PHARMACOKINETIC CHANGES OF A NEW CARBAPENEM, DA-1131, AFTER INTRAVENOUS ADMINISTRATION TO SPONTANEOUSLY HYPERTENSIVE RATS AND DEOXYCORTICOSTERONE ACETATE-SALT-INDUCED HYPERTENSIVE RATS

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ABSTRACT:

The pharmacokinetics of a new carbapenem, DA-1131, were compared after i.v. administration of the drug, 50 mg/kg, to spontaneously hypertensive rats (SHRs) at 16 weeks of age (an animal model for human primary hypertension) and at 6 weeks of age (corresponding to the early phase of the development of hypertension, at which time blood pressure remains within the normotensive range) and their respective age-matched control normotensive Kyoto-Wistar rats (KW rats), and deoxycorticosterone acetate-salt-induced hypertensive rats at 16 weeks of age (an animal model for human secondary hypertension) and their age-matched control Sprague-Dawley rats. The total area under the plasma concentration-time curve from time zero to time infinity (AUC) (4720 versus 7070 μg·min/ml) was significantly smaller, and the nonrenal clearance (CLNR) (5.37 versus 3.57 ml/min/kg) was significantly faster in 16-week-old SHRs than those in their control KW rats. Similar results were also obtained from 6-week-old SHRs in AUC (3800 versus 4680 μg·min/ml) and CLNR (7.73 versus 3.31 ml/min/kg). However, the values were reversed in 16-week-old deoxycorticosterone acetate-salt rats in AUC (5310 versus 3870 μg·min/ml) and CLNR (2.57 versus 4.90 ml/min/kg). The significantly faster CLNR of DA-1131 in both 6- and 16-week-old SHRs could be supported at least partly by the results of the in vitro metabolism with kidney homogenate and considerably greater total renal dehydropeptidase-I activity. The data above indicated that the significantly faster CLNR of DA-1131 in 16-week-old SHRs than that in their age-matched control KW rats was due to any hereditary characteristics of SHRs and was not due to the hyperpertensive state itself.

(1R,5S,6S)-(2S,4S)-2-[(E)-3-methansulfonylamino-1-propenyl] pyrrolidin-4-ylthiol-6-[(R)-1-hydroxyethyl]-1-methyl-1-carbapen-2-yl-3-carboxylic acid (DA-1131) (Fig. 1), a new carbapenem antibiotic, has a broad spectrum of activity against both the Gram-positive and Gram-negative organisms (Kim et al., 1996a). DA-1131 was relatively stable against hydrolysis by renal dehydropeptidase-I (DHP-I) compared with imipenem and meropenem (Kim et al., 1996b) and was resistant to degradation by various types of b-lactamas (Choi et al., 1996). DA-1131 was unstable when incubated in low and high pH solutions, human plasma, rat liver homogenate, and human gastric juice (Kim et al., 1995). The plasma-to-blood cell concentration ratios of DA-1131 were independent of DA-1131 rabbit blood concentrations; the values were 4.61 to 5.80 at initial DA-1131 blood concentrations of 2 to 10 μg/ml (Kim et al., 1995). After i.v. administration of DA-1131 to mice (20–200 mg/kg), rats (50–500 mg/kg), rabbits (20–200 mg/kg), and dogs (10–200 mg/kg), the pharmacokinetic parameters of the drug seemed to be independent of DA-1131 doses studied in all four animal species (Kim et al., 1998d). However, the renal clearance (CLr) and percentages of i.v. dose of DA-1131 excreted in 24-h urine as unchanged drug decreased significantly in rabbits (from 200 mg/kg) and dogs (from 100 mg/kg) due to reduced kidney function induced by DA-1131 (Kim et al., 1998d). The nephroprotective effect of betamipron was observed on DA-1131-induced nephrotoxicity in rabbits (Kim et al., 1999a). DA-1131 was mainly excreted in urine by glomerular filtration in rats (Kim et al., 1999b); however, renal tubular secretion and reabsorption of the drug were observed in rabbits (Kim et al., 1999b) and dogs (Kim et al., 1998c), respectively. Significant linear relationships were obtained between log (total body clearance, CL) and log (body weight); log (CLr) and log (body weight); and log (volume of distribution at steady state, VSS) and log (body weight) after i.v. administration of DA-1131, 50 mg/kg, to rats, rabbits, dogs, and/or mice (Kim et al., 1998a). Pharmacokinetics of DA-1131 were changed in disease models of animals; CLr, and nonrenal clearance (CLNR) were significantly slower in uranyl nitrate-induced acute renal failure rats (Kim et al., 1998e) and alloxa-induced diabetes mellitus rats (Kim et al., 1998b), and CLNR and CLR were significantly slower and faster, respectively, in endotoxin-induced pyrexia rabbits (Kim et al., 1997a). DA-1131 is now being evaluated in preclinical study.

