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Pharmacokinetics and pharmacodynamics of a sustained-release biodegradable pellet containing imidapril, a new angiotensin-converting enzyme inhibitor in spontaneously hypertensive rats

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Abstract

The pharmacokinetics and pharmacodynamics (PK/PD) of a sustained-release biodegradable pellet containing imidapril, a new angiotensin-converting enzyme (ACE) inhibitor, were investigated in comparison with those of an osmotic pump in male spontaneously hypertensive rats (SHRs). A pellet was prepared from copolymer of DL-lactic acid and glycolic acid by the melt-pressing technique. Imidapril was released in vitro from the pellet at an approximately zero-order rate and the release profile was similar to that of the osmotic pump. Imidapril was administered subcutaneously via a pellet or an osmotic pump implanted under the skin in the back of SHRs. Plasma concentrations of imidaprilat as an active metabolite of imidapril, plasma ACE activity and systolic blood pressure (SBP) were determined periodically. The plasma concentration of imidaprilat during the administration of a pellet was maintained for 4 weeks, and the plasma concentration profile was close to that of the osmotic pump. Both groups of pellet and osmotic pump significantly inhibited plasma ACE activity and reduced SBP for 4 weeks, and these action profiles were similar in both groups. In addition, in vivo release profile of the pellet was close to the in vitro release profile, and the in vivo release profiles of the pellet and the osmotic pump were similar to each other. From these results, it was found that the PK/PD of a biodegradable pellet were close to those of the osmotic pump, and it was shown that the pellet may be a useful system to maintain the plasma concentration of imidaprilat for a long time.

Keywords: Imidapril; Imidaprilat; Angiotensin-converting enzyme (ACE); Sustained-release biodegradable pellet; Pharmacokinetics and Pharmacodynamics (PK/PD); Spontaneously hypertensive rat (SHR)

1. Introduction

Imidapril, a new angiotensin-converting enzyme (ACE) inhibitor, is an oral prodrug and is converted into a de-esterified active metabolite, imi-

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daprilat, in vivo [1]. Imidapril has potent antihypertensive action after oral administration once a day [2], and it also reduces damage of organs (heart, brain and kidney) associated with hypertension [3–5]. It has some unique advantage to reduce the frequency of dry cough [6–9].

It is important to investigate the relationship between plasma concentrations and pharmacologic effects of imidapril from a viewpoint of efficacy and safety. The pharmacokinetics and pharmacodynamics (PK/PD) of imidaprilat have been studied in subcutaneous infusion or oral administration to spontaneously hypertensive rats (SHRs) and the usefulness of subcutaneous infusion using an osmotic pump was demonstrated [10]. In this paper, the PK/PD of a sustained-release biodegradable pellet in subcutaneous administration were investigated in comparison with those of an osmotic pump, and the usefulness of the system was discussed.

2. Experimental

2.1. Materials and reagents

2.1.1. Animals

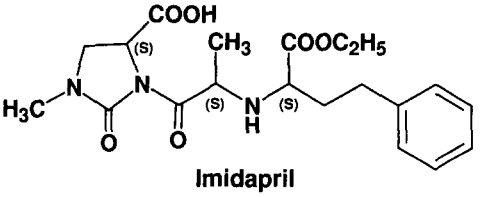
Male spontaneously hypertensive rats (SHRs), each 28 weeks old, (body weight (BW): 380–435 g, systolic blood pressure (SBP): > 200 mmHg) were purchased from Charles River Japan (Kanagawa, Japan). The animals were housed singly under controlled temperature of 23 ± 1°C, humidity of 55 ± 5% and a light 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

