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# Pharmacokinetic and pharmacodynamic study of imidaprilat, an active metabolite of imidapril, a new angiotensin-converting enzyme inhibitor, in spontaneously hypertensive rats

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**Abstract**

The pharmacokinetics and pharmacodynamics (PK/PD) of imidaprilat, an active metabolite of imidapril, a new angiotensin-converting enzyme (ACE) inhibitor, were investigated. Imidapril was infused subcutaneously for 4 weeks via an osmotic pump implanted under the skin in the back of male spontaneously hypertensive rats (SHRs). Plasma concentration of imidaprilat, systolic blood pressure (SBP), and plasma ACE activity were determined periodically. The plasma concentration of imidaprilat increased in proportion to the infusion rates and was maintained for 4 weeks. The SBP and ACE activity did not decrease in proportion to the infusion rates due to the saturation of the pharmacologic effects, but these actions also were maintained for 4 weeks. The PK/PD of imidaprilat were not influenced by aging of SHRs. The antihypertensive action in subcutaneous infusion of imidapril was as potent as that in oral administration at the same dose, although the maximum plasma concentration of imidaprilat in subcutaneous infusion was one-eightieth times of that in oral administration. The action was also maintained 28 times longer than that in oral administration, indicating that subcutaneous infusion is useful as an administration route. Furthermore, good correlation between plasma imidaprilat concentration and SBP was observed in subcutaneous infusion, indicating that plasma concentration may be a useful marker of pharmacologic action. © 1997 Elsevier Science B.V.

*Keywords:* Imidapril; Imidaprilat; Spontaneously hypertensive rats (SHRs); Pharmacokinetics and Pharmacodynamics (PK/PD); Angiotensin-converting enzyme (ACE) inhibitor; Systolic blood pressure (SBP)

## 1. Introduction

Angiotensin-converting enzyme (ACE) inhibitors are widely accepted by Joint National Committee [1] and WHO/ISH Committee [2] as one of the first or second choice drugs for the treatment of hypertension. ACE inhibitors are also useful drugs for the treatment of congestive heart failure, but it has been reported that ACE inhibitors induce dry cough [3,4].

A new ACE inhibitor, imidapril [5] is an oral prodrug and is converted into a de-esterified active metabolite, imidaprilat in vivo. It has a po-

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tent antihypertensive action after oral administration once a day [6], and it also reduces damage of organs (heart, brain and kidney) associated with hypertension [7–9]. It has some unique advantage to reduce the frequency of dry cough [10–12].

It is important to investigate the relationship between plasma concentrations and pharmacologic effects of imidapril, but it has been little discussed in the literature. Therefore, we investigated the effects of infusion rate and aging on the steady-state pharmacokinetics and pharmacodynamics (PK/PD) of imidaprilat during subcutaneous infusion of imidapril to spontaneously hypertensive rats (SHRs). In addition, the PK/PD relationship was compared between subcutaneous infusion and oral administration, and the usefulness of subcutaneous infusion was discussed.

## 2. Experimental

### 2.1. Materials and reagents

#### 2.1.1. Animals

Male spontaneously hypertensive rats (SHRs, systolic blood pressure (SBP) > 200 mmHg) were purchased from Charles River Japan (Kanagawa, Japan). 12 week old (body weight (BW): 265–350 g), 53 week old (460–495 g) and 80 week old (433–488 g) SHRs were used in this study. The animals were housed singly under controlled temperature of 23 ± 1°C, humidity of 55 ± 5%, and light period of 12 h light-dark cycle, and they had free access to water and laboratory chow (CRF-1, Oriental Yeast, Tokyo, Japan).