In many other studies, spontaneously hypertensive rats (SHRs) (Okamoto and Aoki, 1963; Han et al., 1993; Sakane et al., 1993; Jang...
et al., 1994a,b; Yoon et al., 1997) and deoxycorticosterone acetate-salt-induced hypertensive rats (DOCA-salt rats) (Beilin et al., 1970; Han et al., 1993; Sakane et al., 1993; Jang et al., 1994a; Yoon et al., 1997) have been used as animal models for human primary (essential) and secondary hypertension, respectively. The pharmacokinetic differences of drugs between SHRs and their age-matched control Kyoto-Wistar rats (KW rats) and DOCA-salt rats and their age-matched control Sprague-Dawley rats have been reported. For example, the mean plasma concentrations of both M2 and M4 [the metabolites of a new doxorubicin (Adriamycin) analog, DA-125] were significantly higher, and the resultant area under the plasma concentration-time curve from time zero to the last measured time in plasma was significantly greater in 16-week-old SHRs and DOCA-salt rats than in their respective age-matched control rats after i.v. administration of DA-125 (Yoon et al., 1997). However, the values were not significantly different between 6-week-old SHRs and their age-matched control KW rats (Yoon et al., 1997). The mean plasma concentrations of YJA-20379-8, a new reversible proton pump inhibitor, were significantly lower or tended to be lower, and CL was significantly faster in 6-week-old SHRs, 16-week-old SHRs, and 16-week-old DOCA-salt rats than those in their respective control rats (our unpublished data). The CL of thiopan was significantly slower in DOCA-salt rats than their age-matched control Sprague-Dawley rats; however, the CL was not significantly different between SHRs and their age-matched control KW rats (Sakane et al., 1993). Aforementioned data suggested that the pharmacokinetics of DA-1131 could be changed in 6-week-old SHRs and/or 16-week-old SHRs and DOCA-salt rats. Therefore, the present study was performed because the various hypertensive patients could use DA-1131 to treat bacterial infection.

The purpose of the present study was to investigate whether any differences observed in the pharmacokinetics of DA-1131 (especially the differences in CL\textsubscript{MR} of DA-1131 in 16-week-old SHRs) was caused by either the hereditary characteristics of SHRs (between 6-week-old and 16-week-old SHRs) or the hypertensive state itself (between SHRs and DOCA-salt rats at 16 weeks of age). In the present study, the pharmacokinetics and tissue distribution of DA-1131 were evaluated after i.v. administration of the drug, 50 mg/kg, to 16-week-old SHRs following chronic exposure to hypertension (Sladek and Blair, 1984) and to their age-matched control normotensive KW rats. Similar studies were also performed in 16-week-old DOCA-salt rats and their age-matched control Sprague-Dawley rats and in 6-week-old SHRs (corresponding to the early phase of the development of hypertension, at which time the blood pressure remains within the normotensive range; Sladek and Blair, 1984) and their age-matched control KW rats.

**Materials and Methods**

**Chemicals.** DA-1131 (as an HCl salt) was kindly donated by Research Laboratory of Dong-A Pharmaceutical Co. (Yongin, South Korea). DOCA, reduced form of nicotinamide adenine dinucleotide phosphate, uridine diphosphoglucuronic acid, and glycyldehydrophenylalanine were products of Sigma Chemical Co. (St. Louis, MO). Other chemicals were of reagent grade or HPLC grade and used without further purification.

**Animals.** Male SHRs at 5 or 15 weeks of age and their age-matched control KW rats and Sprague-Dawley rats at 12 weeks of age were purchased from Charles River Co. (Atsugi, Japan). At 6 and 16 weeks of age, systolic blood pressure of SHRs and their control KW rats were measured using tail cuff plethysmography (Narcolette 40; NBS, Houston, TX). The mean (±S.D.) systolic blood pressure at 16-week-old SHRs and their age-matched control KW rats were 178 ± 11.3 and 126 ± 12.7 mm Hg, respectively, and the corresponding values at 6-week-old SHRs and their age-matched control KW rats were 118 ± 5.4 and 104 ± 9.01 mm Hg, respectively. Sprague-Dawley rats were randomly divided into two groups: DOCA-salt rats and their age-matched control rats. DOCA-salt rats received s.c. injection of 12.5 mg/kg DOCA (dissolved in cotton seed oil, 5 mg/ml) every 3 days and 1% NaCl as drinking water ad libitum during 12 to 16 weeks of age. The control Sprague-Dawley rats were given s.c. injection of the same volume of cotton seed oil every 3 days and tap water ad libitum during 12 to 16 weeks of age. The mean (±S.D.) systolic blood pressure at 16-week-old DOCA-salt rats and their age-matched control Sprague-Dawley rats were 161 ± 6.06 and 119 ± 6.44 mm Hg, respectively.

