The Pharmacokinetic-pharmacodynamic Assessment of the Hypotensive Effect after Coadministration of Losartan and Hydrochlorothiazide in Spontaneously Hypertensive Rats

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Summary: The interactive hypotensive effect of the combination treatment of losartan (LOS) and hydrochlorothiazide (HCTZ) was assessed using a pharmacokinetic-pharmacodynamic (PK-PD) model in spontaneously hypertensive rats. Intravenous coadministration of these drugs showed a prolonged and enhanced time-course of the hypotensive effect. A population PK analysis revealed the delayed elimination of LOS after coadministration. The time-course of the plasma renin activity (PRA) was measured, and showed a more continuative time profile after coadministration compared with the administration of LOS alone. An indirect response model was applied to describe the relationship between the PK of LOS and the PRA profile, and the Emax value for the increase of the PRA by LOS was increased with the dose of HCTZ. Blood pressure was linked to the PRA through an effect compartment. The model successfully described the relationship between the doses of LOS and HCTZ and their interactive hypotensive effect. These results indicate that the interaction for blood pressure in the combination treatment of LOS and HCTZ can be estimated using the doses of the drugs and the PRA-mediated PK-PD model.

Keywords: losartan; hydrochlorothiazide; PK-PD model; plasma renin activity; drug–drug interaction

Introduction

Hypertension is a risk factor for several cardiovascular events. Avoiding the risk of hypertension by lowering blood pressure is important; in fact: "the lower, the better." The 2009 guidelines for treating hypertension by the Japanese Society of Hypertension (JSH2009) reported that middle age patients with hypertension, but without other risks, should maintain a systolic and diastolic pressure of less than 130/85 mmHg. However, in the Japan Home versus Office Blood Pressure Measurement Evaluation (J-HOME) study, blood pressure management was reported as insufficient, and about half of patients treated with a single anti-hypertension drug could not maintain the target blood pressure. Thus, a combination therapy of anti-hypertensive drugs with different mechanisms of actions is recommended to enhance the hypotensive effect.

For combination therapies, angiotensin receptor blockers (ARBs) and thiazide diuretics are widely used, because they enhance each other’s hypotensive effect. ARBs also suppress such adverse effects as hypokalemia and hyperuricemia from thiazide diuretics. Activation of the renin-angiotensin system is responsible primarily to enhance the hypotensive effect after administration of ARBs with diuretics. As a result, the plasma renin activity (PRA) and the plasma concentrations of angiotensin II (Ang II) and angiotensin (1–7) [Ang (1–7)] increase. Furthermore, plasma concentrations of nitric oxide and bradykinin, which are hypotension factors, also increase via the angiotensin type 2 receptor (AT2R) and the Mas receptor. However, little attention has been given to the quantitative relationship among the doses of drugs, the PRA, and the hypotensive effect after administration of ARBs with diuretics. A contribution of a pharmacokinetic (PK) interaction has not been revealed in the enhanced hypotensive effect either.

The objectives of this study are to evaluate the PK and pharmacodynamic (PD) interactions for the hypotensive effect after the coadministration of losartan (LOS) and...
hydrochlorothiazide (HCTZ) in spontaneously hypertensive rats (SHRs), and to help estimation for the time course of blood pressure after coadministration of both drugs. The responsible interactive factor will be revealed using a PK-PD compartmental analysis.

Methods

Chemicals: Losartan potassium (LOS) was purchased from Wako Pure Chemical Industries (Osaka, Japan) and hydrochlorothiazide (HCTZ) was bought from Sigma Chemical Co. (St. Louis, MO). Monoethanolamine was purchased from Nacalai Tesque (Kyoto, Japan). Saline was obtained from Otsuka Pharmaceuticals (Tokyo, Japan). All other reagents and solvents were commercial products of reagent grade.