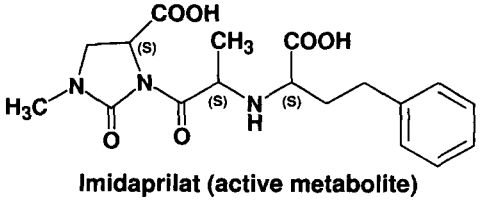
Imidapril hydrochloride ((−)-(4S)-3-[(2S)-2-[[(1S)-1-ethoxycarbonyl-3-phenylpropyl]amino]-propionyl]-1-methyl-2-oxoimidazolidine-4-carboxylic acid hydrochloride), its active metabolite, imidaprilat ((4S)-3-[(2S)-*N*-[(1S)-1-carboxy-3-phenylpropyl]alanyl]-1-methyl-2-oxo-4-imidazolidine carboxylic acid) (Fig. 1) were synthesized at Tabane Seiyaku Company. A copolymer of DL-lactic acid and glycolic acid (PLGA, Medisorb 5050DL-52K:MW = 52 866) was purchased from Du Pont (Cincinnati, Ohio, USA). Hippuryl-L-His-L-Leu was purchased from Sigma (St. Louis, MO, USA). Hippuric acid and *P*-methylhippuric acid were purchased from Katayama Chemicals (Osaka, Japan) and Tokyo Chemicals (Tokyo, Japan), respectively. Sodium pentobarbital (Nembutal® Injection) and heparin sodium were purchased from Abbott Laboratories (North Chicago, IL, USA) and Mochida Pharmaceutical (Tokyo, Japan), respectively. Other chemicals were special grade reagents.

2.1.3. Preparation of pellet

A pellet, cylindrical PLGA parental dosage form containing imidapril, was prepared by the melt-pressing technique [11]. A 38 mg of PLGA and a 2 mg of imidapril hydrochloride were dissolved in 200 μl of methylene chloride, and the solution was evaporated at 40°C and crushed to obtain the homogeneous mixture. The powdered mixture was inserted into a poly(tetrafluoroethylene) tube of 2 mm i.d. and the piston rods from both sides of the tube were inserted under a pressure of 50 kg cm^{-2} at 60°C. The resulting solid formulation in a fine cylindrical form (2 mm in diameter, 4 mm long) was kept at 4°C before use.



Imidapril



Imidaprilat (active metabolite)

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

2.2. Animal experiments

A pellet containing imidapril (2 mg) was implanted under the skin in the back of SHRs (2 mg/rat) under anaesthesia of sodium pentobarbital (50 mg kg^{-1}, i.p.). An osmotic pump (Alzet® model 2ML4: Alza, Palo Alto, CA, USA) containing imidapril solution was also implanted in the same way as a pellet, and imidapril was infused subcutaneously at the rate of 2 mg per rat per 4 weeks. As each control, a pellet containing only PLGA or an osmotic pump containing only saline instead of imidapril solution was implanted, respectively. The osmotic pump was removed under anaesthesia of sodium pentobarbital after 4 weeks. SBP and BW were measured at 4, 7, 14, 21 and 28 day after administration, and 1 ml of each blood sample was collected from the jugular vein with a heparinized syringe under anaesthesia of diethyl ether at the same period after administration. Blood samples were immediately centrifuged at $2000 \times g$ for 10 min, and the plasma samples were stored at −20°C until assay.

2.3. Assay method

Plasma concentrations of imidaprilat were determined by radioimmunoassay [12]. 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 the UV-HPLC method [13] 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. After incubation for 20 min at 37°C, the reaction was stopped by the addition of 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 0.25 nmol ml^{-1} min^{-1} and the assay R.S.D. was below 10%. SBP was measured by the 'tail-cuff' method [14] in conscious SHRs after they were put into a cabin (U-5000, Ueda, Tokyo, Japan) kept at 40°C for about 10 min. The method R.S.D. was below 10%.

2.4. In vitro release test

A pellet (2 mg/pellet) was added to 40 ml of isotonic phosphate buffer (pH 7.4) in a capped tube. The tube was shaken at 60 strokes min^{-1} at 37°C for 4 weeks using a cool bathshaker (ML-10; Taitec, Tokyo, Japan). Samples were withdrawn at 1, 3, 7, 14, 21 and 28 day, and these concentrations were assayed by a UV-spectrophotometer (U-3210; Hitachi, Tokyo, Japan). An osmotic pump designed to release imidapril at a rate of 2 mg per 4 weeks was also used in the in vitro release test.

