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Evaluation of pharmacokinetics and pharmacodynamics of captopril from transdermal hydrophilic gels in normotensive rabbits and spontaneously hypertensive rats

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Abstract

The purpose of this investigation was to assess the pharmacokinetics (plasma concentration) and pharmacodynamics (heart rate, blood pressure (BP), and plasma renin activity (PRA)) of captopril experimental gel in normotensive rabbits and spontaneously hypertensive rats (SHRs) by reference to a short duration intravenous administration of the drug. In normotensive rabbits, the blood concentration versus time course of captopril after transdermal administration could be described well by a two-compartment model, and the maximum plasma concentration (5.68 ± 2.05 μg ml^{-1}) was achieved in about 7 h. The increase in plasma captopril concentration led to increases in PRA and reductions in BP. A simple E_{max} model adequately described the relationship between the percentage change of mean blood pressure (MBP) and the blood concentration of the captopril. The maximum reduction in MBP (E_{max}) was 36.23% and the concentration at half maximum effect (EC_{50}) was 0.24 μg ml^{-1}. The captopril was continuously released from the gel formulation and protected the SHRs in lower BP throughout the period of transdermal therapy. These results indicated that the development of captopril transdermal drug delivery system was possible. Further research was warranted on a modified formulation of captopril, which was optimized for transdermal delivery of the drug.

Keywords: Captopril; Pharmacokinetics; Blood pressure; Heart rate; Plasma renin activity; Spontaneously hypertensive rat

1. Introduction

Transdermal pharmaceutical products, whether ointments, matrix formulations or reservoir systems provide the considerable advantage 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. The rate-controlled transdermal dosage form can provide a precise regulation of drug concentration in plasma and thus a high

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degree of safety and selectivity of action for some drugs (Chien, 1992). In recent years the transdermal therapeutic system has become increasingly popular for administration, e.g. clonidine, fentanyl, glyceryl trinitrate and oestradiol, etc. (Good, 1983; Karim, 1983; Burris and Mroczek, 1986; Seki et al., 1990; Sclar et al., 1991). This dosage form provides a convenient delivery system.

Captopril is an orally effective angiotensin I converting enzyme inhibitor and is used in the 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, 1996). According to the previous research, the oxidation rate of captopril in dermal homogenates is significantly lower than that in intestinal homogenates (Zhou and Li Wan Po, 1994) because the oxidative product of captopril, captopril disulfide is difficulty absorbed from the intestine. Consequently, transdermal drug delivery system (TDDS) may be suitable for captopril as a successful dosage form.

According to the previous in vitro permeation studies using human chest skin as barrier membrane (Kobayashi et al., 1995; Wu et al., 1996), the formulation of captopril gel had attained the serum level (31 ng ml^{-1}) of therapeutic minimum effect using about 10 cm^2 of administered area. This administered area was small enough to develop TDDS. Hence, in order to evaluate the possibility of TDDS development, this captopril gel was selected to perform the in vivo experiment using animal model (normotensive rabbits and spontaneously hypertensive rats (SHRs)) to prove its penetration effect. The objective of the present study was to assess the pharmacokinetics (plasma concentrations) and pharmacodynamics (blood pressure, plasma renin activity (PRA) and heart rate) of captopril from hydrophilic gel dosage form in normotensive rabbits and SHRs by reference to a short duration of intravenous administration of the drug.

2. Materials and methods

2.1. Materials

The following reagents were used — captopril, ascorbic acid and *p*-phenylphenol (Sigma Chemical Company, USA), pentobarbital (Abbott, USA), 4-bromophenacyl bromide (*p*-BPB), ethylenediaminetetraacetic acid trisodium salt (EDTA-3Na)(TCI, Japan). All other chemicals and solvents were of analytical reagent grade.

2.2. Animals

Male New Zealand White rabbits (10–12 weeks old, 2.0–2.5 kg) and male SHRs (10–12 weeks old, 150–200 g) were used in this study. Rabbits and SHRs were anesthetized with 30 mg/kg of sodium pentobarbital injected into a marginal ear vein and intra-abdominal injection, respectively. A catheter was introduced into the femoral artery (for rabbits) or jugular artery (for SHRs), and connected to a pressure transducer to record blood pressure and heart rate at the same time.