#### 2.1.2. Chemicals

Imidapril hydrochloride ((−)-(4S)-3-[(2S)-2[[(1S)-1-ethoxycarbonyl-3-phenylpropyl]amino]-propionyl]-1-methyl-2-oxoimidazolidine-4-carboxylic acid hydrochloride) and its active metabolite, imidaprilat ((4S)-3-[(2S)-*N*-[(1S)-1-carboxy-3-phenylpropyl]alanyl]-1-methyl-2-oxo-4-imidazolidine carboxylic acid) (Fig. 1) were synthesized at Tanabe Seiyaku. Hippuryl-L-His-L-Leu was purchased from Sigma (St. Louis, MO, USA). Hippuric acid and *p*-methylhippuric acid were purchased from Katayama Chemical Industry (Osaka, Japan) and Tokyo Chemical Industry (Tokyo, Japan), respectively. Sodium pentobarbital (Nembutal® Injection) and heparin sodium were purchased from Abbott Laboratories (North Chicago, I11, USA) and Mochida Pharmaceutical (Tokyo, Japan), respectively. Other chemicals were special grade reagents.

### 2.2. Animal experiments

#### 2.2.1. Subcutaneous infusion

Imidapril was dissolved in saline to prepare the solution of 0.125, 0.25, 0.5, 1, 2.5 and 5 mg $ml^{-1}$, and 2 ml of each solution was put into an osmotic pump (Alzet®; model 2ML4, Alza, Palo Alto, CA, USA). As a control, saline was put into pump instead of the drug solution. The osmotic pump was implanted under the skin in the back of SHR under anaesthesia of sodium pentobarbital (50 mg $kg^{-1}$, i.p.), and imidapril was infused subcutaneously at the rates of 7.5, 15, 30, 60, 150 and 300 μg per rat per day for 4 weeks. The pumps were removed after the subcutaneous infusion for 4 weeks. During this study, the animals were housed singly under controlled temperature of 23 ± 1°C, humidity of 55 ± 5%, and light period of 12 h light-dark cycle, and they had free access to water and laboratory chow. SBP and BW were measured at 0, 2, 3, 7, 14, 21, 28, 31 and 42 day

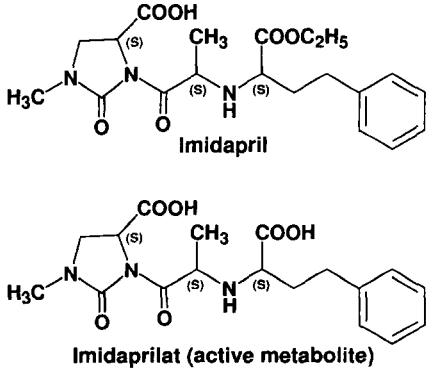


Fig. 1. Chemical structure of imidapril and its active metabolite, imidaprilat.