Animal surgery: Male SHRs (Japan SLC Inc., Shizuoka, Japan, 15–25 weeks old) were used. Rats were housed in constant environmental facilities (temperature: 24 ± 1°C, humidity: 55 ± 10%), exposed to 12:12 h light-dark cycles (06:00 h/18:00 h) for more than 1 week, and allowed free access to the standard diet and tap water. On the day before the experiment, rats were lightly anesthetized with ethyl ether, and were implanted surgically with a PE50 catheter in the jugular vein for drug administration. Both catheters were externalized through the back in the neck region and secured. Unless otherwise specified, all animal experiments were carried out under non-restraining and non-anesthetic conditions and in a fasting state. These animal experiments were approved by the Animal Experimentation Committee of the Osaka University of Pharmaceutical Sciences.

Animal experiments: LOS was dissolved in saline and HCTZ was dissolved in saline containing 1.7% monoethanolamine following the method of Kim et al. Losartan (1–10 mg/kg) and HCTZ (1–10 mg/kg) were administrated through the jugular vein (n = 3–5). Blood samples were withdrawn from the femoral artery at designated postdose intervals. The blood samples were transferred into tubes containing heparin (1 IU), and then centrifuged (10,000 rpm for 3 min). The isolated plasma was stored frozen at −20°C until analysis. The PRA was determined by a radioimmunoassay method (Yamasa, Chiba, Japan).

PK-PD modeling and data analysis: The concentration-time data of the drugs was analyzed by a nonlinear mixed effect model method using NONMEM software (version VI, level 2.0) to evaluate the PK interaction with the coadministration of LOS and HCTZ. The first-order method was employed throughout the analysis. Both 3-compartment and 2-compartment open models, as implemented in the NONMEM-PREDPP library subroutines ADVAN11/TRAN54 and ADVAN4/TRAN54, respectively, were investigated using the Akaike Information Criterion. Inter-individual variability for PK parameters and residual variability were estimated using an exponential error model. Model comparisons were based on the objective function values in NONMEM using the likelihood ratio test. The significance level was set at p < 0.05, which corresponds to a reduction of 3.84 in the objective function value to discriminate between the two nested structural models after inclusion of one additional parameter. Visual predictive checks were performed for the final model.

The plasma drug concentration, PRA and blood pressure were evaluated in different animals. Inter-individual and intra-individual variability for the PD parameters was not characterized using a population analysis. The PK-PD model for the hypotensive effect of LOS was analyzed by a nonlinear regression program, FKDM, based on ordinary least squares. The differential equations were solved by the Runge-Kutta-Gill method.

Table 1. The analytical methods for LOS and HCTZ

<table>
<thead>
<tr>
<th>Method</th>
<th>Conditions</th>
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<tbody>
<tr>
<td><strong>HPLC-UV conditions for LOS</strong></td>
<td></td>
</tr>
<tr>
<td>column</td>
<td>Unison UK-C18 (3 µm)</td>
</tr>
<tr>
<td></td>
<td>(75 mm × 3 mm, Imtakt, Kyoto, Japan)</td>
</tr>
<tr>
<td>UV wavelength</td>
<td>230 nm</td>
</tr>
<tr>
<td>mobile phase</td>
<td>25 mmol/L phosphoric acid:acetonitrile = 80:20 (v/v)</td>
</tr>
<tr>
<td><strong>UPLC-MS/MS conditions for HCTZ</strong></td>
<td></td>
</tr>
<tr>
<td>column</td>
<td>ACQUITY™ UPLC BEH C18 (1.7 µm)</td>
</tr>
<tr>
<td></td>
<td>(Waters Co. Ltd., Milford, NE, USA)</td>
</tr>
<tr>
<td>mass</td>
<td>m/z 295.94 &gt; m/z 269</td>
</tr>
<tr>
<td>mobile phase</td>
<td>10 mmol/L ammonium acetate:methanol = 70:30 (v/v)</td>
</tr>
</tbody>
</table>

From the femoral artery were transferred into tubes containing EDTA-2Na, and then centrifuged (10,000 rpm for 3 min). The isolated plasma was frozen and stored at −20°C until analysis. The PRA was determined by a radioimmunoassay method (Yamasa, Chiba, Japan).
Fig. 1. Diagrammatic representation of the PK-PD model for the hypotensive effect

