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Original Research

Improvement of transdermal permeation of captopril by iontophoresis

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KEY WORDS captopril; iontophoresis; cutaneous administration; hydrogel patch

ABSTRACT

AIM: The feasibility of iontophoresis on the transdermal delivery of captopril was studied. **METHODS**: Iontophoresis was employed for enhancing transdermal transport of captopril through rat skin *in vitro* and *in vivo*. **RESULTS**: It was demonstrated that the iontophoresis-induced flux of captopril was affected by various factors such as pH, ionic concentration, and the concentration of captopril in donor compartments as well as the applied electric current intensity. Electric current could induce several-fold increase in captopril flux with hydrogel. Skin permeation study *in vivo* in rats demonstrated that iontophoresis could effectively promote the transdermal transport of captopril without significant skin irritation. Captopril concentration in plasma reached plateau (~0.9 μg/mL) at 1 h after current application and was maintained at the same level during the experiment. On the contrary, captopril could not be detected in plasma when the current was not applied. No obvious skin irritation was observed after 9-h continuous iontophoresis. **CONCLUSION**: Transdermal delivery of captopril can be effectively improved by iontopophoresis.

INTRODUCTION

In recent years, a number of peptide and protein drugs have been developed. However, oral administration of peptides and proteins is often not practical because of their poor absorption characteristics from the gastrointestinal tract as a result of their extensive degradation by intestinal peptidases and their instability to cross the intestinal mucosa. Alternative routes, including rectal[1], vaginal[2], nasal and pulmonary[3-4], have been investigated for peptide delivery.

Transdermal delivery of peptides is an attractive route due to the painless, controlled input of these agents and is avoidance of the hepatic first-pass effect. However, it is unlikely that peptides and proteins will easily permeate across the skin, particularly the stratum corneum. Furthermore, recent investigations suggested that peptide drugs may also undergo an extensive degradation in the viable skin. It is thus desirable to increase the transport of peptide drugs across the skin by overcoming the problems of their permeability and stability. Among various approaches to improve the transdermal delivery, iontophoresis is a very attractive approach to promote the transdermal transport of peptides and proteins such as insulin, LH-RH and angiotensin-converting enzyme (ACE) inhibitors, which can hardly penetrate through skin without an active driving force[5-8].

Captopril, an ACE inhibitor, has been widely used for treatment of hypertensive disease. Its conventional dosage form is tablet and is administered orally three times daily because its biological half-life is relatively short (~1.7 h). For the transdermal delivery of captopril, Dubey[9] developed its transdermal therapeutic system (TTS) which had the transport rate of 60 - 90 $\mu g \cdot cm^{-2} \cdot h^{-1}$ through human cadaver skin *in vitro*. And Zakzewski[10] also reported that a pulsed direct current iontophoresis device for captopril decreased the blood pressure in hypertensive rabbits.

In this report, we investigated various factors affecting the direct current iontophoresis of captopril to increase the transdermal transport rate, ie, current intensity, pH, ionic concentration, and drug concentration. Based on these results, we chose the optimal conditions for iontophoresis of captopril and performed an *in vivo* transport study in rats.

MATERIALS AND METHODS

Chemicals Captopril was purchased from

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Changzhou Pharmaceutical Co Jiangsu, China). 2,4-Dibromacetophenone (*p*-BPB) was purchased from Merck Co (Germany) and was purified prior to use. Polyvinylalcohol (PVA; saponification value >98 %; average polymerization degree, 1700) was from Shanghai Chemical Industry (Shanghai, China). All other chemicals were of AR grade and were used without further purification.

Preparation of hydrogel patch containing captopril PVA was dissolved in 2 % (w/w) glycerol solution at 95 ℃. After cooling, captopril was added into the solution to yield a concentration of 100 $g \cdot L^{-1}$. The pH of the mixture solution was adjusted to 4.5 with HCl 0.1 $mmol \cdot L^{-1}$. The mixture was degassed and poured into round dies (1.95 cm in diameter and 0.2 cm in height) and then it was frozen at −30 ℃ for 48 h to form gel matrix, and stored at 4 − 5 ℃ for 24 h. The gel patches (100 mg captopril per patch) were taken from the dies and kept in tight package at 25 ℃.

