., 1997). In previous studies (Wu et al., 2002), the anti-irritants including clobetasol and diphenhydramine and enhancer-5 (menthol derivative) possessed potent anti-inflammatory and penetration enhancement in captopril formulation, respectively. Hence, the effect of anti-irritants and penetration enhancer on the stability of captopril was also investigated. As shown in Table 1, there were no significant differences ($P > 0.05$) between the oxidative rate constants in the captopril without and with various concentration of clobetasol, diphenhydramine and penetration enhancer-5, indicating that these additives were compatible with captopril.

In semisolid dosage form, the degradation was also following the first-order kinetic (data not shown). Based on the captopril samples, the shelf life, the time to reach 90% of the original captopril concentrations at 30 °C were calculated to be 56 days as shown in Table 2. The shelf-lives of captopril gel prepared from CMC (29.4 days) and HPC (27.4 days) were similar and higher than others' dosage forms such as absorption cream (7.0 days) and solution (5.6 days). Therefore, the CMC gel was used in the study. According to previous studies (Aioi et al., 1993; Trivedi et al., 1995), tocopherol (vitamin E) possesses anti-irritant, antioxidant and penetration enhancer properties so that the effects of two lipophilic tercopherol (α form and δ form) on the stability were investigated in this study. As shown in Table 2, the addition of lipophillic tercopherol had no effect or a negative effect on the stability of

Table 2
Stability studies of 5% captopril formulations at 30 °C and 70% RH

Formulations	Estimated shelf-life (day)
Solution	5.61
Absorption cream	7.01
CMC	29.39
HPC	27.38
2.5% α-tocopherol[a]	14.33
5.0% α-tocopherol[a]	14.06
10% α-tocopherol[a]	14.15
2.5% δ-tocopherol[a]	21.35
5.0% δ-tocopherol[a]	20.40
10.0% δ-tocopherol[a]	21.49
1.0% Acetic acid[a]	57.38
1.0% EDTA[a]	320.51
1.0% EDTA and 1.0% acetic acid[a]	418.44

[a] Gel prepared with 5% CMC. EDTA, ethylenediaminetetraacetic acid.

captopril. This effect may be due to a decrease in polarity of gel because of captopril was less stable in lower polar solvent than that in the polar vehicle. Timmins et al. (1982) reported that metal ions are the most effective catalysts of the oxidation reaction, and they have been found in formulation additives, closure, container and manufacturing equipment. EDTA had the potent antioxidant effect in captopril solution. Similar results were found in gel dosage form. EDTA could obviously increase the shelf-life up to 320 days, about 11-fold than that of control gel. Moreover, the shelf-life was increased up to 418 days by adding 1% acetic acid into the gel containing EDTA. However, these results indicated that chelating agent EDTA and pH value of gel play the greatest role in the stability of captopril gel dosage form.

3.2. Adverse dermatologic reaction evaluation

The adverse dermatologic reaction including the integrity of stratum corneum and erythema induced by captopril were quantitatively evaluated by TEWL and skin color change (change chroma (ΔE) and difference in color (ΔC)) measurement, respectively. Table 3 presents the maximum values of TEWL, ΔE and ΔC measured on the third day (the other days' data not shown). The values of TEWL, ΔE and ΔC of formulation F1 were significantly ($P < 0.05$) greater than that of vehicle without captopril indicating that the active ingredient possesses potential irritant and anti-irritants required to prevent or improve the adverse dermatological reaction via topical administration. The values of TEWL, ΔE and ΔC were significantly ($P < 0.05$) reduced by incorporating diphenhydramine and clobetasol compared with the gel without anti-irritant, indicated that both diphenhydramine and clobetasol had signifi-

Table 3
The values of TEWL and erythema after application of captopril gel without and with anti-irritant in rabbit by bioengineering methods ($n = 3$)