**Intravenous Study.** In the early morning at the end of 6 or 16 weeks (after overnight fasting with water ad libitum), the carotid artery and the jugular vein were catheterized with polyethylene tube (Clay Adams, Parsippany, NJ) under light ether anesthesia. Both cannulas were exteriorized to the dorsal side of the neck, wherein each cannula terminated with the long Silastic tube (Dow Corning, Midland, MI). The two Silastic tubes were covered with a wire to allow free movement of the rat. Each rat was housed individually in a rat metabolic cage (Daejong Scientific Co., Seoul, South Korea) and allowed 4 to 5 h to recover from anesthesia before the study. They were not restrained at any time during the study. DA-1131 (dissolved in 0.9% NaCl-injectable solution), 50 mg/kg, was administered by i.v. infusion in 1 min via the jugular vein (total injection volume was 1.5 ml) of each of SHRs (n = 7), KW rats (n = 12), DOCA-salt rats (n = 8), and Sprague-Dawley rats (n = 7) at 16 weeks of age and SHRs (n = 7) and KW rats (n = 10) at 6 weeks of age. Blood samples (0.12 ml) were collected via the carotid artery before (to serve as a control) and at 1 (at the end of the infusion), 5, 15, 30, 45, 60, 90, 120, 180, 240, and 360 min after i.v. administration of DA-1131. Heparinized 0.9% NaCl-injectable solution (20 U/ml), 0.25 ml, was used to flush the cannula after each blood sampling to prevent blood clotting. Blood samples were centrifuged immediately to minimize the “blood storage effect” (the change in plasma concentration of DA-1131 due to the time elapsed between collection and centrifugation of the blood sample) of plasma concentrations of DA-1131 (Kim et al., 1995); and a 50-μl aliquot of each plasma sample was frozen in the −70°C freezer (Reuvo ULTR 1490 D-N-S; Western Medicis, Ashevile, NC) until HPLC analysis (Kim et al., 1997b). At 8 h, a large volume of blood was collected through the carotid artery, and each rat was sacrificed by cervical dislocation. Blood samples were centrifuged immediately, and an aliquot of each plasma sample was collected for the measurement of creatinine. At the same time, the metabolic cage was rinsed with 20 ml of distilled water. This, along with the washings of the cut bladder, was combined with 8-h urine. After measuring the exact volume of the combined 8-h urine, two 0.1-ml aliquots of the combined 8-h urine were collected and frozen in the −70°C freezer (Reuvo ULTR 1490 D-N-S) until HPLC analysis of DA-1131 (Kim et al., 1997b). At 8 h, a large volume of blood was collected through the carotid artery, and each rat was sacrificed by cervical dislocation. Blood samples were centrifuged immediately, and an aliquot of each plasma sample was collected for the measurement of creatinine. At the same time, the metabolic cage was rinsed with 20 ml of distilled water. This, along with the washings of the cut bladder, was combined with 8-h urine. After measuring the exact volume of the combined 8-h urine, two 0.1-ml aliquots of the combined 8-h urine were collected and frozen in the −70°C freezer (Reuvo ULTR 1490 D-N-S) until HPLC analysis of DA-1131 (Kim et al., 1997b).

**In Vitro Disappearance of DA-1131 in Homogenates of Kidney and Liver.** The procedures are similar (Kim et al., 1993) to the reported method (Litterst et al., 1975). In the early morning, SHRs (n = 3), KW rats (n = 3), DOCA-salt rats (n = 3), and Sprague-Dawley rats (n = 3) at 16 weeks of age and SHRs (n = 3) and KW rats (n = 3) at 6 weeks of age were sacrificed by cervical dislocation. Approximately 1 g of liver or kidney was excised, rinsed with 50 mM Tris–HCl buffer (pH 7.4), blotted dry with tissue paper, and weighed. All subsequent procedures were conducted at 4°C on an ice bath. Each tissue was minced with scissors and homogenized with 4 volumes of cold 0.25 M sucrose in a tissue homogenizer (Ultra-Turrax, T25; Janke & Kunkel,

![Fig. 1. Chemical structure of DA-1131.](image-url)
IKA-Labortechnik, Staufen, Germany). The homogenate was then centrifuged using a Beckman model J2-21 (Palo Alto, CA) at 9000g for 20 min, and the supernatant fraction was collected.

