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Pharmacokinetics of a New Antigastritic Agent, Eupatilin, an Active Component of Stillen®, in Rats

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Abstract – Pharmacokinetics of eupatilin (an active components of Stillen®, a new antigastritic agent) were investigated after both intravenous and oral administration at a dose of 30 mg/kg to rats. After intravenous administration, the plasma concentrations of unchanged eupatilin declined rapidly with a mean terminal half-life of 0.101 h. Eupatilin was eliminated fast in rats; the total body clearance was 121 mL/min/kg. Eupatilin was mainly metabolized in rats; the percentage of intravenous dose of eupatilin excreted in 24 h urine and feces as unchanged eupatilin was only 2.5 and 0.919%, respectively. Eupatilin was mainly metabolized to form its glucuronide conjugate; after intravenous administration, 15.9 and 51.7% of intravenous dose was excreted in 24 h urine and feces, respectively, as eupatilin plus its glucuronide. After oral administration, the absolute bioavailability was only 3.86% based on $AUC_{0\text{-}24\,h}$ of eupatilin plus its glucuronide. Approximately 68.5% of oral dose was not absorbed from the entire gastrointestinal tract. Therefore, it could be concluded that the superior effect of eupatilin in experimental animal models of gastric ulcer and inflammatory bowel disease after oral administration could be due to the local action of eupatilin. Further pharmacokinetic studies to elucidate the local action of eupatilin are required.

Keywords □ eupatilin, Stillen®, pharmacokinetics, glucuronide conjugation, biliary excretion, rat

Eupatilin (5,7-dihydroxy 3',4'-6-trimethoxyflavone) is a major active component of Stillen® (*Artemisia herba* extract) having a potent antigastritic effect (Oh, *et al.*, 1996). Its ED_{50} after oral administration to rats with gastric ulcer induced by HClethanol was 0.48 mg/kg (Oh, *et al.*, 1996). General pharmacology and toxicology of *Artemisia herba extract* have also been evaluated (Lee, *et al.*, 1996; Kim, *et al.*, 1996). The exact mechanism of its mucosal protective effect has not been fully elucidated, but stimulation of mucus and bicarbonate secretion, increase in mucosal prostaglandins and glutathione, and enhancement of mucosal blood flow rate were considered as its pharmacological action. (Oh, *et al.*, 1996; Oh, *et al.*, 1997). *Artemisia herba extract* also shows beneficial effects on experimental models of inflammatory bowel disease through decreasing oxidative stress and attenuating cytokines involved in colonic inflammation (Ahn, *et al.*, 1997). With successful results of doubleblinded comparative phase III clinical trial, Stillen® has been marketed as a potent antigastritic agent in Korea.

The purpose of this study is to report pharmacokinetic characteristics of eupatilin after intravenous and oral administrations at a dose of 30 mg/kg to rats. Due to the rapid disappearance of eupatilin from blood, 30 mg/kg was selected for effective HPLC quantitation.

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MATERIALS AND METHODS

Animals

Male SprageDawley rats (weighing 250-310 g, 8 weeks of age) were purchased from Charles River Company Korea (Biogenomics, Seoul, Korea). Animals were housed in an air conditioned room at a temperature of 23± 2°C, a relative humidity of 55± 10%, an illumination intensity of 150-300 lux, a frequency of air ventilation of 15-20 times/h, and 12 h illumination (07:00 -19:00). Food and water were supplied *ad libitum*. Rats were fasted for 18 h before experiments with the exception of free access to water.

Chemicals

Eupatilin was synthesized by Research Laboratory of Dong-A Pharmaceutical Company. Biochainin A (an internal standard of

HPLC assay) and β-glucuronidase (Helix pomatia, glucuronidase activity of 110,700 units/ml and sulfatase activity of 7,500 units/mL) were purchased from Sigma Chemical Company (St. Louis, MO). Other chemicals were of reagent grade or HPLC grade, and therefore, were used without further purification.