In order to investigate the percutaneous absorption of captopril gel, a dose of 20 mg kg^{-1} was administered by bolus injection via the ear vein of normotensive rabbits. Subsequently, the captopril gel including 5% captopril, 5% hydroxypropyl cellulose, 5% enhancer 5 and 10% propylene glycol (300 mg per 30 cm^2) was applied on the shaved abdomen by the occlusive dressing technique (ODT) (Naito and Tsai, 1981; Hsu et al., 1991). After both administrations, blood samples (for the measurement of plasma unchanged captopril and PRA) were taken at appropriate intervals and the BP and heart rate were recorded during the experimental period.

In SHRs, the captopril gel (50 mg per 4 cm^2) was applied, and then the BP and heart rate were recorded during the experimental period.

2.3. HPLC analysis of unchanged captopril in plasma

The plasma captopril concentration was analyzed by HPLC methods modified from that developed by Kawahara et al. (1981), Klein et al.

(1990). The artery blood samples (2 ml) were mixed with 0.05 ml of a solution of EDTA-3Na (0.2 M) and ascorbic acid (0.2 M) and centrifuged immediately at 5000 rpm for 7 min at 4°C. A 1-ml aliquot of plasma was added to a 16×125 mm screw-cap glass tube containing 0.1 ml of a derivative agent 4-bromophenacyl bromide (*p*-BPB, 2 mg ml^{-1} in acetonitrile) and 1 ml of phosphate buffer (pH 7.4). The tube was vortexed for 30 s and then left at room temperature for 30 min. After this, 0.2 ml 2 N HCl was added, and the resulting plasma samples were frozen at −20°C until assayed. After defrosting, a 0.1-ml aliquot of *p*-phenylphenol (used as internal standard, 1 μg ml^{-1} in acetonitrile) was added and the tube was vortex-mixed for 15–20 s and then 6 ml of 1:1 mixture of ethyl acetate/benzene was used as extracting solvent. The tube was vortexed for 30 s and then shaken gently for 10 min. After centrifugation, the organic layer was removed, and evaporated to dry under reduced pressure. The residue was reconstituted in 0.2-ml acetonitrile and aliquots of 0.02 ml were injected into the HPLC system.

The HPLC analysis were performed on a Waters system consisting of model M-45 pumps, a model 470 UV detector, a SIC chromatocorder 12 integrator, a 125×4 mm i.d. stainless steel column with LichroCART C-18 column (E. Merk) was used. The mobile phase for captopril–*p*-BPB consisted of 40% acetonitrile and 0.02% acetic acid in water. The operating temperature was 37 ± 0.5°C, and the flow rate was 1.0 ml min^{-1} with UV absorbency monitoring at 260 nm. The coefficients of variation (%, $n = 5$) of the assay method were 5.7 and 5.3% for plasma concentrations of 300 and 5000 ng ml^{-1} captopril, respectively. The limitation of detection was 50 ng ml^{-1} captopril.

2.4. Measurement of plasma renin activity (PRA)

PRA samples taken in EDTA tube were stored at −20°C until assay using a radioimmunoassay (berthold Multi-crystal counter LB2104) with 1 h incubation as recommended by the manufacturer. Values were calculated as mg l^{-1} s^{-1}.

2.5. Data analysis

The relationship between the effect on MBP and the blood levels of captopril in rabbits were analyzed using a simple E_{max} model (Holford and Sheiner, 1981)

$$E = E_{max} \frac{C}{EC_{50} + C}$$

where E is the reduction in MBP (%), E_{max} (%) the maximum reduction in MBP, C the captopril concentration (μg ml^{-1}) and EC_{50} the captopril concentration yielding 50% of the maximum decrease in MBP.

3. Results and discussion

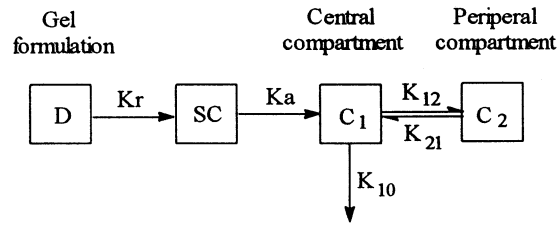
3.1. In normotensive rabbits

3.1.1. Pharmacokinetics

Firstly, the intravenous dosage form of captopril was administered in rabbits (20 mg kg^{-1}), in order to investigate the pharmacokinetics of percutaneous absorption of hydrogel captopril. The plasma concentrations of captopril after i.v. administration declined in a biexponential manner. The plasma levels of captopril adequately described using a two-compartment open model (AIC: 5.57) are compared with one-compartment open model (AIC: 8.74) by utilizing the least-squares fit program (PCNONLIN, SCI, Software, USA) and the parameters calculated are listed in Table 1.