**Theoretical:** Figure 1 represents the PK-PD model for the hypotensive effect after administration of LOS. The PK of LOS in plasma was described by a linear three-compartment open model, as follows:

\[
\begin{align*}
\frac{dA_{LOS1}}{dt} &= Lk_{21} \cdot A_{LOS2} + Lk_{31} \cdot A_{LOS3} - \left( Lk_{12} + Lk_{13} + Lk_{10} \right) \cdot A_{LOS1} \\
\frac{dA_{LOS2}}{dt} &= Lk_{12} \cdot A_{LOS1} - Lk_{21} \cdot A_{LOS2} \\
\frac{dA_{LOS3}}{dt} &= Lk_{13} \cdot A_{LOS1} - Lk_{31} \cdot A_{LOS3} \\
C_{LOS1} &= \frac{A_{LOS1}}{LV}
\end{align*}
\] (1)

where \( A_{LOS1} \) is the amount of LOS (\( \mu g \)) in the central compartment; \( A_{LOS2} \) and \( A_{LOS3} \) are the amount of LOS concentrations in the peripheral compartments; \( LV \) is the distribution volume of LOS in the central compartment; \( Lk_{12}, Lk_{21}, Lk_{13}, Lk_{31}, \) and \( Lk_{10} \) are the first-order rate constants (\( min^{-1} \)) of LOS; \( C_{LOS1} \) is the plasma LOS concentration (\( \mu g/mL \)); and at \( t = 0 \), \( A_{LOS1} = \) Dose and \( A_{LOS2} = A_{LOS3} = 0 \). In the PK-PD analysis, the estimates of the population mean were used in the PK parameters, because animals were individually applied in the PK and pharmacological experiments.

A hybrid model of an indirect response model and an indirect link model was used to describe a sequence of variations in the PRA and the hypotensive effect after LOS administration. In this study, we focused on the contribution of the PRA to the hypotensive effect of LOS. Then, we assumed the following:

a) LOS increases the PRA via the accumulative delay compartments of X1 and X2. b) The influx rate into the X1/X2 compartment is negligible because LOS influx follows a first-order process but the received mass is negligible compared to the PK model. c) Increased PRA decreases blood pressure through the effect compartment \( C_E \). The differential equations of the model are as follows:

\[
\begin{align*}
\frac{dC_{X1}}{dt} &= Xk_{in} \cdot C_{LOS1} - Xk_{out} \cdot C_{X1} - Xk_{12} \cdot C_{X1} + Xk_{21} \cdot C_{X2} \\
\frac{dC_{X2}}{dt} &= Xk_{12} \cdot C_{X1} - Xk_{31} \cdot C_{X2} \\
\frac{dA_{PRA}}{dt} &= Rk_{in} \cdot S(t) - Rk_{out} \cdot A_{PRA} \\
S(t) &= 1 + \frac{RE_{max} \cdot C_{X1}}{REC_{50} + C_{X1}}
\end{align*}
\] (2)

where \( C_{X1} \) and \( C_{X2} \) are the concentrations (\( \mu g/mL \)) in the compartments X1 and X2, respectively; \( Xk_{12}, Xk_{21}, Xk_{in}, \) and \( Xk_{out} \) are first-order constants (\( min^{-1} \)); \( A_{PRA} \) is the plasma renin activity (\( \mu g/\text{I/mL/h} \)) in the PRA compartment; \( RE_{max} \) is the maximum effect of the PRA (\( \mu g/\text{I/mL/h} \)); \( REC_{50} \) is the LOS concentration (\( \mu g/mL \)) producing 50% of \( RE_{max} \); \( Rk_{in} \) is the zero-order constant (\( \mu g/\text{I/mL/h} \)) concerned with the PRA production; and \( Rk_{out} \) is the first-order constant (\( min^{-1} \)) concerned with the PRA degradation. Before LOS is administered, the baseline level of \( A_{PRA} \) is maintained as \( A_{PRA0} \) (\( \mu g/\text{I/mL/h} \)), which is determined by \( Rk_{out} = Rk_{in} \cdot A_{PRA0} \). The PRA decreases blood pressure as follows:
\[
\frac{dC_E}{dt} = P_{kin} \cdot A_{PRA} - P_{kout} \cdot C_E
\]

