Pharmacokinetics and safety of JTE-522, a novel selective cyclooxygenase-2 inhibitor, in healthy male volunteers

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Aims The pharmacokinetics and safety profile of JTE-522, 4-(4-cyclohexyl-2-methyloxazol-5-yl)-2-fluorobenzenesulphonamide, a novel selective cyclooxygenase-2 inhibitor were investigated in healthy male volunteers.

Methods Initially, as a pilot study, five groups of two subjects were given oral doses of 3–100 mg of JTE-522. After safety assessment, subjects were given 150 and 200 mg of JTE-522. The effect of food-intake on the pharmacokinetics of JTE-522 at a dose of 150 mg was examined. In the multiple-dose study, subjects were given 150 mg of JTE-522 once a day for 7 days. Concentrations of unchanged JTE-522 in plasma, blood and urine were determined by high performance liquid chromatography (h.p.l.c.). Concentrations of metabolites were estimated with h.p.l.c. chromatograms and calibration curves for quantification of unchanged JTE-522.

Results In the course of this study, no serious abnormality attributable to the test drug was observed, suggesting that JTE-522 was well tolerated in healthy subjects. In a single-dose study, the concentrations of JTE-522 in blood were much higher than the corresponding concentrations in plasma. JTE-522 was readily distributed to blood cells and percentage distribution into blood cells was more than 99.0%. However, the values of $C_{\max}$ in blood at doses of 100, 150, 200 mg JTE-522 were 15241, 20445 $\pm$ 3918 (16333–24556), 20965 $\pm$ 3260 (17544–24386) ng ml⁻¹, respectively. These findings suggest that JTE-522 has a high affinity for blood cells and the distribution into blood cells is limited at the higher doses of over 100 mg. In a multiple dose study, pharmacokinetic parameters including $t_{1/2}$ and AUC after the fourth administration were comparable with that of the seventh administration. Thus, these findings suggest the absence of accumulation on the multiple-dosing of JTE-522.

Conclusions These results indicate that JTE-522 has an acceptable pharmacokinetic profile for clinical use without any serious adverse events as we verified in healthy young male volunteers.

Keywords: cyclooxygenase-2 inhibitor, JTE-522

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit the activity of cyclooxygenase (COX) and thus prostaglandin synthesis [1, 2]. Cyclooxygenase has two isoforms, constitutive COX-1, which is present in many tissues,

particularly the stomach, kidneys, and platelets and stimulates the physiological generation of prostaglandins, and inducible COX-2, which is expressed in response to an inflammatory stimulus [3–6]. Agents that selectively inhibit COX-2 have the theoretical advantage that they should be potent inhibitors of the inflammatory response but have a low potential for renal and gastric adverse effects [2, 7]. Thus, selective COX-2 inhibitors have been developed [8–11].

JTE-522, 4-(4-cyclohexyl-2-methyloxazol-5-yl)-2-fluorobenzenesulphonamide, is a recently developed orally active selective COX-2 inhibitor, with an IC_{50} of 0.085 μ M (Figure 1) [12]. This compound is highly stable

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Figure 1 Chemical structures of JTE-522 and its metabolites.

and well absorbed after oral administration to rats. JTE-522 is metabolized to M-II and M-IIIb (Figure 1), which have IC_{50} s for COX-2 of 3 and more than 100 μ M, respectively. The safety of JTE-522 has been established at doses up to 3 mg kg⁻¹ in repeated dosing toxicity studies including the repeated reproduction toxicity studies. Furthermore, its anti-inflammatory, analgesic, antipyretic, and bone destruction-inhibitory effects were found to be potent in various acute or chronic inflammation models, and its ulcerogenic effect was negligible.

In this study, the pharmacokinetics and safety of JTE-522 in healthy Japanese male volunteers after single and multiple oral doses have been investigated.

Methods

Chemicals

JTE-522 was supplied by Japan Tobacco Inc., as uncoated tablets.

Subjects

The study protocol was approved by the Institutional Review Board of Shinpukai Maruyama Hospital on Eth-

Table 1 The study design.