Number	Anti-irritant	%	TEWL (g/m^2 per h)	ΔE	ΔC
F0	– (No captopril)	0.0	20.43 ± 1.59	3.46 ± 0.38	2.25 ± 0.56
F1	–	0.0	95.03 ± 5.39^{a}	8.10 ± 1.11^{a}	6.85 ± 1.93^{a}
F2	EDTA	1.0	96.31 ± 3.63	9.12 ± 1.64	6.32 ± 2.43
F3	Clobetasol	0.05	68.10 ± 4.11^{b}	5.14 ± 1.13^{b}	3.76 ± 0.39^{b}
F4	Diphenhydramine	0.2	74.47 ± 6.92^{b}	5.09 ± 1.01^{b}	3.51 ± 0.34^{b}
F5	Diphenhydramine	0.20	$45.62 \pm 2.32^{b,c}$	4.38 ± 2.11^{b}	3.42 ± 1.21^{b}
	Clobetasol	0.05			
F6	Diphenhydramine	0.20	$46.31 \pm 1.23^{b,c}$	5.01 ± 1.12^{b}	4.11 ± 1.57^{b}
	Clobetasol	0.05			
	EDTA	1.0			

[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 among F3, F4, F5 and F6 (ANOVA, $P < 0.05$).

cant inhibition skin irritation effect including the barrier function integrity (reduction in TEWL) and erythema (reduction in ΔE and ΔC) improvement. According to previous study, the skin rash caused by captopril may be the result of the direct pharmacological action of captopril on the inhibition of kinas-II, the same enzyme as ACE (Wilkin et al., 1980). 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 dermatologic reaction. 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 suppression metabolic activity (Aalto-Korete and Turpeinen, 1995). Anaizi and Swenson (1993) reported that the oxidative product, captopril disulfide, possesses significant bradykinin-potentiating activity but no ACE-inhibiting activity. From above stability study, EDTA could inhibit the oxidation reaction of captopril, hence the effect of EDTA on the skin irritation was evaluated. As shown in Table 3, there was no significant differences in the TEWL, ΔE and ΔC between the captopril gel with or without 1% EDTA, indicating that the EDTA had no inhibition irritation effect and also did not induce irritation by itself. The result was not consistent with our expectation. It might be speculated that the control gel was stable during the period of experiment or the molecule of disulfide was too large to penetrate the skin to induce the irritation. In addition, the values of TEWL, ΔE and ΔC of captopril gel containing the diphenhydramine and clobetasol without and with EDTA (F5 and F6) also showed no difference ($P > 0.05$), indicated that EDTA did not retard the inhibition skin irritation effect of these anti-irritants.

3.3. In vitro skin penetration experiments

The cumulative amounts of drug penetration through the skin were plotted against time as shown in Fig. 3, by which a linear relationship was obtained for each formulation ($R^2 >$ 0.92214), showing that the penetration of captopril from the gel was well described by the zero-order kinetics. There were no significant differences ($P > 0.05$) in flux between the captopril gel without and with anti-irritants, which indicated that the anti-irritants, diphenhydramine or/and clobetasol, did not influence the percutaneous absorption of captopril.

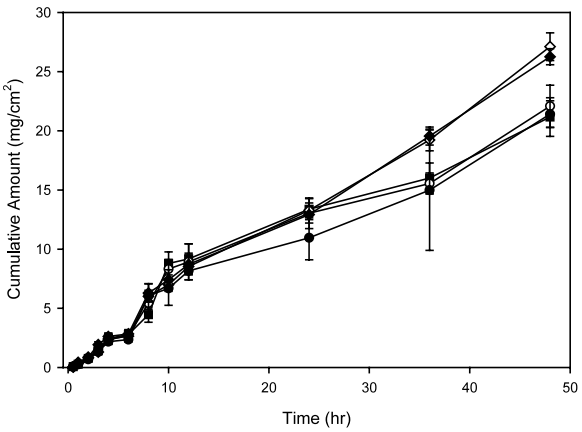

Fig. 3. Permeation-time profile of captopril gel without and with additives through excised rabbit skin ($n = 3$). ■ control; ○ EDTA; ● EDTA and acetic acid; ◇ EDTA, clobetasol and diphenhydramine; ◆ EDTA, clobetasol, diphenhydramine and acetic acid.

According to previous study (Kobayashi et al., 1995; Wu et al., 1996), the flux of captopril through rabbit skin was about 4.8-fold higher than that of through human skin and the required flux of captopril transdermal delivery system to maintain the minimum effective concentration was 1488 μg/cm^2 per h through human skin. The flux of captopril gel containing anti-irritants (diphenhydramine and clobetasol) and EDTA was 539.45 μg/cm^2 per h and the required minimum administration area was about 13.24 cm^2, indicating that the formulation can possibly be developed for a transdermal drug delivery system.

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