The metabolic activity was initiated by adding 1 ml of the above 9000g supernatant of each tissue to a glass test tube containing 25 μl (50 μg) of DA-1131 aqueous solution, 100 μl (1 mM) reduced form of nicotinamide adenine dinucleotide phosphate, 1.9 ml (100 mM) of Tris-HCl buffer, pH 7.4, and 25 μl (3.3 mM) of uridine diphosphoglucuronic acid. The mixture was then thoroughly mixed by hand and shaken in a water-bath shaker kept at 37°C and at a rate of 50 oscillations/min for 30 min. After centrifugation, two 100-μl aliquots of the supernatant were collected and frozen in the −70°C freezer (Revco; ULT 1490 D-N-S) until HPLC analysis of DA-1131 (Kim et al., 1997b).

Measurement of Renal DHP-I Activity. Renal DHP-I activity of SHRs (n = 4) and KW rats (n = 5) at 6 weeks of age and SHRs (n = 4), KW rats (n = 3), DOCA-salt rats (n = 3), and Sprague-Dawley rats (n = 4) at 16 weeks of age was measured by the slight modification of the reported method (Campbell et al., 1963; Mikami et al., 1982). Glycyldehydrophenylalamine was used as an assay substrate. Enzyme-catalyzed hydrolysis was measured by observing the fall in optical density of a solution of 5 × 10⁻⁵ M in 3-(N-morpholino)propanesulfonic acid buffer (50 mM, pH 7.1) at 275 nm. The unit and specific activity were expressed as micromoles of glycyldehydrophenylalamine hydrolyzed per minute and unit per milligram of protein, respectively. Protein concentrations were determined by the reported method (Lowry et al., 1951) using the crystallized bovine serum albumin as a protein standard.

Tissue Distribution Study. DA-1131 (dissolved in 0.9% NaCl-injectable solution), 50 mg/kg, was administered i.v. to SHRs (n = 4) and KW rats (n = 5) at 6 weeks of age and SHRs (n = 5), KW rats (n = 6), DOCA-salt rats (n = 5), and Sprague-Dawley rats (n = 4) at 16 weeks of age. At 30 min after i.v. administration of the drug, a large volume of blood was collected through the carotid artery, and each rat was sacrificed by cervical dislocation. Blood samples were centrifuged immediately, and plasma was collected. Approximately 1 g of each brain, fat, heart, lung, stomach, small intestine, large intestine, liver, kidney, mesentery, muscle, and spleen was excised, rinsed with cold 0.9% NaCl-injectable solution to eliminate blood remaining in the tissues, blotted dry with paper tissue, and homogenized with 4 volumes of distilled water using tissue homogenizer (Ultra-Turrax T25). After centrifugation, two 50-μl aliquots of the supernatant were frozen in the −70°C freezer (Revco ULT 1490 D-N-S) until HPLC analysis of DA-1131 (Kim et al., 1997b). Plasma samples were also diluted with 4 volumes of distilled water. All the procedures were conducted at 4°C on an ice bath.

HPLC Analysis of DA-1131 and Measurement of Creatinine. The concentrations of DA-1131 in the biological samples above were analyzed within 7 days by the reported HPLC method developed from our laboratory (Kim et al., 1997b). The mobile phase, 0.015 M KH₂PO₄-acetonitrile (9:1, v/v, pH 5.0), was run through reversed-phase column at a flow rate of 0.8 ml/min, and the retention of DA-1131 was approximately 8.0 min. The detection limits of DA-1131 in human plasma and urine were lower than 8.39%.

Pharmacokinetics of DA-1131 after Intravenous Administration. After i.v. administration to 16-week-old SHRs and their age-matched control KW rats, the plasma levels of DA-1131 declined rapidly for both groups of rats (Fig. 2), and the mean terminal half-life of 15.7 min in 16-week-old SHRs was significantly shorter (38% decrease) than 25.2 min in their control KW rats (Table 1). The plasma concentrations of DA-1131 were significantly lower in 16-week-old SHRs than those in control KW rats (Fig. 2), and this resulted in a significantly smaller AUC (33% decrease) in 16-week-old SHRs (Table 1). The MRT was also significantly shorter (28% decrease) in 16-week-old SHRs (Table 1). The CL (50% increase), CL_r (38% increase), and CL_m (50% increase) were significantly faster; however, creatinine clearance was significantly slower (21% decrease) in 16-week-old SHRs (Table 1). The kidney weight increased significantly (5.6% increase) in 16-week-old SHRs (Table 1). However, the VSS, total amount of unchanged DA-1131 excreted in 8-h urine (Ae)], and liver weight were not significantly different for both groups of rats (Table 1). There were no significant histological changes in both the kidney and liver for both groups of rats based on tissue microscopy.