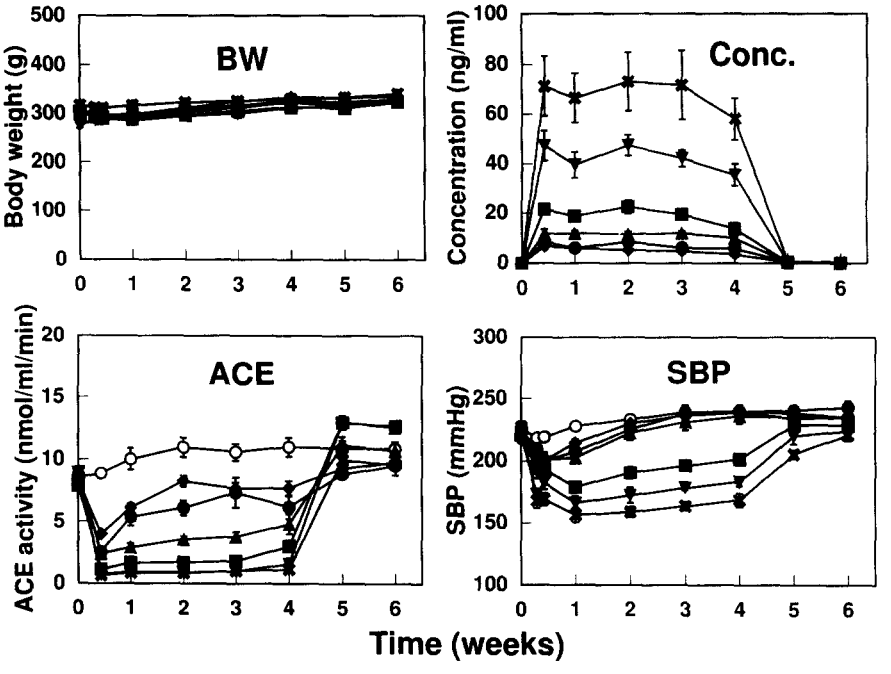


Fig. 2. Effect on body weight, plasma concentration and pharmacologic actions during subcutaneous infusion of imidapril to SHRs (mean ± S.E., $n = 4–10$). -○-: control, -◆-: 7.5 μg per rat per day, -●-: 15 μg per rat per day, -▲-: 30 μg per rat per day, -■-: 60 μg per rat per day, -▼-: 150 μg per rat per day, - × -: 300 μg per rat per day.

after administration, and thereafter 1 ml of each blood sample was periodically collected from the jugular vein by venous puncture with a heparinized syringe. Blood samples were immediately centrifuged at 2000 × *g* for 10 min, and the separated plasma samples were stored under −20°C until assay.

The subcutaneous infusion is useful to precisely evaluate the relationship between plasma concentrations and pharmacologic actions of the ACE inhibitors, since this route maintained steady-state plasma concentration for long time. Also, it is convenient to compare directly the substantial pharmacologic effect of several ACE inhibitors which show different bioavailability.

### 2.2.2. Oral administration

An aqueous solution of imidapril was administered orally to SHRs under fasting condition at doses of 0.15, 0.6, 2.5, 3 and 15 mg per rat. At the dose of 2.5 mg per rat, SBP was measured at 0, 1, 2, 4, 6, 18 and 24 h after administration. Also, at the dose of 0.15, 0.6, 3 and 15 mg per rat, SBP was measured at 0, 2, 6 and 24 h after administration. Immediately after the measurement, blood sample was collected from abdominal aorta with heparinized syringe, and the SHRs were sacrificed by exsanguination under ethyl ether anaesthesia. Blood samples were immediately centrifuged at 2000 × *g* for 10 min, and the separated plasma samples were stored under −20°C until assay.

### 2.3. Assay method

Plasma concentrations of imidapril and imidaprilat were determined by radioimmunoassay [13]. The quantitative limits were 0.1 ng $ml^{-1}$ and the assay relative standard deviations (R.S.D.) were below 10%. Plasma ACE activity was determined by UV-HPLC method [14] with a slight modification. Plasma sample and internal standard solution (*p*-methylhippuric acid) were added to the substrate solution (Hippuryl-L-His-L-Leu) in Tris-HCl buffer (pH 7.4) containing NaCl.

Table 1
Pharmacokinetic and pharmacodynamic parameters in SHR in subcutaneous infusion of imidapril

| Parameter | Infusion rate[a] | | | | | |
|---|---|---|---|---|---|---|
| | 7.5 | 15 | 30 | 60 | 150 | 300 |
| $C_{max}$ (ng ml$^{-1}$) | 6.9 ± 0.5 | 9.0 ± 0.5 | 16.4 ± 2.5 | 24.3 ± 2.2 | 54.3 ± 5.1 | 96.5 ± 12.0 |
| $AUC_{4weeks}$ (ng × week ml$^{-1}$) | 20.2 ± 2.6 | 25.5 ± 4.6 | 43.7 ± 2.3 | 74.4 ± 4.0 | 161.0 ± 10.1 | 264.2 ± 28.4 |
| $\Delta ACE_{max}$ (%) | 51.6 ± 2.5 | 70.3 ± 1.4 | 77.2 ± 2.3 | 85.4 ± 0.4 | 92.5 ± 0.5 | 92.8 ± 0.9 |
| $\Delta SBP_{max}$ (%) | 11.9 ± 1.5 | 11.5 ± 4.2 | 15.1 ± 1.5 | 20.1 ± 1.9 | 28.1 ± 0.6 | 35.9 ± 1.9 |

(Mean ± S.E., $n = 5–10$).
[a]μg per rat per day.