Intravenous and Oral Administration of Eupatilin to Rats

The femoral artery (for blood sampling) and the femoral vein (for drug administration only in the intravenous study) of each rat were cannulated with polyethylene tubing (SP-45, Natume, Japan) under light ether anesthesia. Eupatiln [eupatilin powder was dissolved in dimethylacetamide : PEG 600 (1 : 1, v/v)] at a dose of 30 mg/kg (n=6) was infused via the femoral vein over 1-min (total injection volume was 2 mL/kg). Approximately 0.25-mL aliquot of blood sample was collected via the femoral artery at 0 (to serve as a control), 1(at the end of infusion), 5, 15, 30, and 45 min, and 1, 1.5, 2, 4, 6, 8 and 24 h after the beginning of the infusion. After centrifugation of blood samples, a 0.1-mL aliquot of plasma sample was collected, and stored in a -20°C freezer until HPLC analysis of eupatilin. Urine (0-24 h) sample was also collected. After measuring the exact volume of urine sample, two 0.1-mL aliquot of urine sample were stored in the -20°C freezer until HPLC analysis of eupatilin. Feces (0-24 h) were again collected. After drying in the hood, the exact weight of feces was measured. Each feces was homogenized with 20-volume of methanol and two 0.1-mL aliquots of the supernatant were collected and stored in a 20°C freezer until HPLC analysis of eupatilin. At the end of experiment (24 h), each rat was sacrificed by cervical dislocation and the abdomen was opened. The entire gastrointestinal (GI) tract (including its contents and feces) was removed, transferred into a beaker containing 50 mL of methanol (to facilitate the extraction of eupatilin), and cut into small pieces using scissors. After stirring with a glass rod, two 0.1-mL aliquots of the supernatant were collected from each beaker and stored in a -20°C freezer until HPLC analysis of eupatilin.

Eupatilin (eupatilin powder was suspended in 0.5% methylcellulose) at a dose of 30 mg/kg (n=6) was administered orally (total oral volume was 5 mL/kg) using a feeding tubing after 18 h overnight fasting. Blood samples were collected at 0, 5, 15, 30, and 45 min, and 1, 1.5, 2, 4, 6, 8 and 24 h. Other procedures were similar to those in the intravenous study.

Biliary Excretion after Intravenous Administration of Eupatilin to Rats.

The femoral vein (for drug administration) was cannulated with polyethylene tubing (SP-45, Natume) and the bile duct was also cannulated with polyethylene tubing (SP-10, Natume) under light ether anesthesia. Eupatilin (the same solution as used in the intravenous study) at a dose of 30 mg/kg (n=4) was injected via the femoral vein over 1-min (total injection volume was 2 mL/kg). Bile samples were collected between -30-0 (to serve as a control), 0-30 min, 30 min-1 h, 1-1.5 h , 1.5-2 h, 2-4 h, 4-6 h, 6-8 h, and 8-24 h. After measuring the exact volume of each bile sample, two 0.1-mL aliquots of bile sample were stored in a -20°C freezer until HPLC analysis of eupatilin.

HPLC Analysis of Eupatilin

The concentrations (or amount) of eupatilin in the above samples were analyzed by the slight modification of HPLC method developed from our laboratories (Kim, *et al*, 1997). To a 0.1-mL aliquot of biological sample, a 0.1-mL aliquot of 0.2 M $Ba(OH)_2$, a 0.1-mL aliquot of 0.15 M $ZnSO_4$, and a 0.1-mL aliquot of acetonitrile containing 10 μg/mL of biochainin A (an internal standard) were added. After vortex-centrifugation at 10,000 rpm for 3 min, a 0.1-mL aliquot of the supernatant was injected directly onto a reversed-phase column (particle size, 5 μm; 4.6 mm, i.d. ×150 mm, *l*; Inertsil ODS-2, GL Science Inc., Japan). The mobile phase, 1% ammonium acetate buffer (0.5% acetic acid) : acetonitrile (58 : 42, v/v), was run at a flow rate of 1.5 mL/min and column effluent was monitored by a UV detector set at 340 nm at room temperature. The retention times of eupatilin and the internal standard (biochainin A) were approximately 7.2 and 12.0 min, respectively. The quantitation limit of eupatilin was 10 ng/mL.

The concentrations (or amount) of eupatilin plus its glucuronide in the biological samples were also analyzed. Incubation with β-glucuronidase are as follows. To a 0.1-mL aliquot of biological sample, a 0.1-mL aliquot of 1 M sodium acetate buffer (pH 5.6), and a 0.02-mL aliquot of β-glucuronidase were added. After 2 h of incubation at 37°C, a 0.1-mL aliquot of the internal standard (biochainin A, 1 μg/mL in ethanol) and a 1.5-mL aliquot of diethyl ether were added. After vortex-centrifugation at 12,000 rpm for 3 min, the supernatant was collected, and dried under gentle stream of nitrogen gas. A 0.13-mL aliquot of the mobile phase was added to reconstitute to the residue and a 0.1-mL aliquot was injected directly onto a reversed-phase column.