2.5. PK/PD parameters

The maximum plasma concentration (C_{max}) was determined from observed plasma concentrations 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 (ΔACE_{max}) and antihypertensive action (ΔSBP_{max}) were determined from ΔACE and ΔSBP, respectively. The area under the plasma concentration-time curve (AUC) and the area under the ΔSBP-time curve (AUC of ΔSBP) in subcutaneous administration were calculated by the trapezoidal rule.

2.6. Statistical analysis

Statistical analysis was performed by Student's unpaired *t*-test in two groups.

3. Results and discussion

3.1. In vitro release profiles of pellet and osmotic pump

As shown in Fig. 2, imidapril (2 mg/pellet) was released from the pellet at an approximately zero-order rate for 4 weeks, and imidapril in the pellet

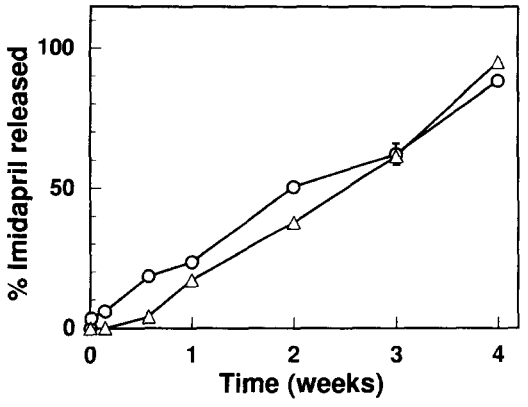


Fig. 2. In vitro release profiles of imidapril from a pellet or an osmotic pump containing imidapril (mean ± S.E., $n = 3$). -○-: pellet (2 mg/pellet), -△-: osmotic pump (2 mg/4 weeks). The points without vertical bars have smaller S.E. than the symbols.

was completely released within 4 weeks. Also, the release profile was similar to that of an osmotic pump.

3.2. Plasma concentration of active metabolite during subcutaneous administration of imidapril to SHRs

Plasma concentration of the active metabolite (imidaprilat) during the subcutaneous administration of a pellet at a dose of 2 mg/rat was determined. As shown in Fig. 3, the plasma

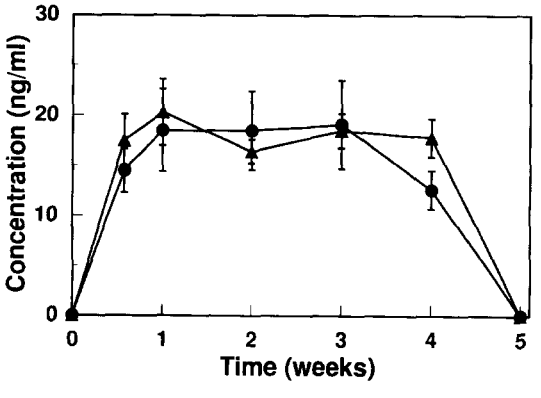


Fig. 3. Plasma concentrations of imidaprilat during subcutaneous administration of a pellet or an osmotic pump containing imidapril to SHRs (mean ± S.E., $n = 3$–6). -●-: pellet (2 mg/rat), -▲-: osmotic pump (2 mg/rat/4 weeks).