(9)

Blood pressure = \(E_0 - \frac{P_{E\text{max}} \cdot C_E}{P_{E50} + C_E}\)

(10)

where \(C_E\) is the value of the PRA (ng-Ang I/mL/h) in the effect compartment, and \(P_{kin}\) and \(P_{kout}\) are first-order constants (min\(^{-1}\)); \(P_{E\text{max}}\) is the maximum hypotensive effect of the PRA (mmHg); \(P_{E50}\) is the PRA (ng-Ang I/mL/h) producing 50% of \(P_{E\text{max}}\); and \(E_0\) is the basal level of blood pressure (mmHg).

**Results**

Pharmacokinetic and pharmacological interaction in the combination treatment: After i.v. bolus coadministration of LOS and HCTZ in each individual animal, the plotting points in **Figure 2A** show the effect of coadministered HCTZ on the time-course of plasma LOS concentration. A two- or three-exponential decline in plasma was observed after administration of LOS alone. Since linearity was shown in these dose-normalized time-courses for LOS (5, 10, 20 mg/kg, data not shown), a linear three-compartment model was selected to describe the PK of LOS. As shown by covariate adjustment, coadministered HCTZ, as a covariate, significantly and dose-independently decreased the elimination clearance of LOS by about 25%, but did not affect the distribution volume (Table 2, solid lines in **Fig. 2A**). The goodness-of-fit of the final model is shown in **Figure 3**. The plotting points in **Figure 2B**

<table>
<thead>
<tr>
<th>Table 2. Population pharmacokinetic parameters of LOS and HCTZ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fixed effect</strong></td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Losartan (LOS)</strong></td>
</tr>
<tr>
<td>(C_{LLOS} \text{ (mL/min)}) = 2.54 \times 0.759^\text{HT}</td>
</tr>
<tr>
<td>(Q_{2LOS} \text{ (mL/min)}) = 0.938</td>
</tr>
<tr>
<td>(Q_{3LOS} \text{ (mL/min)}) = 7.38</td>
</tr>
<tr>
<td>(V_{1LOS} \text{ (mL)}) = 44.2</td>
</tr>
<tr>
<td>(V_{2LOS} \text{ (mL)}) = 210</td>
</tr>
<tr>
<td>(V_{3LOS} \text{ (mL)}) = 75</td>
</tr>
<tr>
<td><strong>SIGMA_{LOS}</strong> = 0.0847 CV (%) = 29.1</td>
</tr>
<tr>
<td>HT: administration with HCTZ = 1, administration without HCTZ = 0,</td>
</tr>
<tr>
<td>(CL_{LOS,\text{ETA}}) and (CL_{HCTZ,\text{ETA}}) the total clearances for LOS and HCTZ; (Q_{2LOS}, Q_{3LOS}, Q_{HCTZ}): the clearances for LOS and HCTZ for the peripheral compartments; (V_{1LOS}, V_{2HCTZ}): the distribution volume of the central compartment for LOS and HCTZ; (V_{3LOS}, V_{2HCTZ}): the distribution volume of the peripheral compartment for LOS and HCTZ; (CL_{LOS,\text{ETA}}, CL_{HCTZ,\text{ETA}}): variance of the random effect for (CL_{LOS}) and (CL_{HCTZ}); (V_{1LOS,\text{ETA}}, V_{2HCTZ,\text{ETA}}): variance of the random effect for (V_{1LOS}); CV: coefficient of variance; (SIGMA_{LOS}, SIGMA_{HCTZ}): residual variability for LOS and HCTZ.</td>
</tr>
</tbody>
</table>