Study	Dosing condition	Step	Dose (mg)	Number of subjects
Single dose (Preliminary)	In fasting state	1	3	2
		1b	3	2
		2	10	2
		3	30	2
		4	100	2
Single dose (Main)	In fasting state	5	150	8*
		6	200	8*
	Post-prandial	7	150	8*
Multiple dose	Post-prandial	8	150	8*

*Including 2 placebo-treated subjects.

ics of human experimentation. Forty-two healthy male Japanese volunteers gave written informed consent to participate in the study after being fully informed of the purpose and risks involved. The subjects were aged 24–44 years and weighed between 53.3 and 85.6 kg. They were judged to be fit for the study based on a physical examination and standard biochemical, haematological,

and urinalysis screening tests. All subjects were free of other medications for at least 1 week prior and during the study. Alcohol and caffeine-containing beverages were not allowed during the study.

Study design

Based on the principles of 'less than 1/60th' of no toxic effective dose which is 3 mg kg^{-1} in the rat during a 3 month repeated oral toxicity study, an initial JTE 522 dose of 3 mg was chosen (Table 1).

The drug was taken at 09.00 h after 14 h supervised fast. In the first study (preliminary single dose), five groups of two subjects were given single 3, 3, 10, 30 and 100 mg doses. In the second study (main single dose), there were three groups of eight subjects, six of whom were given active treatment and two placebo. The treatments for the three groups were 150 mg fasting, 150 mg postprandial and 200 mg fasting all given as a single dose. The third study (multiple dose) involved eight subjects six of whom were given active treatment and two placebo. Subjects were given 150 mg once daily at 09.00 h. In step 7 (see Table 1) and the multiple dose study, drug was administered 30 min after breakfast. In step 7 and on days 1, 4, and 7 of the multiple-dose study, breakfast consisted of two pieces of bread, one piece of margarine, one piece of cheese, one boiled egg, 100 ml of orange juice and 150 ml of milk. On the other days in the multiple-dose study, a normal Japanese breakfast was served.

Blood samples were collected via venepuncture into heparinized vacutainers. In the single-dose study, blood samples were collected before dosing and at the following times after dosing: 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48 and 192 h in step 1; 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48 and 168 h in step 1b, 2, 3 and 4; 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 168 and 336 h in steps 5, 6 and 7. Urine samples were collected before dosing and at 0–4, 4–8, 8–12, 12–24, and 24–48 h after dosing. In the multiple-dose study, blood samples were collected at the following times: before dosing and 1, 2, 4, 8, 12 h after dosing on days 1 and 4; immediately before dosing and 1, 2, 4, 8, 12, 24, 168 and 336 h after final dosing on day 7. On days 2, 3, 5, and 6, blood samples were also collected immediately before dosing and 4 h after dosing. Urine samples were collected before the first dose and at 0–4, 4–8, 8–12, 12–24, 24–48, 144–168, and 312–336 h after dosing on day 1 and at 0–12 and 12–24 h after dosing on days 2, 3, 4, 5 and 6. Samples were stored at -30°C until analysis. It has been established previously that the blood-to-plasma concentration ratio of JTE-522 does not depend on temperature.

JTE-522 in plasma, urine and blood was quantified with high performance liquid chromatography (h.p.l.c.).

The parent drug and metabolites were separated on a C18 analytical column, YMC-Pack ODS AM-302 (YMC, Kyoto, Japan) using acetonitrile/water (40/60) as mobile phase at a flow rate of 1.0 ml min^{-1} . The limits of quantification were 1.0 ng ml^{-1} for plasma, and 10 ng ml^{-1} for urine and blood. Detection of the parent drug and metabolites was performed using a fluorescence-spectrophotometer with an excitation wavelength of 288 nm and an emission wavelength of 368 nm.