After i.v. administration to 6-week-old SHRs and their age-matched control KW rats, the plasma concentrations of DA-1131 also declined...
rapidly for both groups of rats (Fig. 3), with mean terminal half-lives of 15.5 and 16.3 min for 6-week-old SHRs and their control KW rats, respectively (Table 2). They were not significantly different. The plasma concentrations of DA-1131 were significantly lower up to 60 min in 6-week-old SHRs than those in control KW rats (Fig. 3), and this resulted in a significantly smaller AUC (19% decrease) (Table 2). The CL (23% increase) and CLNR (134% increase) were significantly faster in 6-week-old SHRs. In 6-week-old SHRs, the Ae g, 0–8 h decreased (36% decrease) significantly (Table 2). However, the MRT, CL R, VSS, kidney and liver weight, and creatinine clearance were not significantly different for both groups of rats. There were significant histological changes in the liver of 6-week-old SHRs based on liver microscopy; the mild centrilobular hepatocytic degeneration was observed. However, there were no significant histological changes in the kidney for both groups of rats and in the liver for 6-week-old KW rats.

After i.v. administration to 16-week-old DOCA-salt rats and their age-matched control Sprague-Dawley rats, the plasma concentrations of DA-1131 again declined rapidly in both groups of rats (Fig. 4), and the mean terminal half-lives of 22.9 min in 16-week-old DOCA-salt rats was significantly longer (39% increase) than 16.5 min in their control SPRague-Dawley rats (Table 3). The plasma concentrations of DA-1131 in 16-week-old DOCA-salt rats were significantly higher than those in their age-matched control Sprague-Dawley rats (Fig. 4), and this resulted in a significantly greater AUC (37% increase) (Table 3). The CL (27% decrease) and CLNR (48% decrease) and creatinine clearance (27% decrease) were significantly slower in 16-week-old DOCA-salt rats (Table 3). MRT was significantly longer (56% increase) and kidney weight (30% increase) increased significantly in 16-week-old DOCA-salt rats (Table 3). However, the CL R, VSS, and liver weight were not significantly different for both groups of rats. Impaired liver function in 16-week-old DOCA-salt rat was observed based on liver microscopy; mild centrilobular hepatocytic degeneration was developed. However, there were no significant histological changes in the kidney for both groups of rats and in the liver in control Sprague-Dawley rats based on tissue microscopy.

**In Vitro Disappearance of DA-1131 in Homogenates of Kidney and Liver.** The mean amount of DA-1131 remaining per gram of liver after 30-min incubation was significantly smaller (48% decrease) in 16-week-old SHRs than those in their age-matched KW rats (Table 4). However, the mean value of liver was significantly greater in 6-week-old SHRs (865% increase) and 16-week-old DOCA-salt rats (238% increase) than those in their respective age-matched control rats (Table 4).

**Measurement of Renal DHP-I Activity.** In 16-week-old SHRs, the renal DHP-I activity increased significantly (44% increase), and total renal DHP-I activity was considerably greater (50% increase, p < .123) than those in their age-matched control KW rats (Table 5). In 6-week-old SHRs, the total DHP-I activity (105% increase, p < .05; **p < .01; ***p < .001.

TABLE 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>KW Rats a (n = 12)</th>
<th>SHRs a (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminal half-life (min)</td>
<td>25.2 ± 3.76</td>
<td>15.7 ± 1.54***</td>
</tr>
<tr>
<td>AUC (μg · min/ml)</td>
<td>7070 ± 862</td>
<td>4720 ± 785***</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>20.1 ± 2.04</td>
<td>14.4 ± 1.13***</td>
</tr>
<tr>
<td>CL (ml/min/kg)</td>
<td>7.07 ± 0.871</td>
<td>10.6 ± 1.98***</td>
</tr>
<tr>
<td>CLR (ml/min/kg)</td>
<td>3.41 ± 0.615</td>
<td>4.69 ± 1.79***</td>
</tr>
<tr>
<td>CLR (ml/min/kg)</td>
<td>3.57 ± 0.594</td>
<td>5.37 ± 1.26***</td>
</tr>
<tr>
<td>VSS (ml/kg)</td>
<td>141 ± 19.6</td>
<td>152 ± 31.3</td>
</tr>
<tr>
<td>Ae g, 0–8 h (% of i.v. dose)</td>
<td>48.9 ± 5.77</td>
<td>48.0 ± 11.3</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min/kg)</td>
<td>3.98 ± 0.776</td>
<td>3.15 ± 0.756**</td>
</tr>
<tr>
<td>Liver (% of body weight)</td>
<td>3.14 ± 0.164</td>
<td>3.12 ± 0.209</td>
</tr>
<tr>
<td>Kidney (% of body weight)</td>
<td>0.718 ± 0.0379</td>
<td>0.758 ± 0.0147*</td>
</tr>
</tbody>
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a P values are as follows: *p < .05; **p < .01; ***p < .001.
crease) increased significantly (Table 5). In 16-week-old DOCA-salt rats, 206 total renal DHP-I activity (Table 5).