***In-vitro* skin permeation of captopril by iontophoresis** Full-thickness abdominal skin excised from a male Wistar rat (150 − 200 g) was used. Horizontal-type diffusion cells with an effective diffusional area of 3.0 cm^2, donor volume of 1.0 mL and receiver volume of 2.0 mL were used. After removal of hair with a hair clipper and subcutaneous fat, the skin was mounted on the diffusion cell with the epidermal side facing the donor cell. The two needle type carbon electrodes were separately inserted into the donor and receiver compartments. The donor compartment (cathode) was filled with 1.0 mL of captopril solution and receiver (anode) was filled with 0.9 % NaCl solution. The apparatus was maintained at 37 ℃. At designated intervals, the receiver medium was drawn out entirely and immediately replaced with fresh 0.9 % NaCl solution. The samples were determined by HPLC.

***In-vitro* iontophoresis experiment with captopril-containing patch** Commercially available Franz-type diffusion cells (Hanson Research, USA) with an effective diffusional area of 2.26 cm^2 and receiver volume of 7.5 mL were used. After removal of hair and subcutaneous fat, the skin was mounted on a Franz-type diffusion cell with the epidermal side facing the donor cell. A needle-type carbon anode and a disk-type cathode were put into the receiver compartment and onto the skin, respectively. During the experiment, temperature was maintained at 37 ℃ and the current intensity was applied at 170 $\mu A/cm^2$.

In some experiments, we investigated the release of captopril from the hydrogel patch with the use of the Franz-type diffusion cell. In this case, no current was introduced.

***In vivo* skin absorption experiment by using iontophoresis** Under anesthesia induced by ip injection of pentobarbital 50 mg/kg, the abdominal hair of male Wistar rat weighing 200 − 220 g was removed and two hydrogel patches were attached to the abdominal surface at a distance of 10 cm. Two disk type electrodes were put onto each patch, namely, one electrode was on the captopril-containing hydrogel patch as a cathode and the other was put onto the hydrogel patch without captopril as an anode. Iontophoresis was undertaken during the experiment at current intensity of 170 $\mu A/cm^2$. Plasma samples were collected at 1, 2, 3, 5, 7, and 9 h from rat tail and were subjected to the assay.

Analysis The concentrations of captopril were determined according to the method of Yukinori[11]. Briefly, captopril was treated with p-BPB at pH 7.0 for 30 min at room temperature and assayed by reversed phase HPLC on a YWG $10C_{18}$ column (150 mm × 4.6 mm). The mobile phase was a mixture of methanol-water-acetic acid (67:32.5:0.5) and was run at a flow rate of 1.5 mL/min. The eluate was monitored with a UV detector at 254 nm. The limit of detection for captopril was 50 $\mu g/L$.

Statistic analysis Data were expressed as $\bar{x} \pm s$ and compared with t-test, a two-tailed value of $P < 0.05$ was taken to indicate statistical significance.

RESULTS AND DISCUSSION

Various factors affecting the *in-vitro* skin penetration of captopril by iontophoresis With the use of the horizontal-type diffusion cell, we investigated several factors for electrophoresis which might affect the flux of captopril through the rat skin as summarized in Tab 1.

We investigated the iontophoresis-induced skin penetration of captopril at two different pH conditions, pH 2.0 and pH 4.0. When iontophoresis was applied to the rat skin *in vitro*, the flux of captopril at pH 4.5 was 2.4-fold larger than that at pH 2.0. The difference of the flux of captopril between pH 2.0 and 4.5 could be explained by the difference of its dissociated form. Namely, since captopril is a weak acid (pKa 3.7), the fraction of ionization form of captopril varied at different pH conditions. At pH 4.5, about 86 % of captopril is expected to be in ionized form in the solution, while only

Tab 1. The influence of donor composition on the captopril flux. Current intensity 170 μA/cm², pH 2.0 or 4.5, drug concentration 10, 50, or 117 g/L (saturated), NaCl ionic concentration added 0, 0.14, or 0.39 mol/L. $n = 3$ replicates. $\bar{x} \pm s$.