After incubation for 20 min at 37°C, the reaction was stopped by the adding ice cold methanol. The liberated hippuric acid was determined by HPLC with UV detection at 228 nm. Calibration curve for hippuric acid was linear in the concentration range 5–400 nmol ml$^{-1}$ with correlation coefficient > 0.999. The recovery was approximately 100%. The quantitative limit was 5 nmol ml$^{-1}$ and the assay R.S.D. was below 10%. SBP was measured by the 'tail cuff' method [15] in conscious SHRs after they were put into a cabin (UR-5000; Ueda, Tokyo, Japan) kept at 40°C for 10 min. The R.S.D. of the method was below 10%.

### 2.4. PK/PD parameters

The maximum plasma concentration ($C_{max}$) was determined from plasma concentration of imidaprilat. The plasma ACE inhibitory effect (ΔACE) and the antihypertensive action (ΔSBP) were calculated as the percentage of change in ACE activity and SBP relative to pretreatment with the drug, respectively. The maximum ACE inhibitory effect ($\Delta ACE_{max}$) and antihypertensive action ($\Delta SBP_{max}$) were determined from ΔACE and ΔSBP, respectively. The areas under the plasma concentration-time curve (AUC) and the areas under the ΔSBP-time curve (AUC of ΔSBP) were calculated by the trapezoidal rule.

In oral administration, 36 SHRs were randomly numbered from 1 (1 h) to 6 (24 h) at each sampling time (1, 2, 4, 6, 18 and 24 h). Six SHRs (No. 1–6) at different times were considered as one group (total six groups), and the mean ± S.E. of $C_{max}$, AUC, $\Delta SBP_{max}$ and AUC of ΔSBP were calculated from the six group data.

### 2.5. Statistical analysis

In the case of uniform variance, the significance of the difference was determined by Scheffe's multiple comparison following ANOVA. Otherwise, comparisons were made using a Scheffe-type multiple comparison following the Kruskal-Wallis analysis.

## 3. Results and discussion

### 3.1. Plasma concentration of active metabolite and pharmacologic actions in subcutaneous infusion of imidapril to SHRs

#### 3.1.1. Body weight

The body weight (BW) of SHRs before and in the 4th week after subcutaneous infusion were 302.1 ± 2.7 and 324.4 ± 2.4 g (mean ± S.E., $n = 50$), respectively. Little change of BW was observed among control, 7.5, 15, 30, 60, 150 and 300 μg per rat per day groups (Fig. 2).

#### 3.1.2. Plasma concentration and pharmacologic actions

Plasma concentration of the active metabolite (imidaprilat) was determined. As shown in Fig. 2, the plasma concentration of imidaprilat increased according to the infusion rate and was maintained for 4 weeks. Imidaprilat disappeared from plasma after the removal of the pump in the 4th week.