JTE-522 was extracted from plasma (200 μl) with 5 ml of diethyl ether after addition of 10 μl of 40% acetonitrile/water, 10 μl of internal standard solution (JTE-503 $1.0 \mu\text{g ml}^{-1}$, 40% acetonitrile/water solution), and 1.0 ml of 0.1 M K_2HPO_4 solution. After evaporation of 4 ml of the organic layer, the residue was dissolved in 200 μl of acetonitrile and 300 μl of water. A 50 μl aliquot of the solution was injected onto the h.p.l.c. The coefficients of variation (CV) for the intra- and interday assay were 3.3–5.3% and 1.5–6.0% at relevant concentrations, respectively. The accuracy was ± 0.0 – $+4.0\%$.

JTE-522 was extracted from urine (200 μl) with 5 ml of diethyl ether after addition of 10 μl of 40% acetonitrile/water, 10 μl of internal standard solution (JTE-503 $1.0 \mu\text{g ml}^{-1}$, 40% acetonitrile/water solution), and 1.0 ml of 0.1 M K_2HPO_4 solution. After evaporation of 4 ml of the organic layer, the residue was dissolved in 400 μl of acetonitrile and 600 μl of water. A 15 μl aliquot of the solution was injected onto the h.p.l.c. The CVs for the intra- and interday assay were 2.6–4.6% and 0.0–7.8% at relevant concentrations, respectively. The accuracy was -1.0 – $+1.5\%$.

JTE-522 was extracted from blood (200 μl) with 900 μl of methanol after the addition of 10 μl of 40% acetonitrile/water, 10 μl of internal standard solution (JTE-503 $1.0 \mu\text{g ml}^{-1}$, 40% acetonitrile/water solution). After evaporation of 0.2 ml of the supernatant, the residue was dissolved in 200 μl of 40% acetonitrile/water. A 50 μl aliquot of the solution was injected onto the h.p.l.c. The CVs of the intra- and interday assay were 2.0–3.4% and 1.5–10.0% at relevant concentrations, respectively. The accuracy was -2.6 – $+3.4\%$.

Metabolites, M-II and M-III in plasma, urine and blood were estimated by monitoring their chromatographic peaks during the analysis of unchanged JTE-522.

Peak assignment of metabolites was performed using retention time. M-IIIa (*cis*-4'-ol isomer), M-IIIb (*trans*-4'-ol isomer) and M-IIIc (3'-ol isomer) were identified as M-III, because those isomers cannot be separated from each other under the analytical conditions used for the quantification of unchanged JTE-522. The concentrations of metabolites were estimated from the calibration equations for unchanged JTE-522 using the internal standard method, assuming that the detector response for each metabolite was the same as unchanged JTE-522.

In the single-dose study, subjective symptoms and objective and vital signs including blood pressure, heart rate and body temperature were measured before and periodically up to 48 h after drug administration. Routine laboratory tests including haematology, blood biochemistry and urinalysis were performed before and 22 h after administration and a 12-lead electrocardiogram was recorded before and at 1 and 22 h after drug administration. Faecal occult blood was tested for within 3 days before administration and 22 h after administration and thereafter. In steps 1b to 4, capillary vessel resistance was measured before and 2, 22 and 48 h after administration, and bleeding time, platelet aggregation test, coagulation and fibrinolysis were measured—before and 2 and 22 h after administration. In steps 2–4, the erythrocyte deformability test was performed before and 2, 22 and 48 h after administration. In steps 2–7, erythrocyte osmotic pressure resistance was measured before and 2, 22 and 48 h after administration.

In the multiple dose study, subjective symptoms and objective and vital signs including blood pressure, heart rate and body temperature were examined before and periodically up to 48 h after the final drug administration. Routine laboratory tests including haematology, blood biochemistry and urinalysis were performed before the first administration, 2 h before administration on day 4 and 46 h after the final administration (day 9) and a 12-lead electrocardiogram was recorded before and 4 h after the first administration, 4 h after administration on days 2–7, and 23 and 46 h after the final administration. Intraocular pressure was determined on the day before the first administration, 5 h after administration on day 3, and 5 h after administration on day 7. Creatinine clearance and urinary electrolytes (Na^+ , K^+ and Cl^-) were measured 24–0 h before the first administration and 0–24 h after administration on days 4 and 7. Faecal occult blood was tested for within 3 days before administration, from day 4 (after administration) until day 7 (before administration), and after the final administration. An exercise tolerance test was performed on the day before the first administration and 5 h after administration on day 5. If any abnormality was observed in these tests, the subject was followed up until resolution.