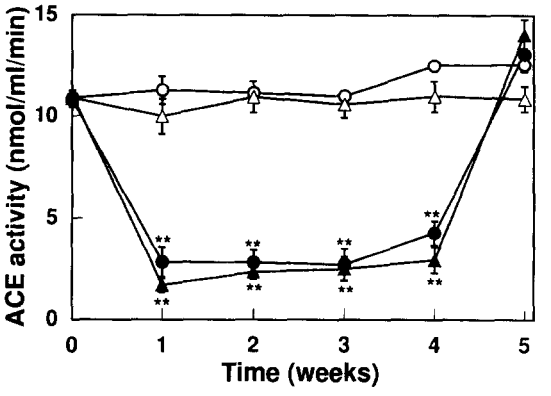


Fig. 4. Plasma ACE activity during subcutaneous administration of imidapril pellet or an osmotic pump containing imidapril to SHRs (mean ± S.E., $n = 3$–6). -○-: control (pellet), -△-: control (osmotic pump), -●-: pellet (2 mg/rat), -▲-: osmotic pump (2 mg/rat/4 weeks). Significantly different from the control group: $^{**}P < 0.01$.

concentration of imidaprilat increased and was maintained at about 20 ng ml^{-1} for 4 weeks. Imidaprilat disappeared from the plasma on the 5th week after the administration. Also, the plasma concentration profile was similar to that of the osmotic pump at the same dose.

The plasma concentration of imidapril was too low in subcutaneous administration of a pellet or an osmotic pump, in comparison with the concentration of imidaprilat, since imidapril was quickly hydrolyzed to imidaprilat with esterase (data not shown).

3.3. Plasma ACE activity and systolic blood pressure during subcutaneous administration of imidapril to SHRs

Body weight (BW) of SHRs before administration was 411.3 ± 3.6 g, and BW of SHRs on the fourth week after administration were 405.0 ± 3.7, 401.0 ± 8.0 and 401.0 ± 8.7 g (mean ± S.E., $n =$ 5–6) in three groups of control, pellet and osmotic pump, respectively. Little change of BW was observed among the three groups.

As shown in Fig. 4, the pellet group significantly inhibited plasma ACE activity in comparison with the control group, and the action was maintained for 4 weeks. The plasma ACE activity increased to the control level on the fifth week

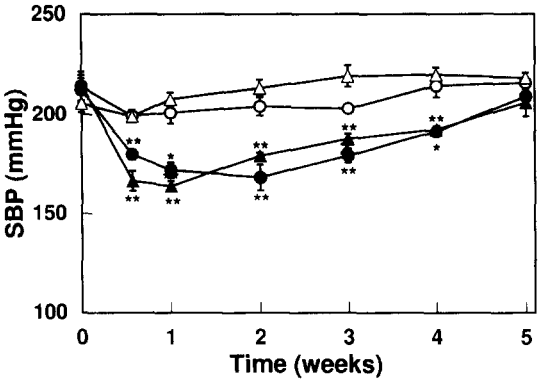


Fig. 5. Systolic blood pressure during subcutaneous administration of imidapril to SHRs (mean ± S.E., $n = 3$–6). -○-: control (pellet), -△-: control (osmotic pump), -●-: pellet (2 mg/rat), -▲-: osmotic pump (2 mg/rat/4 weeks) $^{*}P < 0.05$, $^{**}P < 0.01$.

after the administration, and the plasma ACE activity profile of the pellet was quite close to that of the osmotic pump.

As shown in Fig. 5, the pellet group significantly decreased SBP in comparison with the control group, and the antihypertensive action was maintained for 4 weeks. The SBP increased to the control level on the fifth week after the administration. Also, the SBP profile of the pellet group was similar to that of the osmotic pump group.

Table 1
Pharmacokinetic and pharmacodynamic parameters of imidaprilat

Parameter	Pellet	Osmotic pump
C_{max} (ng ml^{-1})	26.1 ± 1.7	24.4 ± 1.8
AUC_{4weeks} (ng × week ml^{-1})	63.5 ± 2.7	67.0 ± 3.5
ΔACE_{max} (%)	84.0 ± 2.0	86.9 ± 2.2
ΔSBP_{max} (%)	20.8 ± 3.3	24.8 ± 2.1
AUC_{4weeks} of ΔSBP (% × week)	54.4 ± 7.8	61.3 ± 7.6

(mean ± S.E., $n = 5$)