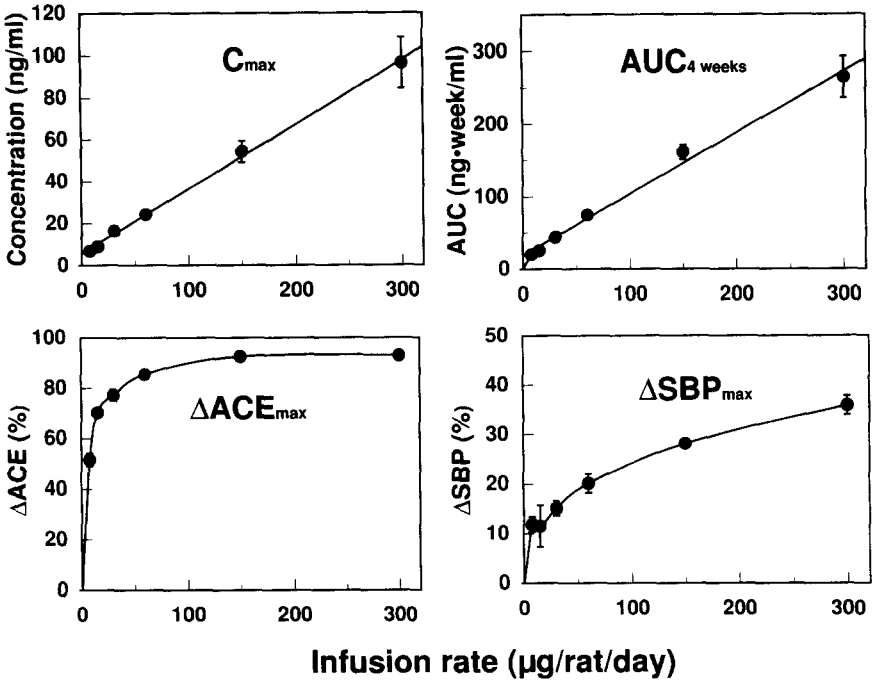


Fig. 3. Relationship between infusion rate and pharmacokinetic and pharmacodynamic parameters in subcutaneous infusion of imidapril (means ± S.E., $n = 5–10$).

The plasma concentration of imidapril was too low in any infusion rate, in comparison with the concentration of imidaprilat, since imidapril was quickly hydrolyzed to imidaprilat with esterase (data was not shown).

The plasma ACE activity and SBP of imidapril group decreased according to the increase of infusion rate, compared with the control group, and were maintained for 4 weeks (Fig. 2). The plasma ACE activity and SBP increased to the control levels after the removal of the pump in the 4th week.

### 3.1.3. PK/PD parameters

Table 1 shows the PK/PD parameters for imidaprilat in subcutaneous infusion of imidapril. The $C_{max}$ were 6.9 ± 0.5, 9.0 ± 0.5, 16.4 ± 2.5, 24.3 ± 2.2, 54.3 ± 5.1 and 96.5 ± 12.0 ng $ml^{-1}$ (mean ± S.E., $n = 5–10$) at the infusion rates of 7.5, 15, 30, 60, 150 and 300 μg per rat per day, respectively. The $AUC_{4weeks}$ were 20.2 ± 2.6, 25.5 ± 4.6, 43.7 ± 2.3, 74.4 ± 4.0, 161.0 ± 10.1 and 264.2 ± 28.4 ng week $ml^{-1}$ (mean ± S.E., $n = 5–10$), respectively. The $\Delta ACE_{max}$ were 51.6 ± 2.5, 70.3 ± 1.4, 77.2 ± 2.3, 85.4 ± 0.4, 92.5 ± 0.5 and 92.8 ± 0.9%, (mean ± S.E., $n = 5–10$), respectively, and the $\Delta SBP_{max}$ were 11.9 ± 1.5, 11.5 ± 4.2, 15.1 ± 4.2, 20.1 ± 1.9, 28.1 ± 0.6 and 35.9 ± 1.9% (mean ± S.E., $n = 5–10$), respectively.

Fig. 3 shows the relationship between infusion rates and PK/PD parameters. The $C_{max}$ and $AUC_{4weeks}$ increased in proportion to infusion rate ($r = 0.990$ and 0.983, respectively), but the $\Delta ACE_{max}$ and $\Delta SBP_{max}$ did not increase in proportion to the infusion rate, as expected. That is, the saturation phenomenon was observed between doses and pharmacologic actions.

### 3.2. Effect of ageing on plasma concentration and pharmacologic actions in subcutaneous infusion of imidapril to SHRs

#### 3.2.1. Plasma concentration and pharmacologic actions in 12, 53 and 80 week old SHRs

In order to investigate the effect of ageing on PK/PD plasma concentration of imidaprilat,