Table 1
Pharmacokinetic parameters of captopril following intravenous administration (20 mg kg^{-1}) in rabbits ($n = 3$)

Parameters	Estimate
α (h^{-1})	4.72 ± 0.33
β (h^{-1})	0.97 ± 0.70
$t_{1/2}$ (h)	2.51 ± 2.79
k_{10} (h^{-1})	3.74 ± 1.07
k_{12} (h^{-1})	1.21 ± 0.86
k_{21} (h^{-1})	1.15 ± 0.87
$AUC_{0-\infty}$ (μg·h ml^{-1})	8.03 ± 2.02
Clearance (l h^{-1})	5.19 ± 0.09
V_d (l)	1.51 ± 0.43



Scheme 1. Pharmacokinetic model for percutaneous absorption where k_r is the release rate; k_a the absorption rate constant; k_{12} the rate constant from the central to tissue compartment; k_{21} the rate constant from the tissue to central compartment and k_{10} the elimination rate constant from the central compartment.

The pharmacokinetics of percutaneous absorption of drugs have been widely discussed and several effective models have been developed for understanding the absorption behavior of drugs through the skin (Naito and Tsai, 1981; Guy et al., 1982; Ogiso et al., 1989; Takayama and Nagai, 1991; Huang et al., 1993). The model (Scheme 1) which consisted the zero-order release rate and first-order absorption rate constant was employed to explain the plasma–time data for captopril after topical administration in this study. The plasma concentration of captopril is given as follows (Huang et al., 1993)

$$C_l = \frac{k_r}{V_p k} + \frac{k_r k_a}{V_p}\left[\frac{(\alpha - k_{21})e^{-a(t-t_L)}}{\alpha(\beta-\alpha)(k_a-\alpha)} + \frac{(\beta - k_{21})e^{-\beta(t-t_L)}}{\beta(\alpha-\beta)(k_a-\beta)} + \frac{(k_a - k_{21})e^{-k_a(t-t_L)}}{k_a(k_a-\alpha)(k_a-\beta)}\right]$$

where C_l is the plasma concentration, t_L the lag time, k_r the release rate and k_a the absorption rate constant. The details of other parameters, such as α, β, V_p, and k_{21} are the same as those given in Table 1. As depicted in Fig. 1, the lag time was about 1.11 h and the maximum plasma captopril concentration was 5.68 ± 2.05 μg ml^{-1} achieved in about 7 h. The correlation coefficient was 0.963 ($P < 0.05$) that indicated this equation was adequate to describe the in vivo transdermal data. The k_o, k_a and $AUC_{0-12\ h}$ were 33.23 ± 9.56 mg h^{-1}, 0.41 ± 0.01 h^{-1} and 47.18 ± 14.02 μg h ml^{-1}, respectively. Since k_r is higher than k_a, it would be reasonable to assume that k_o was the main rate-limiting factor in this model.

3.1.2. Pharmacodynamics

As shown in Figs. 2 and 3, the heart rate increased slightly in early stages after intravenous and transdermal administration of captopril. There were no significant differences ($P > 0.05$) between heart rate in both the routes of administration. The results are consistent with previous reports on captopril (Katzung, 1992; McEvoy, 1996) which indicated when captopril is used the cardiac output and heart rat are not significantly changed because captopril does not result in reflex