The contribution of biliary excretion of unchanged DA-1131 to CL_{NR} of DA-1131 was negligible; less than 1.76% of i.v. dose of DA-1131, 200 mg/kg to six Sprague-Dawley rats, was excreted in 8-h bile as unchanged DA-1131 (Kim et al., 1998d). Therefore, the CL_{NR} of DA-1131 could represent metabolic clearance of DA-1131 in rats. The significantly faster CL_{NR} of DA-1131 in 16-week-old SHRs could represent faster nonrenal metabolism of DA-1131 in SHRs. The faster metabolism of DA-1131 in the kidney of SHRs at 16 weeks of age could be supported at least partly by the result of the in vitro incubation of 50 μg of DA-1131 with the 9000g supernatant fraction of kidney homogenates; the mean amount of DA-1131 remaining per gram of kidney after a 30-min incubation was considerably smaller in 16-week-old SHRs (Table 4). The greater metabolic activity of DA-1131 in the kidney of 16-week-old SHRs may also be supported by the significantly greater renal DHP-I activity and considerably greater total renal DHP-I activity (Table 5). It has been reported that the carbapenems, such as imipenem (Kropp et al., 1982) or meropenem (Mouton and van den Anker, 1995), are mainly metabolized by renal DHP-I. The faster metabolism of DA-1131 in the liver of SHRs of 16 weeks of age could be supported by liver homogenate study; the amount of DA-1131 remaining per gram of liver decreased significantly (Table 4). Therefore, it could be concluded that the significantly faster metabolism of DA-1131 in 16-week-old SHRs could be due to faster metabolism of DA-1131 in both the kidney and liver. It has been reported (Kim et al., 1995) that rat liver and kidney were the main disappearing (mainly due to metabolism) organs for DA-1131 based on in vitro tissue homogenate study.

To determine whether the significantly faster CL_{NR} of DA-1131 in 16-week-old SHRs than that in their control KW rats (Table 1) was due to the hereditary characteristics of SHRs or other factors (such as hypertension itself), DA-1131 was i.v. administered to 6-week-old SHRs—at which time the blood pressure remains within the normotensive range—and their age-matched control KW rats (Table 2). The faster metabolism of DA-1131 in the kidney of SHRs of 6 weeks of age could be supported at least partly by the results of the in vitro incubation of 50 μg of DA-1131 with the 9000g supernatant fraction of kidney homogenate; the mean amount of DA-1131 remaining per gram of kidney after a 30-min incubation was significantly smaller in 6-week-old DOCA-salt rats and their age-matched control Sprague-Dawley rats. The significantly faster CL of DA-1131 in 6-week-old DOCA-salt rats and their age-matched control Sprague-Dawley rats could be due to significantly faster metabolism of DA-1131 in both the kidney and liver. It has been reported (Kim et al., 1995) that rat liver and kidney were the main disappearing (mainly due to metabolism) organs for DA-1131 based on in vitro tissue homogenate study.

However, 206 tended to increase, and the total protein in kidney (60% increase) increased significantly (Table 5). In 16-week-old DOCA-salt rats, the renal DHP-I activity (27% decrease, p < .0698) decreased considerably, and total protein in kidney (69% increase) increased significantly (Table 5).