Drug/ g·L⁻¹	pH	NaCl ionic concentration/ mol·L⁻¹	Flux/ μg·cm⁻²·h⁻¹
50	2.0	0	88 ± 6
50	4.5	0	210 ± 42
50	4.5	0	210 ± 43
50	4.5	0.14	110 ± 21
50	4.5	0.39	71 ± 18
10	4.5	0.14	43 ± 6
50	4.5	0.14	110 ± 21
117	4.5	0.14	353 ± 17

2 % of captopril is in ionized form at pH 2.5 (Tab 1).

The addition of NaCl to the donor compartment decreased the iontophoresis-induced flux of captorpil in a concentration dependent manner. It is well known that when co-ion of the drug is added to the donor compartment the iontophoresis-induced drug flux is reduced. In our experiment, chlorine ion might act as co-ion for the ionized captopril and might reduce its flux through the rat skin (Tab 1).

The iontophoresis-induced flux of captopril increased along with its increasing concentrations in the donor compartment. The flux of captopril was (353 ± 17) μg·cm⁻²·h⁻¹ at a concentration of 117 g·L⁻¹ which is the apparenu saturation concentration of captopril at pH 4.5 (Tab 1).

Next we studied the effect of current intensity on the iontophporeis of captopril. Fig 1 shows the cumulative amount of captopril permeating at various current intensities. When the iontophoresis was not applied, the flux of captoril through the rat skin was very low [(37 ± 4) μg·cm⁻²·h⁻¹]. On the other hand, the application of iontophoresis significantly enhanced the captopril flux across the rat skin [(197 ± 9) μg·cm⁻²·h⁻¹ at 33 μA/cm² and (353 ± 17) μg·cm⁻²·h⁻¹ at 170 μA/cm²].

***In-vitro* iontophoresis with captopril-containing hydrogel patch** PVA hydrogel patch is a non-dissolving and water-swollen matrix with high mechanical strength and water content. We prepared three kinds of captopril patches with different PVA contents, 5 %, 10 %, and 15 %, and investigated the release of captopril from the patches into saline solution (Fig 2). The release rate of captopril from the patch was decreased with

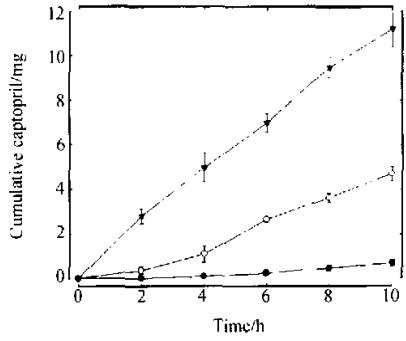

Fig 1. The effect of electric current density on the iontophoretic transdermal delivery of captopril. Drug concentration 117 mg/ml, pH 4.5, NaCl ionic concentration added 0.14 mol/L. ▼ 170 μA/cm², ○ 33 μA/cm², ● 0 μA/cm². $n = 3$ replicates. $\bar{x} \pm s$.

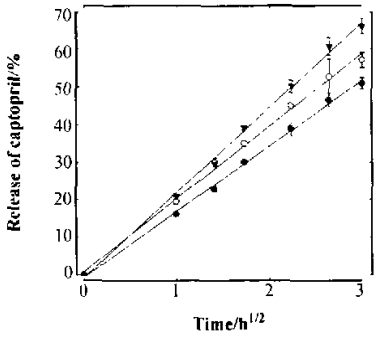

Fig 2. *In vitro* release profile of captopril hydrogel with different PVA patch content. ▼ 5 % PVA, ○ 10 % PVA, ● 15 % PVA. $n = 6$ replicates. $\bar{x} \pm s$.

increasing PVA content. There was a linear relationship between the release of captopril from the patches and square-root of time as shown in Fig 3. The release pattern thus appears to follow the Higuchi's equation and the rate-limiting step of the captoril release from the PVA hydrogel matrix might be its diffusion in the gel matrix.