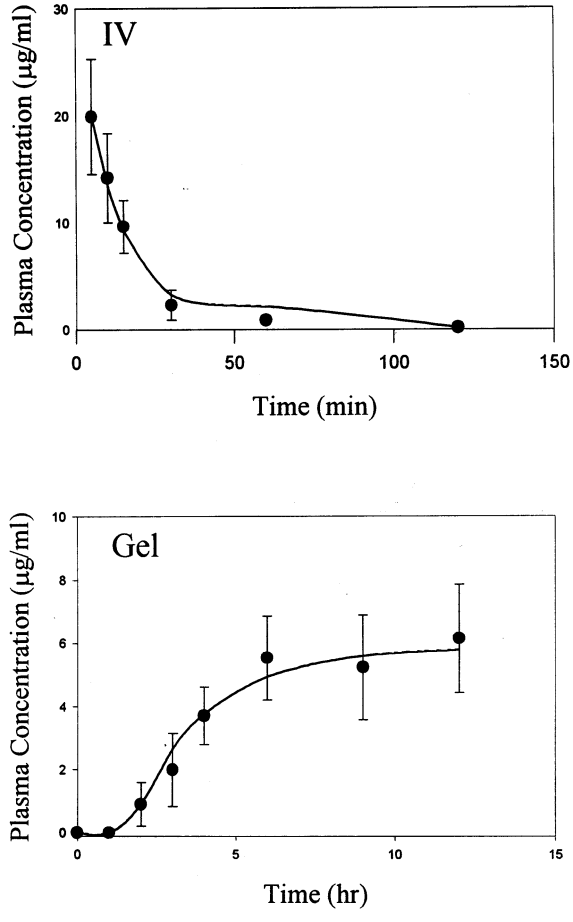


Fig. 1. Plasma concentration–time profile of captopril following intravenous (20 mg kg^{-1}) and transdermal (300 mg per 30 cm^2) administration in normotensive rabbits. The solid line shows simulated curve for captopril.

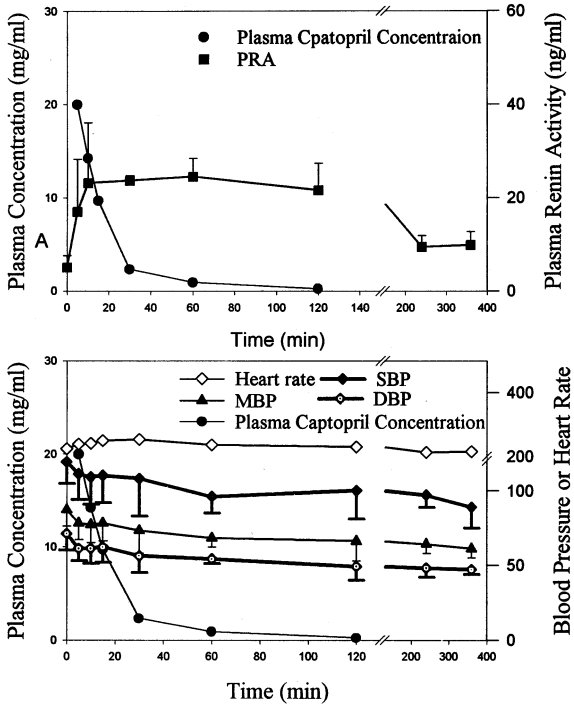


Fig. 2. Correlation over time of plasma captopril concentration with plasma renin activity (PRA) and heart rate and blood pressure after intravenous (20 mg kg^{-1}) administration in normotensive rabbits ($n = 3$).

sympathetic activation and, therefore, it can be used safely in persons with ischemic heart diseases.

The influence of blood pressure (including SBP, DBP and MBP) and plasma rennin activity (PRA) with plasma captopril concentration after intravenous and topical administration is depicted in Figs. 2 and 3. The blood pressure (including SBP, DBP and MBP) slowly decreased with time after intravenous administration. The PRA increased rapidly within the first 10 min, subsequently remained stable for 2 h, and then slowly decreased with time to the initial concentration before intravenous administration. This result is consistent with a previous research (McEvoy, 1996), which reported a positive correlation between PRA and long-term response to captopril. The mechanism of action of captopril has not been fully elucidated. However, the major mechanism of captopril is to decrease plasma angiotensin II (a potent vasoconstrictor) concentration and, consequently, blood pressure may be reduced in part through decreased vasoconstriction. Furthermore, captopril could also increase PRA as a result of a decrease in blood pressure.