In the single and the multiple dose studies, a medical examination was performed 7 and 14 days after the last drug administration in most cases. Parameters measured were subjective symptoms, objective findings, blood pressure, heart rate, body temperature, standard 12-lead electrocardiography, and laboratory tests.

Pharmacokinetic analysis

Pharmacokinetic parameters were assessed by using model-independent analysis. The peak plasma, blood and

blood cell level values (C_{max}) and the corresponding time (t_{max}) were observed experimental values. In order to calculate the concentration in blood cells, the haematocrit values estimated from one sample were used.

Half-lives were calculated using the elimination rate constant (K_e) determined from the slope of mono-exponential decline in the logarithm of concentration vs time profile. When biexponential decline was observed, half-lives in each phase were calculated. $\text{AUC}(0,24 \text{ h})$ was calculated from time 0–24 h by the trapezoidal rule. $\text{AUC}(0,\infty)$ was calculated from the following equation:

$$\text{AUC}(0,\infty) = \text{AUC}(0,t) + C_t/K_e$$

where $\text{AUC}(0,t)$ is the area to the last measurable time point, t , and C_t is the concentration at the last measurable time point.

Results

Tolerability

In the single dose study at a dose of 3 mg, a yellowish spot was found on the skin surface of the right upper arm of one subject 8 days after administration of JTE-522 and a subcutaneous haemorrhagic spot and a yellowish spot on the skin surface of right upper arm were found in another subject 8 days after administration. No other subjects showed dermatological signs. There were no abnormal findings in any of the haematological tests, suggesting that JTE-522 has no deleterious effect on the microvascular bed or on erythrocyte formation. In the 150 mg single dose study, one subject complained of muscle pain 29 days after drug administration. In the 200 mg single dose study, one subject had epigastric pain for 2 h, 55 h after administration. This subject had a positive response to the faecal occult blood test on the day after administration, although the test results were negative before administration, and 2, 3 and 5 days after administration. Thus, the upper gastrointestinal tract of this subject was examined endoscopically 7 days after administration, but an ulcer or an erosion in the gastric or duodenal wall was not observed. In the 100 mg single dose study, one subject felt a slight dull headache and in the postprandial 150 mg single dose study, one subject had a slight headache, diarrhoea and abdominal pain at defaecation. In the multiple dose study, two subjects had a mild headache and one subject had a mild dull headache. Three subjects including one placebo treated subject had transient soft stool after breakfast. In the multiple dose study, intraocular pressure and urine pH were determined, and an exercise tolerance test was performed,

Figure 2 a) Plasma, b) blood and c) blood cell concentrations *vs* time of JTE-522 after single oral administration. Data represent the means $\pm$ s.d. ($n = 6$). ■ step 5 (150 mg), ▲ step 6 (200 mg), □ step 7 (150 mg after meal).

none of which gave abnormal findings. There were no other abnormal findings in objective symptoms and laboratory tests including blood pressure, heart rate, electrocardiogram, body temperature, haematology, blood chemistry and urinalysis. All adverse events observed in this study were classified as of no causality.

Single-dose pharmacokinetics

The changes in mean plasma, blood and blood cell concentrations of JTE-522 with time are shown in Figure 2. The pharmacokinetic parameters in plasma, blood and blood cell are shown in Tables 2 and 3. The distribution of JTE-522 into blood cells is shown in Figure 3.

Figure 3 Distribution of unchanged drug *vs* time after single oral administration of JTE 522. Data represent the means $\pm$ s.d. ($n = 6$). ■ step 5 (150 mg), ▲ step 6 (200 mg), □ step 7 (150 mg after meal).