Pharmacokinetic Analysis

The area under the plasma concentrationtime curve of eupatilin from time zero to time infinity ($AUC_{0-\infty}$) or up to the last

measured time in plasma, at 24 h ($AUC_{0\text{-}24\,h}$) was calculated by the trapezoidal rule-extrapolation method. This method employed the logarithmic trapezoidal rule recommended by Chiou (1978) for the calculation of area during the declining plasma-level phase and the linear trapezoidal rule for calculation of area during the rising plasma-level phase. The area from the last datum point to time infinity (for the calculation of $AUC_{0\text{-}\infty}$) was estimated by dividing the last measured plasma concentration the by terminal rate constant. The time-averaged total body (CL), renal (CL_R), and nonrenal (CL_{NR}) clearances, first moment of AUC (AUMC), mean residence time (MRT), apparent volume of distribution at steady state (Vd_{ss}), and extent of absolute oral bioavailability (F) were calculated by the standard methods (Gibaldi and Perrier, 1982).

$$CL = Dose / AUC$$

$$CL_R = Ae_{0\text{-}24\,h} / AUC$$

$$CL_{NR} = CL - CL_R$$

$$AUMC = \int_0^\infty t \times C_p dt$$

$$MRT = AUMC / AUC$$

$$Vd_{ss} = CL \times MRT$$

$$F = AUC_{oral} / AUC_{iv}$$

when Cp is the plasma concentration of eupatilin at time t and $Ae_{0\text{-}24\,h}$ is total amount of unchanged eupatilin excreted in 24-h urine.

The harmonic mean method was employed for the calculation of the mean values of terminal half-life (Eatman, Colburn *et al.*, 1977), each clearance (Chiou, 1980), and Vd_{ss} (Chiou, 1979).

Statistical Analysis

A p value of less than 0.05 was considerable to be statistically significant using an unpaired t-test.

RESULTS

Pharmacokinetics of Eupatilin after Intravenous Administration to Rats

The mean arterial plasma concentration-time profiles of unchanged eupatilin and eupatilin plus its glucuronide after intravenous administration of eupatilin at a dose of 30 mg/kg to rats are shown in Fig. 1; the relevant pharmacokinetic parameters were listed in Table I. The plasma concentrations of unchanged

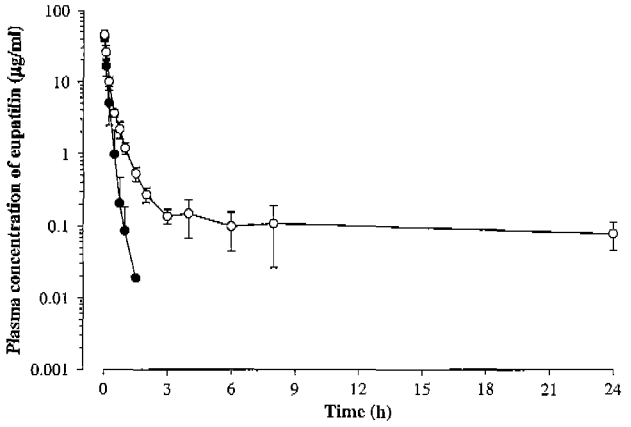

Fig. 1. Mean plasma concentrationtime profiles of unchanged eupatilin (●) and eupatilin plus its glucuronide (○) after intravenous administration of eupatilin at a dose of 30 mg/kg to rats ($n = 3$). Bar represents standard deviation.

Table I. Mean (± standard deviation) pharmacokinetic parameters of unchanged eupatilin and eupatilin plus its glucuronide after intravenous administration of the drug at a dose of 30 mg/kg to rats ($n = 3$ or 6).