![Fig. 3. Goodness-of-fit for the final population pharmacokinetic model for LOS](image-url) Plots are individual data.
represent the effect of LOS in the plasma HCTZ concentration–time course after i.v. bolus coadministration of LOS and HCTZ in each individual animal. The plasma concentration of HCTZ was fitted to a two-compartment model (Table 3, solid line in Fig. 2B). LOS did not affect any PK parameters of HCTZ. The time-courses of SBP after i.v. bolus administration of LOS and HCTZ are shown in Figures 4A and 4B, and the dose-dependency of the hypotensive effect in the AUE is shown in Figures 4C and 4D. After i.v. bolus administration of LOS (5 mg/kg) or HCTZ (5 mg/kg), SBP decreased slightly and continued for about 72 h (Figs. 4A and 4B). After coadministration of LOS and HCTZ, SBP is remarkably decreased up to 24 h after dosing and a lower SBP profile is maintained for about 144 h. This interactive enhancement of the hypotensive effect depended on the doses of LOS and HCTZ (Figs. 4C and 4D). This hypotensive interaction was also synergistic; e.g. AUE was 2,144.4 mmHg h with a combination of LOS 5 mg/kg and HCTZ 5 mg/kg compared to 662.6 mmHg h and 521.1 mmHg h, respectively, for each drug alone.

**The effect of the combination treatment on the plasma renin activity:** The time-course of the PRA after i.v. bolus coadministration of LOS and HCTZ is represented in Figure 5. The PRA temporally increased and then gradually recovered to baseline up to 72 h after administration, although the eliminations of LOS and HCTZ from plasma were rapid. Thus, there was a typical counter-clockwise hysteresis between plasma LOS concentrations and the PRAs (data not shown). Dose-dependent increases in the PRA were observed in LOS and HCTZ coadministration studies (Fig. 5).

**PK-PD analysis for PRA enhancement:** To describe the time-course of the PRA after administration of LOS with HCTZ, an indirect response model was applied to the PK-PD model for LOS as shown in Figure 1 and Eqs. (1)–(7). However, the compartments X1 and X2 were

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Estimates</th>
</tr>
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<tbody>
<tr>
<td>( X_{c1} ) (min(^{-1}))</td>
<td>0.00732 ± 0.0168</td>
</tr>
<tr>
<td>( X_{c2} ) (min(^{-1}))</td>
<td>0.0123 ± 0.000720</td>
</tr>
<tr>
<td>( X_{c3} ) (min(^{-1}))</td>
<td>0.0162 ± 0.0264</td>
</tr>
<tr>
<td>REC(_{50}) (( \mu )g/mL)</td>
<td>0.585 ± 0.0717</td>
</tr>
<tr>
<td>RE(_{50}) (h(^{-1}))</td>
<td>3.14 ± 26.5</td>
</tr>
<tr>
<td>RE(_{max}) (HCTZ 1 mg/kg)</td>
<td>14.0 ± 2.52</td>
</tr>
<tr>
<td>RE(_{max}) (HCTZ 5 mg/kg)</td>
<td>15.7 ± 2.36</td>
</tr>
<tr>
<td>RE(_{max}) (HCTZ 10 mg/kg)</td>
<td>21.5 ± 3.61</td>
</tr>
<tr>
<td>( P_{c1} ) (min(^{-1}))</td>
<td>0.0244 ± 0.000440</td>
</tr>
<tr>
<td>PEC(_{50}) (ng Ang 1/mL/h)</td>
<td>85.5 ± 118</td>
</tr>
<tr>
<td>PE(_{max}) (mmHg)</td>
<td>180 ± 208</td>
</tr>
</tbody>
</table>

For E-3174

\( a^* \) (min\(^{-1}\)) = 0.0228 ± 0.0016

\( M_{c1} \) (min\(^{-1}\)) = 0.0110 ± 0.0285

\( M_{c2} \) (min\(^{-1}\)) = 0.00590 ± 0.00211

\( M_{c3} \) (min\(^{-1}\)) = 0.0219 ± 0.00205

*Parameters of the model for E-3174 (see Fig. 11). Data represent the computer-fitted value ± SD.