The concentration of unchanged drug in plasma at doses of 30 mg or less reached a peak ($C_{\max}$) at 2.5–6 h, and then declined with a $t_{1/2}$ of 45–75 h, whereas those at doses of 100 mg or higher reached $C_{\max}$ 4.0–4.7 h after dosing, and then followed a biphasic decline with a $t_{1/2, \lambda_1}$ of 11–14 h and a $t_{1/2, \lambda_2}$ of 32–39 h. The values of $C_{\max}$ and $AUC(0, \infty)$ in plasma increased proportionally with increasing doses up to 30 mg. At doses of 100–200 mg, the normalized values of $C_{\max}/\text{dose}$ in plasma increased with increasing dose, although marked increases were not observed in the normalized $AUC(0, \infty)/\text{dose}$ values. There were no appreciable differences in the $t_{\max}$ and $t_{1/2}$ (and $t_{1/2, \lambda_2}$) values between each dose.

In whole blood, the concentrations of JTE-522 were much higher than the corresponding values in plasma, and reached $C_{\max}$ 1.5–5 h after dosing. The values of $C_{\max}$ and $AUC(0, \infty)$ in blood increased proportionally with increasing doses up to 100 mg. However, over the dose of 100 mg, the increases of $C_{\max}$ in blood trend to being suppressed, in contrast to those in plasma.

$C_{\max}$ of unchanged drug in red cells occurred at 1.5–5 h after dosing and was 2.2–2.5 times higher than the corresponding concentrations in whole blood. $C_{\max}$ then declined at the almost same rate as that in whole blood. JTE-522 was 99% distributed to blood cells, at almost all points. However, at the dose of 150–200 mg, the extent of distribution into blood cells at the points near $t_{\max}$ was a little lower at 98.0–98.9%.

At the dose of 150 mg, there were no significant differences in the pharmacokinetic parameters between the fed and fasted conditions.

Following oral dosing of JTE-522, minimal unchanged drug was excreted into urine (0.02% or less of the dose within 48 h after dosing).

Table 2 Pharmacokinetic parameters after oral administration of JTE-522 over the dose range 3–100 mg.

Parameter	Pharmacokinetic parameters of unchanged drug					Pharmacokinetic parameters of M-III				
	Step1	Step1-b	Step2	Step3	Step4	Step1	Step1-b	Step2	Step3	Step4
<i>Plasma</i>										
Dose (mg)	3	3	10	30	100				30	100
$C_{\max}$ (ng ml ⁻¹)	1.2	1.3	4.9	18.5	115.7				5.1	53.7
$t_{\max}$ (h)	4	2.5	4	6	4				168	48
$t_{1/2}$ (h)	N.C.	N.C.	74.6	45.0	29.3				N.C.	192.9
AUC(0,∞) (μg ml ⁻¹ h)	N.C.	N.C.	0.52	1.22	5.85				N.C.	16.6
<i>Blood</i>										
Dose (mg)	3	3	10	30	100	3	3	10	30	100
$C_{\max}$ (ng ml ⁻¹)	466	555	1825	4985	15241	260	321	886	1951	8705
$t_{\max}$ (h)	5	3	4	1.5	3	192	168	168	48	48
$t_{1/2}$ (h)	68.4	87.3	77.8	45.2	37.8	418.6	441.6	371.8	316.8	411.4
AUC(0,∞) (μg ml ⁻¹ h)	49.6	69.5	216.5	372.5	871.3	192.3	252.4	675.6	1141.4	3648.3
<i>Blood cell</i>										
Dose (mg)	3	3	10	30	100					
$C_{\max}$ (ng ml ⁻¹)	1068	1205	4022	11036	34085					
$t_{\max}$ (h)	5	3	4	1.5	3					
$t_{1/2}$ (h)	68.0	84.6	78.5	46.1	38.6					
AUC(0,∞) (μg ml ⁻¹ h)	114.3	145.2	471.8	811.5	1985.8					

Data represent the mean values, $n = 2$. N.C. Not calculated. Half-life ($t_{1/2}$) was calculated from the time points $t_{\max}$ to the last points. AUC(0,∞) was calculated by the trapezoidal rule and estimation to infinity.