Parameter	Unchanged eupatilin	Eupatilin plus its glucuronide
Terminal half-life (h)	0.101 ± 0.035	22.0 ± 13.1
$AUC_{0\text{-}24\,h}$ (μg h/ml)	4.52 ± 1.67	11.0 ± 1.96
$AUC_{0\text{-}\infty}$ (μg h/ml)	4.52 ± 1.67	14.5 ± 5.64
CL (ml/min/kg)	121 ± 42	N.C.
Vd_{ss} (l/kg)	0.894 ± 0.079	N.C.
CL_R (ml/min/kg)	4.35 ± 3.18	6.77 ± 2.18
CL_{NR} (ml/min/kg)	116 ± 38.2	N.C.
$Ae_{0\text{-}24\,h}$ (% of dose)	2.50 ± 1.61	15.9 ± 3.17
$Feces_{0\text{-}24\,h}$ (% of dose)	0.919 ± 2.02	0.871 ± 1.88
$GI_{24\,h}$ (% of dose)	12.1 ± 10.3	51.7 ± 24.2

*$p < 0.05$.
N.C.: Not Calculable.

eupatilin declined rapidly with a mean terminal half-life of 0.101 h (6 min). The CL, CL_R, CL_{NR}, and Vd_{ss} of unchanged eupatilin were 121 mL/min/kg, 4.35 mL/min/kg, 116 mL/min/kg, and 0.894 L/kg. The percentage of intravenous dose of eupatilin excreted in 24-h urine ($Ae_{0\text{-}24\,h}$) and feces ($Feces_{0\text{-}24\,h}$) and recovered from the entire GI tract at 24 h ($GI_{24\,h}$) were 2.50, 0.919, and 12.1%, respectively. The plasma concentrations of eupatilin plus its glucuronide were higher and prolonged than those of unchanged eupatilin. This resulted in a significantly greater $AUC_{0\text{-}\infty}$ (221% increase) and $AUC_{0\text{-}24\,h}$ (143% increase) and significantly longer terminal half-life (217 times increase) of eupatilin plus its glucuronide than those of unchanged eupatilin. The percentage of intravenous dose of eupatilin excreted

in 24 h urine (536% increase) and recovered from the entire GI tract at 24 h (327% increase) as eupatilin plus its glucuronide were greater than those of unchanged eupatilin. However, $Feces_{0\text{-}24\,h}$ (% of dose excreted in 24 hr feces) was comparable between unchanged eupatilin and eupatilin plus its glucuronide. The above data suggested that considerable amount of eupatilin are circulated in rat blood as its glucuronide.

Pharmacokinetics of Eupatilin after Oral Administration to Rats

After oral administration of eupatilin at a dose of 30 mg/kg to rats, the mean arterial plasma concentration-time profile of eupatilin plus its glucuronide is shown Fig. 2; the relevant pharmacokinetic parameters are listed in Table 2. Unchanged eupatilin was not detected (below detection limit) in plasma (quantitation limit of 10 ng/mL in plasma). The percentage of

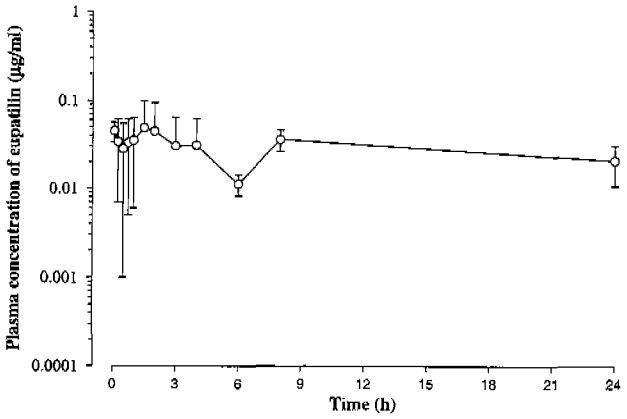

Fig. 2. Mean plasma concentrationtime profiles of unchanged eupatilin (●) and eupatilin plus its glucuronide (○) after oral administration of eupatilin at a dose of 30 mg/kg to rats ($n=3$). Bar represents standard deviation.

Table II. Mean (± standard deviation) pharmacokinetic parameters of unchanged eupatilin and eupatilin plus its glucuronide after oral administration of the drug at a dose of 30 mg/kg to rats ($n=3$ or 6).

Parameter	Unchanged eupatilin	Eupatilin plus its glucuronide
Terminal half-life (h)	N.C.	N.C.
$AUC_{0\text{-}24\,h}$ (μg h/ml)	N.C	0.425± 0.436
C_{max} (μg/ml)	N.C	47.1± 20.3
T_{max} (h)	N.C	31.0± 11.7
CL_R (ml/min/kg)	N.C.	6.20± 4.24
F (%)	N.C	3.86%
$Ae_{0\text{-}24\,h}$ (% of dose)	0.173± 0.151	0.720± 0.368*
$Feces_{0\text{-}24\,h}$ (% of dose)	1.57± 3.41	1.51± 3.32
$GI_{24\,h}$ (% of dose)	68.5± 27.1	90.8± 32.9

*$p<0.05$.
N.C.: Not Calculable.