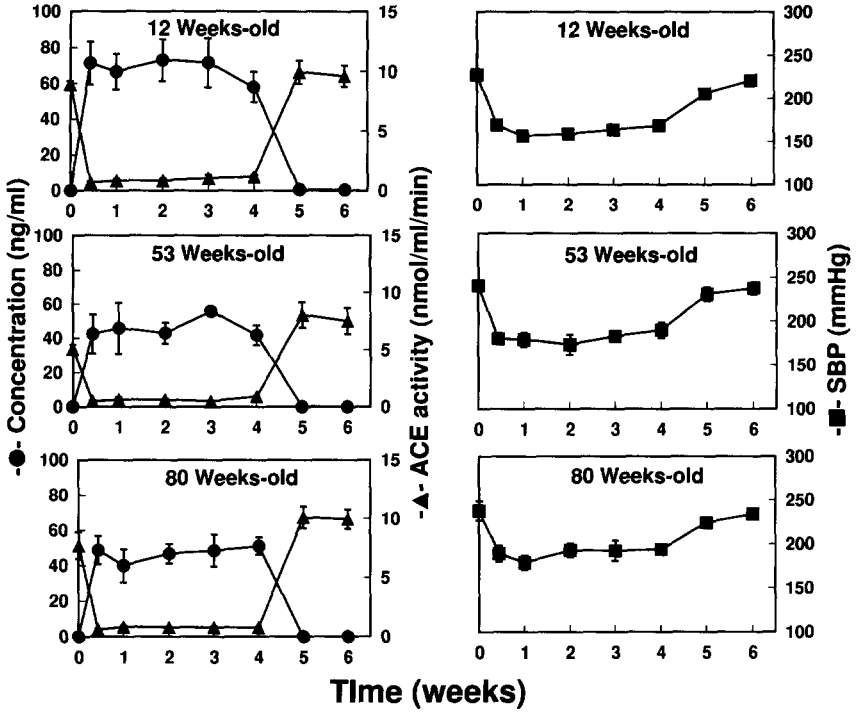


Fig. 4. Effect of ageing on plasma concentration and pharmacologic actions during subcutaneous infusion of imidapril at a dose of 300 μg per rat per day (mean ± S.E., $n = 3–10$).

plasma ACE activity, and SBP were periodically determined in subcutaneous infusion of imidapril to 12, 53 and 80 week old SHRs at the rate of 300 μg per rat per day. As shown in Fig. 4, the plasma concentrations in 12, 53 and 80 week-old SHRs increased to almost the same level and were maintained for 4 weeks. Imidaprilat disappeared from plasma after the removal of the pump in the 4th week in any SHRs. The ACE activity and SBP were also decreased to almost the same level and were maintained for 4 weeks. The ACE activity and SBP were increased to the control levels after the removal of the pump in the 4th week in any SHRs.

Table 2
Effects of ageing on pharmacokinetic and pharmacodynamic parameters

| Parameter | Age (weeks old) | | |
|---|---|---|---|
| | 12 | 53 | 80 |
| $C^{a}_{max}$ (ng ml$^{-1}$) | 96.5 ± 12.0 | 97.4 ± 9.8 | 90.8 ± 10.0 |
| $AUC^{a}_{4weeks}$ (ng × week ml$^{-1}$) | 264.2 ± 28.4 | 279.0 ± 15.0 | 281.1 ± 42.8 |
| $\Delta ACE_{max}$ (%) | 92.8 ± 0.9 | 93.5 ± 0.4 | 92.4 ± 0.9 |
| $\Delta SBP_{max}$ (%) | 35.9 ± 1.9 | 31.3 ± 1.9 | 25.5 ± 5.1 |

[a]Corrected by body weight (300 μg per rat per day, mean ± S.E., $n = 3–10$).

### 3.2.2. PK/PD parameters

Table 2 shows the effects of ageing on PK/PD parameters. $C_{max}$ and $AUC_{4weeks}$ of 53 and 80 week-old SHRs were corrected by mean BW of 12 week old SHRs and the $\Delta ACE_{max}$ and $\Delta SBP_{max}$ were not corrected by BW, because pharmacologic actions showed the saturation at the infusion rate of 300 μg per rat per day. The $C_{max}$ were 96.5 ± 12.0, 97.4 ± 9.8 and 90.8 ± 10.0 ng ml$^{-1}$ (mean ± S.E., $n = 3–10$) in 12, 53, and 80 week