Figure 4 Plasma and blood concentrations *vs* time of unchanged drug and metabolites M-II, M-III after multiple oral administration of 150 mg JTE-522. Data represent the means $\pm$ s.d. ($n = 6$). $\circ$ JTE-522 in plasma, $\bullet$ JTE-522 in blood, $\square$ M-II in plasma, $\blacksquare$ M-II in blood, $\triangle$ M-III in plasma, $\blacktriangle$ M-III in blood.

Multiple-dose pharmacokinetics

Figure 4 shows the mean plasma and blood concentration-time profile obtained from each subject after oral multiple administration of JTE-522. The resulting pharmacokinetic parameters are shown in Table 4. Plasma

concentration of unchanged drug reached a steady state after the second dose and remained constant thereafter. The pharmacokinetic parameters after the fourth administration were comparable with those after the seventh administration. Plasma half-lives ($t_{1/2}$) were about 5 h and AUC(0,24 h) about 8000 ng ml⁻¹ h after both the fourth and seventh doses.

Blood concentration of JTE-522 reached 20 μg ml⁻¹ at 4 h after the first administration. Thereafter C_{4h} in blood declined, and concentrations reached a steady state after the third dose and remained constant. The pharmacokinetic parameters after the fourth administration were comparable with those after the seventh dose. Blood half-lives ($t_{1/2}$) were about 12.5 h and AUC(0,24 h) about 130 μg ml⁻¹ h after both the fourth and seventh dose.

Following oral multiple-dosing of JTE-522, minimal unchanged drug was excreted into urine (0.02% 48 h after the last dose). The drug was not detected in urine at 1 and 2 weeks after the last dose.

Pharmacokinetics of the metabolites of JTE-522

Figure 5 shows the mean plasma and blood concentrations of M-III obtained after oral single administration of JTE-522 and its pharmacokinetic parameters are shown in Tables 2 and 3. The concentrations of M-III in whole-blood were much higher than those in plasma, showing that M-III was distributed into blood-cells. After doses

Table 3 Pharmacokinetic parameters after oral administration of JTE-522 at doses of 150 and 200 mg.