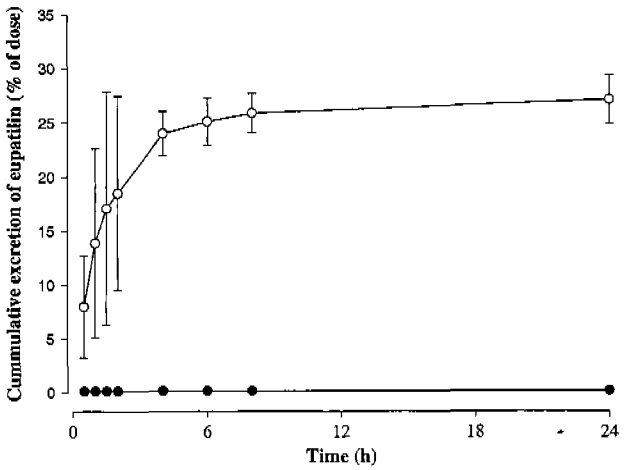

Fig. 3. Mean cumulative (% of dose) biliary excretion of unchanged eupatilin (●) and eupatilin plus its glucuronide (○) after intravenous administration of eupatilin at a dose of 30 mg/kg to rats ($n=4$). Bar represents standard deviation.

oral dose of eupatilin excreted in 24 h urine ($Ae_{0\text{-}24\,h}$) and feces ($Feces_{0\text{-}24\,h}$) and recovered from the entire GI tract at 24 h ($GI_{24\,h}$) as unchanged eupatilin was 0.173, 1.57, and 68.5%, respectively. But eupatilin plus its glucuronide was detected and retained with low level (30-40 ng/mL) in plasma. The F of unchanged eupatilin could not be calculated as mentioned earlier, however, the value was estimated based on $AUC_{0\text{-}24\,h}$ of eupatilin plus its glucuronide; the value was 3.86%. The percentage of oral dose of eupatilin excreted in 24 h urine and feces and recovered from the entire GI tract at 24 h as as eupatilin plus its glucuronide was 0.720, 1.51, and 90.8%, respectively.

Biliary Excretion of Eupatilin after Intravenous Administration to Rats

Biliary excretion of unchanged eupatilin and eupatilin plus its glucuronide after intravenous administration of eupatilin at a dose of 30 mg/kg to rats are shown in Fig. 3. The percentage of intravenous dose of eupatilin excreted in 24 bile as unchanged drug and eupatilin plus its glucuronide were 0.173± 0.143 and 27.2± 0.173%, respectively.

DISCUSSION

After both intravenous and oral administration of eupatilin to rats, most of the eupatilin were metabolized to its glucuronide (Tables I and II). This could be proven by following results. The percentage of dose of eupatilin recovered from the entire GI tract at 24 h as eupatilin plus its glucuronide were 4.27 and 1.36 times greater than those as unchanged eupatilin after

intravenous (Table I) and oral (Table II) administration, respectively. The corresponding values excreted in 24 h urine were 6.36 (Table I) and 4.16 (Table II) times. After intravenous administration, the 24 h biliary excretion of eupatilin plus its glucuronide was 157 times greater than that of unchanged eupatilin (Fig. 3). The $Ae_{0-\infty}$ of eupatilin plus its glucuronide was significantly greater than that of unchanged eupatilin after intravenous (Table I) and oral (Table II) administration, but the corresponding values excreted in 24 h feces were very similar after both intravenous (Table I) and oral (Table II) administration. This suggested that the glucuronide of eupatilin excreted in urine could be mainly formed in the kidney. It has been reported that also glucuronide was formed in kidney and GI tract. And it was considered that conjugated eupatilin was not excreted in feces because of microflora having β-glucuronidase activity.

After intravenous administration of eupatilin, contribution of CL_R of eupatilin to CL of eupatilin was almost negligible; the value was only 3.60% and the percentage of intravenous dose of eupatilin excreted in 24 h urine as unchanged drug was only 2.50% (Table I). This indicated that the CL of eupatilin listed in Table I could represent CL_{NR} of eupatilin in rats. The contribution of biliary (including gastrointestinal) excretion of unchanged eupatilin to CL_{NR} of eupatilin was also not considerable; the unchanged eupatilin recovered from the entire GI tract at 24 h after intravenous administration was 12.1% of intravenous dose (Table I) and only 0.173% of intravenous dose was excreted unchanged in 24 h bile as mentioned earlier. Hence, the CL_{NR} of eupatilin listed in Table 1 could represent metabolic clearance of eupatilin and eupatilin was metabolized almost completely after intravenous administration in rats.