**Tissue Distribution of DA-1131 after Intravenous Administration**. Although DA-1131 was widely distributed in all rat tissues studied, the tissue-plasma ratios were far less than unity in all tissues studied except kidney in all rats and small intestine in SHRs at 16 weeks of age (Table 6). Generally, the amount of DA-1131 recovered from each tissue and/or the tissue-plasma ratios in rat tissues studied were not significantly different between 16-week-old SHRs and their age-matched control KW rats, 6-week-old SHRs and their age-matched control KW rats, and 16-week-old DOCA-salt rats and their age-matched control Sprague-Dawley rats, respectively (Table 6).
indicated that kidney is the main metabolizing organ for DA-1131 in 6-week-old SHRs (n = 3) and KW rats (n = 3) of 6 weeks of age, and SHRs (n = 5), KW rats (n = 3), DOCA-salt-induced hypertensive rats (n = 3), and Sprague-Dawley rats (n = 3) of 16 weeks of age. The data above could be due to liver impairment; the mild centrilobular hepatocytic metabolism of DA-1131 in the liver at 6-week-old SHRs (Table 4) is significantly greater in 6-week-old SHRs (Table 4). The significantly slower CLNR incubation of 50 μg of DA-1131 with liver homogenate was significantly greater in 6-week-old SHRs (Table 4). The significantly slower metabolism of DA-1131 in the liver at 6-week-old SHRs (Table 4) could be due to liver impairment; the mild centrilobular hepatocytic degeneration was observed based on liver microscopy. The data above indicated that kidney is the main metabolizing organ for DA-1131 in 6-week-old SHRs.

It was unexpected based on a comparison with 6-week-old and 16-week-old SHRs that the CL and CL_NA of DA-1131 were significantly slower in 16-week-old DOCA-salt rats than those in their age-matched control Sprague-Dawley rats (Table 3). The significantly slower CL of thiorphan (Sakane et al., 1993) in DOCA-salt rats has also been reported. The significantly slower CL of DA-1131 in DOCA-salt rats could be at least due to slower metabolism of DA-1131 in the liver and kidney. This was supported by the in vitro tissue homogenate studies; the mean amounts of DA-1131 g/g kidney and liver at 30 min after 1-min i.v. infusion of the drug, 50 mg/kg, to 16-week-old DOCA-salt rats was due to significantly slower CLNR in vitro tissue homogenate studies; the mean amounts of DA-1131 g/g kidney and liver at 30 min after 1-min i.v. infusion of the drug, 50 mg/kg, to SHRs (n = 5), KW rats (n = 3), DOCA-salt-induced hypertensive rats (n = 3), and Sprague-Dawley rats (n = 4) of 16 weeks of age 6 Weeks of Age

<table>
<thead>
<tr>
<th>Tissues</th>
<th>6 Weeks of Age</th>
<th>16 Weeks of Age</th>
<th>16 Weeks of Age</th>
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<tr>
<td></td>
<td>KW rats SHRs</td>
<td>KW rats SHRs</td>
<td>Sprague-Dawley rats DOCA-Salt rats</td>
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<tr>
<td>Kidney</td>
<td>7.19 ± 1.72</td>
<td>0.729 ± 0.0970**</td>
<td>1.49 ± 0.775</td>
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<tr>
<td>Liver</td>
<td>1.99 ± 1.24</td>
<td>19.2 ± 2.86**</td>
<td>22.7 ± 3.76</td>
</tr>
</tbody>
</table>

a P values are as follows: * p < 0.05; ** p < 0.01; *** p < 0.001.

b Under detection limit (the detection limit of DA-1131 in tissue homogenate was 0.1 μg/g kidney).

c Mean (± S.D.) amount (μg) of DA-1131 remaining per gram of tissues after 30-min incubation of 50 μg of DA-1131 with 9000g supernatant fraction of kidney and liver homogenates in SHRs (n = 3) and KW rats (n = 3) of 6 weeks of age, and SHRs (n = 5), KW rats (n = 3), DOCA-salt-induced hypertensive rats (n = 3), and Sprague-Dawley rats (n = 3) of 16 weeks of age.