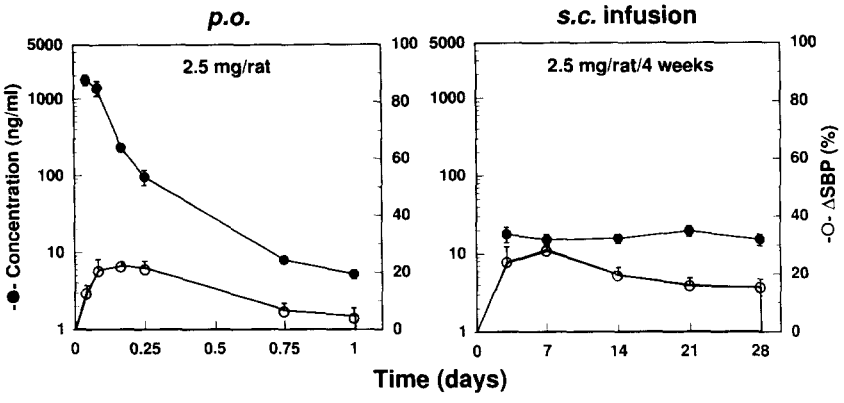


Fig. 5. Comparison between oral administration and subcutaneous infusion on plasma concentration and systolic blood pressure at a dose of 2.5 mg per rat (mean ± S.E., $n = 5$–6).

old SHRs, respectively. The $AUC_{4weeks}$ were 264.2 ± 28.4, 279.0 ± 15.0 and 281.1 ± 42.8 ng week $ml^{-1}$ (mean ± S.E., $n = 3$–10), respectively, and the $\Delta SBP_{max}$ were 35.9 ± 1.9, 31.3 ± 1.9 and 25.5 ± 5.1% (mean ± S.E., $n = 3$–10), respectively. The smaller value of $\Delta SBP_{max}$ in 80 week old SHRs might be due to the decrease of dose per rat according to the increase of BW. Statistically, the difference of $\Delta SBP_{max}$ between 12 and 80 week old were not significant. Thus, the $\Delta ACE_{max}$ and $\Delta SBP_{max}$ also were almost the same in any SHR.

It has been reported that peak pumping ability of heart markedly decreased from 52 to 90 week old SHR [16], and that the activities of drug-metabolizing enzyme and electron transport systems in liver microsomes decreased in 86 week old rats of Wistar strain [17]. However, these factors did not influence the PK/PD of imidaprilat in this study, though the reason is not clear.

Table 3
Pharmacokinetic and pharmacodynamic parameters in oral administration or subcutaneous infusion of imidapril

| Parameter | p.o.[a] | s.c. infusion[b] | s.c./p.o. |
|---|---|---|---|
| $C_{max}$ (ng $ml^{-1}$) | 1840 ± 280 | 24.1 ± 1.3 | 0.013 |
| AUC (ng × day $ml^{-1}$) | 210.7 ± 23.8 | 447.3 ± 45.5 | 2.1 |
| $\Delta SBP_{max}$ (%) | 25.2 ± 1.8 | 30.8 ± 3.7 | 1.2 |
| AUC of ΔSBP (% × day) | 12.6 ± 2.1 | 546.0 ± 56.0 | 43.3 |

Dose: 2.5 mg per rat, (mean ± S.E., $n = 5$–6).
[a]Oral administration.
[b]Subcutaneous infusion.

### 3.3. Comparison of plasma concentration and SBP in subcutaneous infusion and oral administration of imidapril to SHRs

Plasma concentrations of imidaprilat and SBP in subcutaneous infusion of imidapril were compared with those in oral administration of imidapril. As shown in Fig. 5, mean plasma concentration of imidaprilat reached a maximum value, 1759.4 ± 265.1 ng $ml^{-1}$ (mean ± S.E., $n = 6$) at 1 h after oral administration of imidapril at a dose of 2.5 mg per rat and thereafter decreased with an elimination half-life of about 1 h. The ΔSBP(%) increased gradually and reached a maximum value, 21.9 ± 2.7% (mean ± S.E., $n = 6$) at 4 h after the administration. Also, the ΔSBP at 24 h was about one-fifth of the maximum value. On the other hand, maximum mean plasma concentration of imidaprilat in subcutaneous infusion was 19.7 ± 2.9 ng $ml^{-1}$ (mean ± S.E., $n = 5$) on the third week (2.5 mg per 4 weeks per rat), and the concentration was maintained for 4 weeks. Also, the maximum mean antihypertensive action was 28.5 ± 1.8% (mean ± S.E., $n = 5$) on the first