3.4. PK/PD parameters

The PK/PD parameters in subcutaneous administration of the pellet and osmotic pump are summarized in Table 1. The C_{max} of imidaprilat in the pellet and the osmotic pump groups were 26.1 ± 1.7 and 24.4 ± 1.8 ng ml^{-1} (mean ± S.E., $n = 5$), respectively. The AUC_{4weeks} of imidaprilat were 63.5 ± 2.7 and 67.0 ± 3.5 ng week ml^{-1}, respectively (mean ± S.E., $n = 5$). The ΔACE_{max} were 84.0 ± 2.0 and 86.9 ± 2.2% in the pellet and the osmotic pump groups, and the ΔSBP_{max} were 20.8 ± 3.3 and 24.8 ± 2.1% (mean ± S.E., $n = 5$), respectively. Also, the AUC_{4weeks} of ΔSBP were 54.4 ± 7.8 and 61.3 ± 7.6% week (mean ± S.E., $n = 5$), respectively. The statistically significant differences of these parameters were not observed between both groups.

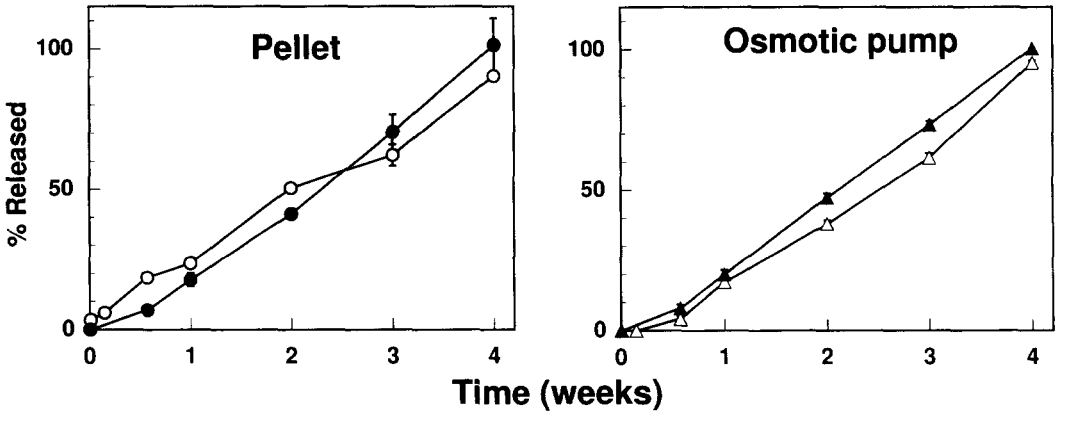


Fig. 6. In vitro and in vivo release profiles of imidapril from a pellet or an osmotic pump containing imidapril (mean ± S.E., n3–5). -○-: pellet (in vitro), -△-: osmotic pump (in vitro), -●-: pellet (in vivo), -▲-: osmotic pump (in vivo). The points without vertical bars have smaller S.E. than the symbols.

3.5. Correlation of in vitro and in vivo release profiles of pellet and osmotic pump

The release of imidapril from the pellet and osmotic pump in vivo was calculated from the individual plasma concentration of imidaprilat in SHRs (Fig. 3) by the Wagner-Nelson method [15]. Fig. 6 shows the in vivo and in vitro release profiles. Imidapril was released in vivo from a pellet at an approximately zero-order rate for 4 weeks, and imidapril in the pellet was completely released within 4 weeks. The in vivo release profile from a pellet was almost close to the in vitro release profile. Also, the in vitro-in vivo release profiles were close in the case of the osmotic pump.

These results suggest that imidapril release and absorption from the pellet or osmotic pump occurs under the skin in the backs of SHRs in a fashion that appears to correlate with in vitro release data.

In conclusion, it was found that the PK/PD characteristics of the pellet were almost close to those of the osmotic pump after subcutaneous administration. The in vivo release profile of imidapril from the pellet and osmotic pump were close to the in vitro release profiles. In addition, it was shown that the pellet may be a useful system to maintain the plasma concentration of imidaprilat for a long time.

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