Fig. 4. Changes in blood pressure (A, B) and AUE (C, D) after coadministration of LOS and HCTZ.

Values represent the mean ± S.E.M. (n = 3–4).
required to explain the delayed profiles of the PRA against plasma LOS concentration profiles. The solid lines, shown in Figure 6, represent the results of fitting the observed data after coadministration of LOS and HCTZ to the model described in the theoretical section. The regression curves fit the observed data well. The estimated PD parameters are listed in Table 3. REmax values increased with the dose of HCTZ (Fig. 7).

**PK-PD analysis for the PRA-mediated hypotensive effect:** The PK-PD analysis for the PRA-mediated hypotensive effect after i.v. bolus administration of LOS was carried out according to Eqs. (1)–(10). Since there was a typical counter-clockwise hysteresis between the PRA level and the hypotensive effect, an additional effect compartment was introduced between the PRA and the hypotensive effect model. The solid lines shown in Figure 8 represent the theoretical hypotensive effect and fit the observed data well. The estimated PD parameters are listed in Table 2. To evaluate the validity of the model, we simulated the time-courses of the PRA and SBP after i.v. bolus administration of 5 mg/kg LOS and 8 mg/kg HCTZ. In this simulation, we

Fig. 5. Time-course of plasma renin activity after coadministration of LOS and HCTZ
Plots represent the mean ± S.E.M. (n = 2–3)

Fig. 6. Comparison of the model-fitted value with the observed data for the PRA after coadministration of LOS and HCTZ
Solid lines are the model-fitted profiles. Plots represent the mean ± S.E.M. (n = 2–3).

Fig. 7. Relationship between the REmax value and the dose of HCTZ for the hypotensive effect after coadministration of LOS with HCTZ
The solid line represents a regression line. Plots represent the model-calculated value.
calculated the value of \( R_{\text{Emax}} \) based on the correlation between \( R_{\text{Emax}} \) and the dose of HCTZ (Fig. 7) and used 19.09 mmHg. The other parameters were used from Table 2. The solid line shown in Figure 9 represents the model-predicted profiles of SBP and explain the observed data well.

**Discussion**

**Pharmacokinetic and Pharmacodynamic interaction:** When HCTZ is coadministered with LOS, no effect is observed for the elimination of HCTZ alone, but the total elimination clearance of LOS decreased by about 25%. (Table 2, Fig. 2). LOS is primarily metabolized by the hepatic cytochrome P450 (CYP2C9) in rats, and then more than 94% of the dose is excreted in the feces; however, most of the administered HCTZ was excreted in the urine as an intact drug without any metabolites. These reports indicate that coadministered LOS and HCTZ do not compete with each other for their metabolism or excretion. The plasma protein binding ratio of LOS is reported to be more than 99%, and that of HCTZ is about 22%. Thus, HCTZ would not affect the protein binding of LOS. On the other hand, 10 mg/kg HCTZ significantly increased the urine volume up to 4 h after oral treatment for 5 days in SHRs. Change in the urine volume was not investigated before and after the drug administration in the present study. However, decrease of the body fluid by the diuretic effect of HCTZ might concentrate the level of LOS in plasma, and then the apparent clearance of LOS would decrease. This speculation also supports the invariable PK of HCTZ after coadministration with LOS.

The interactive hypotensive effect was observed after administration of LOS and HCTZ. It was greater than the aggregate of their respective effects (Fig. 4), even after considering the PK interaction by HCTZ. The hypotensive interaction depended on the doses of LOS and HCTZ, which means there is a synergistic effect.

As shown in Figure 10, LOS blocks the angiotensin type 1 receptor (AT\(_1\)R). This interaction of LOS with the AT\(_1\)R suppresses vasoconstriction and activation of the AT\(_2\)R with the elevated Ang II, and consequently yields a decreased blood pressure. As a result of blocking AT\(_1\)R, plasma renin is also activated through suppressing the negative feedback mechanism for the renin-angiotensin system, and subsequently elevates Ang I, Ang II, and Ang\(_1\)–\(_7\) levels. Continuous increases of the PRA and Ang II level in plasma were observed after administration of valsartan. Ang (1–7) releases nitric oxide and decreases blood pressure after binding to the Mas receptor.