Mean (± S.D.) amount of tissue to plasma (T/P) ratio. The numbers in parentheses represent tissue to plasma (T/P) ratio.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>6 Weeks of Age</th>
<th>16 Weeks of Age</th>
<th>16 Weeks of Age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KW rats SHRs</td>
<td>KW rats SHRs</td>
<td>Sprague-Dawley rats DOCA-Salt rats</td>
</tr>
<tr>
<td>Plasma</td>
<td>27.9 ± 5.54</td>
<td>30.0 ± 14.0</td>
<td>46.9 ± 12.9</td>
</tr>
<tr>
<td>Brain</td>
<td>1.32 ± 0.34</td>
<td>1.87 ± 0.976</td>
<td>2.49 ± 0.486</td>
</tr>
<tr>
<td>Fat</td>
<td>3.96 ± 0.69</td>
<td>2.33 ± 0.959**</td>
<td>3.18 ± 0.519</td>
</tr>
<tr>
<td>Heart</td>
<td>5.44 ± 1.03</td>
<td>5.70 ± 3.87</td>
<td>7.97 ± 1.81</td>
</tr>
<tr>
<td>Kidney</td>
<td>37.2 ± 9.99</td>
<td>64.1 ± 50.9</td>
<td>97.5 ± 31.4</td>
</tr>
<tr>
<td>Large intestine</td>
<td>4.58 ± 0.761</td>
<td>4.29 ± 2.26</td>
<td>10.2 ± 9.99</td>
</tr>
<tr>
<td>Liver</td>
<td>8.64 ± 1.60</td>
<td>8.50 ± 3.48</td>
<td>20.2 ± 4.99</td>
</tr>
<tr>
<td>Lung</td>
<td>4.41 ± 1.77</td>
<td>10.7 ± 10.6</td>
<td>7.68 ± 2.42</td>
</tr>
<tr>
<td>Mesentery</td>
<td>4.12 ± 0.777</td>
<td>7.33 ± 4.86</td>
<td>6.02 ± 2.34</td>
</tr>
<tr>
<td>Muscle</td>
<td>4.10 ± 1.24</td>
<td>8.99 ± 8.02</td>
<td>6.91 ± 3.47</td>
</tr>
<tr>
<td>Small intestine</td>
<td>8.90 ± 3.08</td>
<td>8.05 ± 4.22</td>
<td>14.8 ± 4.70</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.69 ± 0.145</td>
<td>3.84 ± 1.56</td>
<td>4.55 ± 0.971</td>
</tr>
<tr>
<td>Stomach</td>
<td>4.02 ± 0.772</td>
<td>6.94 ± 4.23</td>
<td>7.58 ± 1.58</td>
</tr>
</tbody>
</table>

a P values are as follows: * p < 0.05; ** p < 0.01; *** p < 0.001.

The numbers in parentheses represent tissue to plasma (T/P) ratio.
50 μg of DA-1131 were considerably greater and significantly greater, respectively, in 16-week-old DOCA-salt rats (Table 4). The decreased metabolic activity of DA-1131 in the liver of 16-week-old DOCA-salt rats could be due to impaired liver function; mild centrilobular hepatocytic degeneration was developed based on liver microscopy. The decreased metabolic activity of DA-1131 in the kidney of 16-week-old DOCA-salt rats was supported by impaired kidney function; the creatinine clearance decreased significantly, and the kidney weight increased significantly (Table 3), although there were no histological changes based on kidney microscopy. It has been reported that renal blood flow and glomerular filtration rate were markedly reduced (Roman et al., 1988) and that kidney weight increased significantly (Iversen and Ofstad, 1987) in DOCA-salt rats. The significantly slower CLNR of DA-1131 in 16-week-old DOCA-salt rats was due to significantly slower metabolism of DA-1131 in both the liver and kidney.

Whether the pharmacokinetic changes of drugs in 16-week-old SHRs were due to hereditary characteristics of SHRs and/or a hypertensive state itself were dependent on drugs (depends on main metabolizing organs, main metabolic enzyme systems, and fraction of dose excreted in urine). For example, the significantly lower or tended to be lower plasma concentrations and the significantly faster CL of YJA-20379-8 were due to both hereditary characteristics of SHRs and a hypertensive state itself (our unpublished data). The increased formation of M2 and M4, the metabolites of DA-125, was due to the hypertensive state itself; however, the significantly faster CLNR of DA-1131 was due to hereditary characteristics of SHRs.

In conclusion, the significantly smaller AUC and significantly faster CLNR of DA-1131 in 16-week-old SHRs compared with their age-matched control KW rats was due to any hereditary characteristics of SHRs and was not due to the hypertensive state itself. Although the patients exhibiting hypertension will be usually treated for this first to control blood pressure before being treated with DA-1131, the pharmacokinetics of DA-1131 could be changed in the primary hypertensive patients after controlled blood pressure (if the present rat data could be extrapolated to humans), because the faster CLNR of DA-1131 was due to hereditary characteristics of SHRs. More studies are required whether the modification of the i.v. dose of DA-1131 is necessary in the primary hypertensive patients.

Acknowledgments. We thank Dr. In Chull Lee (Choong-Ang Hospital, Seoul, South Korea) for histological examination on the necessary in the primary hypertensive patients. DA-1131 was due to hereditary characteristics of SHRs. More studies could be extrapolated to humans, because the faster CLNR of DA-1131 could be changed in the primary hypertensive patients after controlled blood pressure (if the present rat data could be extrapolated to humans), because the faster CLNR of DA-1131 was due to hereditary characteristics of SHRs. More studies are required whether the modification of the i.v. dose of DA-1131 is necessary in the primary hypertensive patients.

References