Using these captopril-containing PVA hydrogel we investigated the iontophoresis-induced captopril transport through the rat skin. The flux rate of captopril into the receiver compartment is shown in Fig 4. The application of iontophoresis at current intensity of 170 μA/cm² markedly increased the flux of captopril. In addition, the flux of captopril across the rat skin tended to be slightly decreased by the increasing PVA content, but there is no significant difference. The tendency seems to consistent with the captopril release from the patches.

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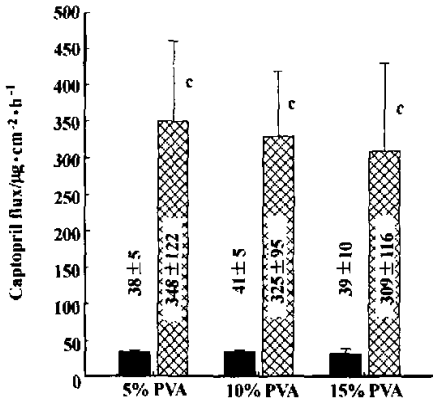

Fig 3. ***In vitro*** **permeation flux of captopril hydrogel patch.** $n=3$ **replicates.** $\bar{x} \pm s$. $^cP<0.01$ ***vs*** **passive diffusion.**

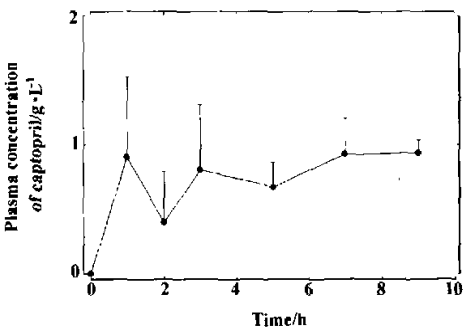

Fig 4. Plasma captopril concentration-time curve of ***rats receiving iontophoretic transdermal administration*** **of captopril hydrogel patch. Current intensity 170 μA/cm².** $n=3$ **replicates.** $\bar{x} \pm s$.

In-vivo **application of captopril-containing hydrogel and iontophoresis** Finally we applied the *captopril-containing hydrogel and iontophoresis on rat in vivo*. Fig 4 shows the plasma concentration profile of captopril during application of iontophoresis. Captopril concentration in plasma reached plateau (about 0.9 mg/L) at 1 h after current application and was maintained at the same level during the experiment. Due to the detection limit of HPLC, no obvious captopril was found during passive diffusion of captopril hydrogel patch. Furthermore, no obvious skin irritation was observed after a 9-h continuous iontophoresis.

CONCLUSION

In summary, we were able to promote the transdermal transport of captopril through the rat skin using combination of iontophoresis and PVA hydrogel patch without skin irritation. The flux rate of captopril on the application of iontophoresis was 10-times larger than that without iontophoresis. The encouraging results of the combination of the iontophoresis and the PVA hydrogel patch might be helpful to develop the potential therapeutic transdermal application of this novel technique.

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离子导入对卡托普利透皮吸收的促进作用

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关键词 卡托普利; 离子透入法; 透皮吸收; 水凝胶贴片

目的: 研究离子导入技术对卡托普利透皮吸收的促进作用. **方法**: 应用离子导入技术研究了卡托普利体外透过大鼠离体皮肤的影响因素, 并进行了卡托普利水凝胶贴片大鼠在体的试验, 测定了血药浓度的变化. **结果**: 离子导入技术可以有效地促进卡托普利的透皮吸收, 透皮速率增加约 7 倍. 药物贮库中的各种因素如 pH, 离子强度, 药物浓度和电流强度均影响药物的透皮速率. 随着 pH 的增加, 离子强度的减小, 药物浓度的增加及电流强度的增加, 透皮速率也增加. 大鼠在体试验也表明用约 1 h 后血药浓度即可达到坪值(约 0.9 μg/mL), 并在整个试验阶段维持稳定. **结论**: 离子导入可以有效地促进卡托普利的透皮吸收.

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