Collister
and Hendel18 reported that the increase of Ang (1–7) in plasma develops the hypotensive effect. Furthermore, the PRA increased after administration of HCTZ.14 In our study, the PRA continuously increased for 72 h after LOS administration (Fig. 5). HCTZ administered with LOS further raised the PRA synergistically. These results suggest that PRA is a pharmacological mediator for PK and the hypotensive effect.

PRA-mediated PK-PD model: The hypotensive effect after i.v. coadministration of LOS and HCTZ was described well using the PK-PD model with PRA enhancement (Fig. 6). In this model, a hypothetical maximum effect for enhancement of the PRA (RE max) increased depending on the dose of HCTZ (Fig. 7). The delay compartment X1/X2 was necessary to describe the change in the PRA, because of the delay for activation of plasma renin against the plasma LOS concentration profile. LOS has an active metabolite, E-3174, which shows a hypotensive effect. E-3174 also has a long half-life and a strong affinity compared to LOS. We evaluated the possibility that the X1/X2 compartment represented the PK of E-3174 using the reported PK data.19 Figure 11 represents the PK model for E-3174 that was modified based on the previous model (Fig. 1). The PK of E3174 in plasma is described by Eqs (11)–(14), as follows:

\[
\frac{dA_{LOS1}}{dt} = L_{k21} \cdot A_{LOS2} + L_{k31} \cdot A_{LOS3} - (L_{k12} + L_{k13} + L_{k10}) \cdot A_{LOS1}
\]

(11)

\[
\frac{dM_1}{dt} = a \cdot C_{LOS1} - (M_{k12} + M_{k_{out}}) \cdot M_1 + M_{k21} \cdot M_2
\]

(12)

\[
\frac{dM_2}{dt} = M_{k12} \cdot M_1 - M_{k21} \cdot M_2
\]

(13)

where M1 and M2 are the concentration of E-3174 (µg/mL); and M_{k12}, M_{k21}, M_{k_{out}}, and a are the first-order rate constants (min⁻¹) of E-3174. These parameter values were estimated using the values shown in Table 2 and the reported plasma concentrations of E-3174 after i.v. bolus administration of LOS without HCTZ. The regression curves fit the literature data well (Fig. 11, lower), and the estimated PK parameters are listed in Table 3. However, as compared with the parameters X_{k12}, X_{k31} and X_{k_{out}} in Table 3, these parameters indicated a faster elimination of the E-3174 profile in plasma. That is, the delay compartments X1/X2 do not represent the PK of E-3174 only. These compartments also include other mediators, such as unknown metabolites and endogenous factors concerned with the PRA production. In addition, this result indicates the validity of the present model based on plasma LOS concentration, and the pharmacokinetic data of E-3174 is not necessary for the estimation of the interactive hypotensive effect.

Furthermore, an additive effect compartment (C_e) was required to link the PRA and the hypotensive effect. PRA raises the plasma Ang II and Ang (1–7) concentrations. Then these Angs bind to the AT2R and the MasR (Fig. 10). As a result, a decrease in blood pressure is observed. Therefore, the delays described by the effect compartment may represent the transit time required for the sequence of these pharmacological events of the renin-angiotensin system. The HCTZ dose-dependency of the maximum hypotensive effect also reasonably predicted the observed data.

In conclusion, the interactive hypotensive effect is shown after i.v. bolus coadministration of LOS and HCTZ, and the
relationship between plasma LOS concentration and blood pressure is described using the PK-PD model. The model quantitatively reveals that HCTZ principally enhances the hypotensive effect of LOS dose-dependently through an increase of the PRA. This relationship can estimate the hypotensive effect for combination treatment using different doses and kinds of ARBs and thiazide diuretics.

References

13) Package insert of Preminent® tablet, ver. 9, Tokyo, MSD K.K., 2011.