After captopril HPC gel topical application (300 mg per 30 cm^2), the effects of captopril on blood pressure and PRA are shown in Fig. 3. The blood pressure decreased following the increase of captopril concentration in plasma during the initial stages of administration. The lowest blood pressure was observed after 2 h of transdermal application, and then the blood pressure remained constant, although the plasma captopril concentration still rose. This result indicated that the BP of normotensive rabbits would not decrease without limit after the increase of plasma captopril concentration. From the result, we suggested the relationship between concentration of captopril

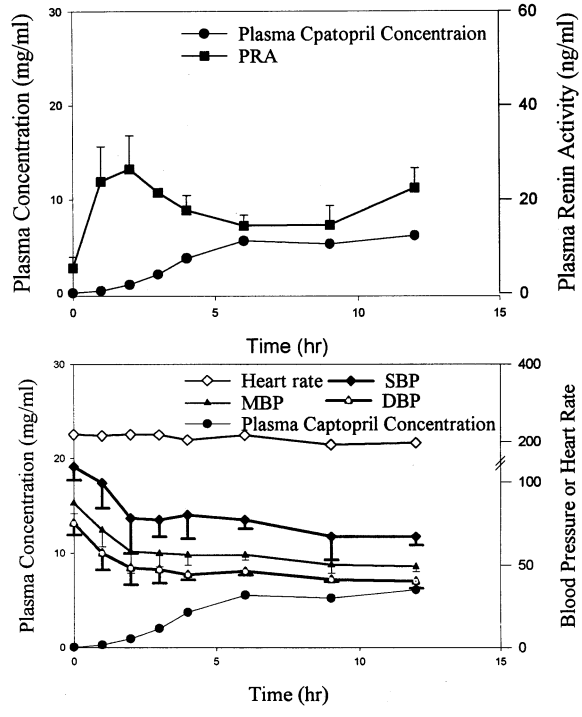


Fig. 3. Correlation over time of plasma captopril concentration with plasma renin activity (PRA) and heart rate and blood pressure after transdermal (300 mg per 30 cm^2) administration in normotensive rabbits ($n = 3$).

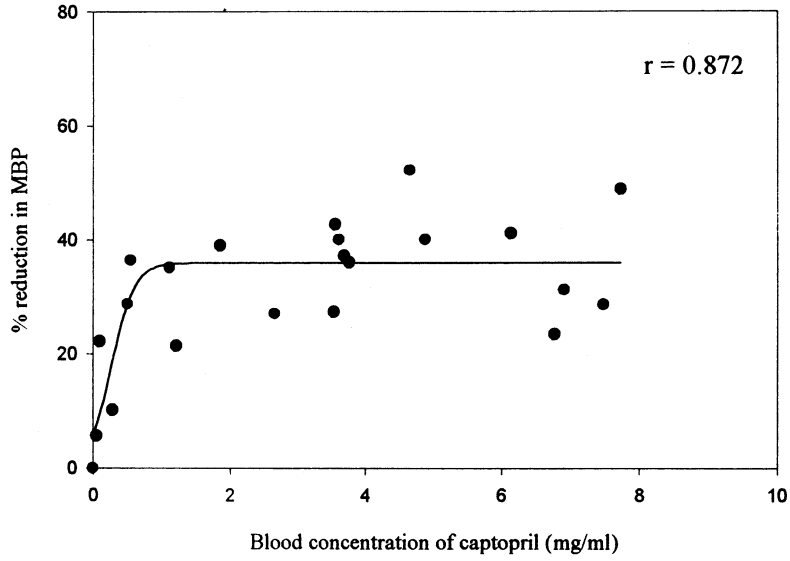


Fig. 4. The individual value of reduction in MBP (%) vs. the blood concentration of captopril after transdermal (300 mg per 30 cm^2) administration in normotensive rabbits ($n = 3$). The solid line represents the model-derived relationship between the blood concentration and effect.

and decrease in BP was nonlinear due to the inhibition effect of captopril for angiotensin converting enzyme was saturated, which restricted the BP only to a limited level. Another possible reason is the natural compensatory reaction, which resulted in the maintenance of BP within a physiological range. According to previous studies (Katzung, 1992; McEvoy, 1996), the mechanism of action of captopril has not been fully elucidated. The drug appears to inhibit the angiotensin converting enzyme, resulting in decreased plasma angiotensin II concentration. Consequently, BP may be reduced in part through decreased vasoconstriction, PRA increases, possibly as a result of loss of feedback inhibition (mediated by angiotensin II) on the release of renin from the kidney and/or stimulation of reflex mechanism via baroreceptor (as a result of the decrease in BP). As observed in Fig. 2, the PRA rapidly increased with an increase in captopril plasma concentration initially, and then persisted at a higher level for 12 h of observation, which was 3-fold higher than that in pretreatment. After the comparison of plasma captopril concentration with BP and PRA, the curve of PRA closely reflected the plasma captopril concentration than that of blood pressure in normotensive rabbits after topical application.