Parameter	Pharmacokinetic parameters of unchanged drug			Pharmacokinetic parameters of M-III		
	Step 5	Step 6	Step 7	Step 5	Step 6	Step 7
<i>Plasma</i>						
Dose (mg)	150 (fast)	200 (fast)	150 (nonfast)	150 (fast)	200 (fast)	150 (nonfast)
$C_{\max}$ (ng mL ⁻¹)	325.0 ± 124.2 (194.7, 455.4)	654.3 ± 248.1 (393.9, 914.7)	370.9 ± 144.1 (219.6, 522.1)	103.5 ± 53.3 (47.6, 159.3)	220.4 ± 52.6 (165.3, 275.6)	121.8 ± 35.4 (84.6, 159.0)
$t_{\max}$ (h)	4.0 ± 0.0 (4.0, 4.0)	4.3 ± 2.0 (2.3, 6.4)	4.7 ± 1.6 (3.0, 6.4)	22.0 ± 4.9 (16.9, 27.1)	16.0 ± 6.2 (9.5, 22.5)	26.0 ± 11.8 (13.6, 38.4)
$t_{1/2,\lambda 1}$ (h)	13.6 ± 3.1 (10.3, 16.8)	10.8 ± 3.1 (7.6, 14.0)	12.2 ± 3.2 (8.9, 15.6)			
$t_{1/2,\lambda 2}$ (h)	33.2 ± 4.5 (27.7, 38.8)	38.9 ± 15.8 (19.4, 58.5)	31.8 ± 3.6 (28.0, 35.6)			
$t_{1/2}$ (h)				169.2 ± 59.4 (106.9, 231.6)	137.1 ± 13.4 (123.1, 151.2)	137.4 ± 63.0 (71.4, 203.5)
AUC (0,∞) (µg mL ⁻¹ h)	7.83 ± 0.88 (6.91, 8.76)	14.36 ± 6.51 (7.53, 21.19)	8.25 ± 2.13 (6.01, 10.48)	17.3 ± 3.2 (14.0, 20.7)	23.1 ± 3.9 (19.0, 27.1)	17.6 ± 4.1 (13.3, 21.9)
<i>Blood</i>						
Dose (mg)	150 (fast)	200 (fast)	150 (nonfast)	150 (fast)	200 (fast)	150 (nonfast)
$C_{\max}$ (ng mL ⁻¹)	20445 ± 3918 (16333, 24556)	20965 ± 3260 (17544, 24386)	19659 ± 2465 (17072, 22246)	10517 ± 1255 (9200, 11834)	12767 ± 2907 (9716, 15817)	13151 ± 1445 (11634, 14668)
$t_{\max}$ (h)	2.3 ± 1.4 (0.9, 3.8)	3.5 ± 2.5 (0.9, 6.1)	3.3 ± 1.0 (2.2, 4.4)	44.0 ± 9.8 (33.7, 54.3)	32.0 ± 12.4 (19.0, 45.0)	44.0 ± 9.8 (33.7, 54.3)
$t_{1/2}$ (h)	40.2 ± 10.3 (29.4, 51.0)	44.5 ± 17.3 (26.4, 62.6)	41.1 ± 3.4 (37.5, 44.7)	321.7 ± 91.2 (226.0, 417.5)	328.7 ± 76.4 (248.5, 408.8)	310.5 ± 57.8 (249.9, 371.2)
AUC (0,∞) (µg mL ⁻¹ h)	925.3 ± 315.3 (594.5, 1256.2)	927.0 ± 449.0 (455.8, 1398.2)	816.1 ± 179.8 (627.4, 1004.7)	3158.1 ± 475.7 (2658.8, 3657.3)	3530.8 ± 658.8 (2839.5, 4222.2)	3713.7 ± 345.3 (3351.3, 4076.1)
<i>Blood cells</i>						
Dose (mg)	150 (fast)	200 (fast)	150 (nonfast)	150 (fast)	200 (fast)	150 (nonfast)
$C_{\max}$ (ng mL ⁻¹)	47932 ± 10284 (37139, 58724)	52105 ± 7088 (44667, 59543)	45482 ± 5534 (39675, 51290)	24317 ± 3648 (20488, 28145)	31794 ± 6547 (24923, 38665)	30289 ± 3735 (26370, 34209)
$t_{\max}$ (h)	2.3 ± 1.4 (0.9, 3.8)	3.3 ± 2.7 (0.5, 6.1)	3.0 ± 1.1 (1.9, 4.1)	44.0 ± 9.8 (33.7, 54.3)	32.0 ± 12.4 (19.0, 45.0)	44.0 ± 9.8 (33.7, 54.3)
$t_{1/2}$ (h)	40.3 ± 10.0 (29.8, 50.8)	44.6 ± 17.2 (26.5, 62.6)	41.5 ± 3.5 (37.8, 45.2)	317.7 ± 92.7 (220.4, 414.9)	330.0 ± 75.8 (250.4, 409.5)	312.3 ± 58.6 (250.7, 373.8)
AUC (0,∞) (µg mL ⁻¹ h)	2151.2 ± 745.6 (1368.7, 2933.7)	2318.3 ± 1164.0 (1096.8, 3539.8)	1892.2 ± 409.2 (1462.8, 2321.7)	7112.5 ± 1169.3 (5885.4, 8339.6)	8840.1 ± 1542.6 (7221.3, 10459.0)	8830.5 ± 1000.9 (7780.1, 9880.8)

Data represent as the mean ± s.d., $n = 6$ Figures in the parentheses represent the 95% confidence interval. $t_{1/2,\lambda 1}$ was calculated from the time point $t_{\max}$ to 48 h. $t_{1/2,\lambda 2}$ was calculated from 48 h to the last measured point. $t_{1/2}$ was calculated from the time point $t_{\max}$ to the last measured point. AUC (0,∞) was calculated by the trapezoidal rule and estimation to infinity.