The mean protein binding of eupatilin to fresh rat plasma (n = 3) at an eupatilin concentration of 10 μg/mL was 95.5± 3.50% using an ultrafiltration method. Considering the mean protein binding value and CL_R value of eupatilin (Table 1) in rats, the estimated renal clearance of eupatilin as free (unbound in plasma proteins) drug was 96.7 mL/min/kg. The value was extremely faster than the reported glomerular filtration rate in rats, 5.24 mL/min/kg (Davies and Morris, 1993) suggesting that renal secretion of eupatilin was considerable in rats. Considering the CL_R value of eupatilin (Table 1) and reported kidney blood flow rate of 36.8 mL/min/kg (Davies and Morris, 1993) and hematocrit of approximately 45% (Mitruka and Rawnsley, 1981) in rats, the estimated renal extraction ratio (CL_R of eupatilin/ kidney plasma flow rate, only for urinary excretion of unchanged eupatilin) was 21.5%. This indicates that eupatilin is a poor renal excretion ratio drug in rats.

After intravenous administration of eupatilin at a dose of 30 mg/kg to rats, the CL value (121 mL/min/kg based on plasma data, Table I) was smaller (approximately 74.3% of cardiac output) than the reported cardiac output in rats, 296 mL/min/kg based on blood data (Davies and Morris, 1993). This suggested that first-pass effect of eupatilin in the lung and heart could be almost negligible, if any, in rats. The CL_{NR} value of eupatilin (Table I) was approximately 4.6 times greater than the reported hepatic blood flow rate of 55.2 mL/min/kg in rats (Davies and Morris, 1993) considering approximately 45% of hematocrit (Mitruka and Rawnsley, 1981) in rats. This suggested that extrahepatic metabolism of eupatilin was considerable in rats. Eupatilin was mostly metabolized to its glucuronide as mentioned earlier (Table I and II). It has been reported that glucuronide are formed in GI tract and kidney.

After oral administration of eupatilin at a dose of 30 mg/kg to rats, unchanged eupatilin was not detected in plasma (Fig. 2). This could be mainly due to poor absorption of the drug. The F value based on $AUC_{0-24\,h}$ of eupatilin plus its conjugate was 3.86%. This could be proven by the following explanation. After oral administration of eupatilin at a dose of 30 mg/kg, the percentage of oral dose recovered from the entire GI tract at 24 h was 68.5% of oral dose (Table II). It is possible that this unchanged eupatilin, 68.5%, might be partly attributed to the biliary (including gastrointestinal) excretion of the absorbed drug. Based on the linear pharmacokinetics, the mean true fraction of dose unabsorbed (F_{unabs}) in this study may be estimated by the following equation (Lee and Chiou, 1983);

$$0.685 = F_{unabs} + (0.0386 \times 0.121)$$

where 0.0386 and 0.121 are the F (Table 2) and mean fraction of intravenous dose of eupatilin recovered from the entire GI tract at 24 h as unchanged eupatilin, respectively (Table 1). The calculated F_{unabs} was close to 68.0% indicating that the contribution of biliary (including gastrointestinal) excretion of the drug to the total drug recovered from the entire gastrointestinal tract following oral administration was almost negligible. Hence, 68.0% of orally administered eupatilin at a dose of 30 mg/kg was not absorbed from gastrointestinal tract. Therefore, it could be considered that the superior effect of eupatilin in experimental animal models of gastric ulcer and inflammatory bowel disease after oral administration could be due to the local action of eupatilin. Further pharmacokinetic studies of eupatilin to elucidate the local action of eupatilin are required.

In conclusion, a new antiulcer agent, eupatilin, an active components of Stillen®, was rapidly disappeared after intrave-

nous administration to rats. The oral absorption of eupatilin was extremely low; the *F* was only 3.86% and percentage of oral dose of eupatilin excreted in 24 h urine plus feces as unchanged eupatilin were less than 5% (Table II). Most of the eupatilin after oral administration were remained in gastrointestinal tract (65% of oral dose was not absorbed) and this could enhance the local action of eupatilin.

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