From the above results of intravenous and topical administration, there was a trend that the increased concentration of captopril in plasma led rapidly to increased PRA, but the PRA was maintained in plasma for a longer period of time than the captopril concentration. Simultaneously, there was a clear correlation between the rise of captopril plasma concentration and the decrease of BP after both routes of administration. However, there was no clear relationship between the rise of PRA and antihypertensive response. These results were similar to earlier researches (Hutchinson et al., 1980; Al-Furasih et al., 1991; Levy et al., 1991), which indicated that although converting enzyme inhibitors are most effective under conditions associated with high PRA, there is no clear correlation between subjects in PRA and antihypertensive responses.

3.1.3. Pharmacokinetics/pharmacodynamics

The individual values of the percentage change in MBP versus the blood concentration of captopril at the time of effect recording are shown in Fig. 4. The solid line represents the fit of the E_{max}

model to the pooled data. According to the model, the maximum reduction in MBP (E_{max}) was 36.23% and the concentration at half maximum effect (EC_{50}) was 0.24 μg ml^{-1}.

3.2. In spontaneously hypertensive rats (SHRs)

The influence of systolic and diastolic BP and heart rate after transdermal administrations are shown in Fig. 5. The heart rate of SHRs increased slightly, which was similar to that of normotensive rabbits. The BP of SHRs decreased significantly at 1 h after administration, and then longer steady state lower BP persisted for 9 h of observation. These results demonstrated that captopril could continuously release from the gel formulation and it protected SHRs at lower BP throughout the period of transdermal therapy.

4. Conclusion

A two-compartment open model was the best model to describe the blood concentration versus time course of captopril in this study. After topical application, the maximum plasma captopril concentration 5.68 ± 2.05 μg ml^{-1} was achieved at about 7 h. The increase in plasma captopril concentration led to increases in PRA and reductions in BP after intravenous and transdermal administration in normotensive rabbits. Heart rate increased slightly after captopril administration and the relationship between the pharmacodynamic response (percentage change in MBP) and the blood concentration of captopril could be described adequately using a simple E_{max} model.

However, captopril continuously released from the gel formulation and protected the SHRs at lower arterial blood pressure (including SBP, MBP and DBP) throughout the period of transdermal therapy. These results indicated that the development of captopril as a TDDS was possible. Further research warranted on a modified formulation of captopril, is optimizing transdermal delivery of the drug.

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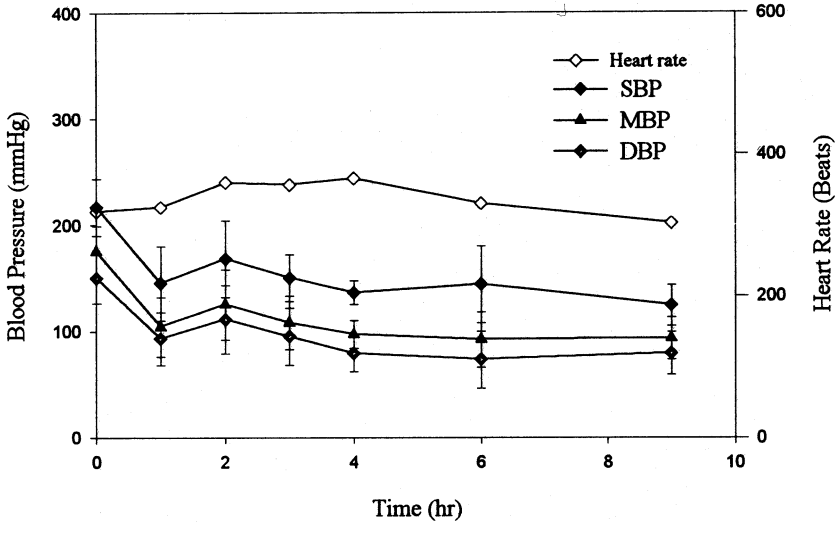

Fig. 5. The heart rate and blood pressure vs. time curve after transdermal (50 mg per 4 cm^2) administration in SHRs ($n = 3$).

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