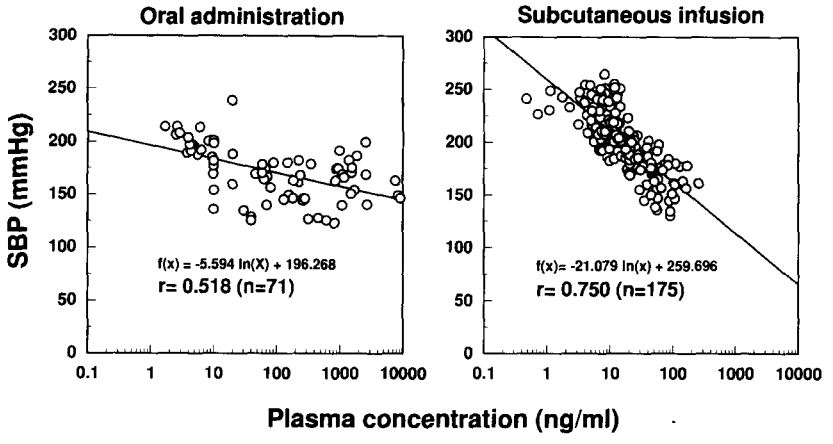


Fig. 6. Relationship between plasma concentration and systolic blood pressure in oral administration or subcutaneous infusion of imidapril to SHRs.

week, and the ΔSBP was maintained for 4 weeks. The action was maintained 28 times longer than that in oral administration.

The PK/PD parameters of both administration routes were summarized in Table 3. The $\Delta SBP_{max}$ was the same in both administration, although $C_{max}$ in subcutaneous infusion was approximately one-eightieth of that in oral administration. Also, AUC of $\Delta SBP_{max}$ was 43.3 times larger than that of oral administration. From these results, it was found that the high plasma concentration is not always necessary to maintain the pharmacologic action, and that the subcutaneous infusion is a useful administration route to maintain pharmacologic action for long time.

### *3.4. Relationship between plasma concentration and SBP in subcutaneous infusion or oral administration to SHRs*

Relationship between plasma concentration and SBP was examined in subcutaneous infusion (7.5, 15, 30, 60, 150 and 300 μg per rat per day) or oral administration (0.15, 0.6, 2.5, 3 and 15 mg per rat). As shown in Fig. 6, there was a correlation between logarithm of plasma concentration and SBP in both administration routes. The correlation coefficient ($r = 0.750$) in subcutaneous infusion was higher than that ($r = 0.518$) in oral administration, and the slope of regression line in subcutaneous infusion was higher than that in oral administration. Also, the correlation and the slope for the points ($n = 42$) below 100 ng $ml^{-1}$ in oral administration became 0.614 and $-13.7$, respectively and were close to the values in subcutaneous infusion. Therefore, it was deduced that one of the reasons for the lower correlation and poor slope of regression line in oral administration may be due to the saturation of pharmacologic action in high plasma concentration. From these results, it was found that plasma concentration of imidaprilat in subcutaneous infusion is useful as a marker of antihypertensive action.

In conclusion, the plasma concentration of imidaprilat increased in proportion to the infusion rate in subcutaneous infusion of imidapril, and the $\Delta SBP_{max}$ and $\Delta ACE_{max}$ did not increase in proportion to the infusion rate due to the saturation of the pharmacologic effects. There was little effect of ageing on PK/PD in SHRs. The antihypertensive action in subcutaneous infusion was as potent as that in oral administration at the same dose, and the action was maintained 28 times longer than that in oral administration. Furthermore, good correlation between plasma imidaprilat concentration and SBP were obtained in subcutaneous infusion, and it was shown that plasma concentration of imidaprilat may be a useful marker of pharmacologic action.

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