of 3 and 10 mg, the concentrations in blood reached a peak at 7 or 8 days ($t_{\max}$) and M-III was eliminated with the half-lives ($t_{1/2}$) of 15–18 days. After doses of 30–200 mg, blood concentrations of M-III peaked at 1.3–2 days and then declined with a $t_{1/2}$ of 13–17 days. $C_{\max}$ and AUC(0,∞) increased with increasing doses up to 100 mg. At 100–200 mg, there were no significant changes in $C_{\max}$ and AUC(0,∞) in blood. At each dose, the concentrations of M-II in plasma and blood were much lower than those of M-III.

The mean plasma and blood concentrations of JTE-522, M-II and M-III after oral multiple administration are shown in Figure 4, and its pharmacokinetic parameters

are shown in Table 5. The C_{4h} and AUC(0,24 h) of M-III in plasma increased during repeated medication, although trough concentrations reached steady state after the fourth administration. The trough concentrations of M-III in blood reached steady state after the second dose, and the C_{4h} concentrations between the 3rd to 7th dose were constant.

Discussion

In the course of this study, no serious adverse effects attributable to the test drug were observed with respect to subjective symptoms, objective and vital signs, and

Table 4 Pharmacokinetic parameters of unchanged drug after oral multiple administration of JTE-522.

	C_{4h} (ng mL ⁻¹)	C_{24h} (ng mL ⁻¹)	$t_{1/2}$ (h)	AUC (μg mL ⁻¹ h)
Plasma				
Day 1	334.2 ± 83.1 (247.0, 421.4)	93.0 ± 10.9 (81.6, 104.5)	11.09 ± 3.17# (7.76, 14.42)	4.5 ± 0.8* (3.7, 5.3)
Day 2	947.0 ± 261.3 (672.7, 1221.2)	78.4 ± 18.4 (59.1, 97.7)		
Day 3	787.1 ± 260.0 (514.3, 1060.0)	67.4 ± 20.5 (45.9, 88.9)		
Day 4	903.8 ± 131.5 (765.9, 1041.8)	65.8 ± 16.8 (48.2, 83.4)	5.43 ± 0.64# (4.75, 6.10)	8.0 ± 1.6* (6.3, 9.7)
Day 5	675.8 ± 314.8 (345.4, 1006.2)	71.6 ± 16.6 (54.2, 89.0)		
Day 6	793.0 ± 237.1 (544.2, 1041.8)	69.7 ± 12.4 (56.7, 82.7)		
Day 7	1045.9 ± 193.9 (842.4, 1249.3)	60.9 ± 16.4 (43.6, 78.1)	5.08 ± 0.73# (4.31, 5.84)	8.5 ± 1.4* (7.1, 9.9) 9.4 ± 1.5** (7.9, 10.9)
	C_{4h} (μg mL ⁻¹)	C_{24h} (μg mL ⁻¹)	$t_{1/2}$ (h)	AUC (μg mL ⁻¹ h)
Blood				
Day 1	20.0 ± 2.2 (17.6, 22.3)	8.2 ± 1.7 (6.4, 9.9)	16.14 ± 3.20# (12.78, 19.50)	305.6 ± 34.2* (269.7, 341.5)
Day 2	12.2 ± 2.0 (10.1, 14.3)	3.9 ± 1.0 (2.8, 4.9)		
Day 3	9.4 ± 1.4 (7.9, 10.8)	3.1 ± 0.9 (2.1, 4.1)		
Day 4	9.3 ± 1.2 (8.0, 10.6)	3.0 ± 0.9 (2.0, 4.0)	12.48 ± 2.27# (10.10, 14.86)	132.6 ± 25.5* (105.8, 159.4)
Day 5	8.7 ± 1.5 (7.2, 10.3)	3.2 ± 1.0 (2.2, 4.3)		
Day 6	9.4 ± 2.1 (7.2, 11.5)	3.2 ± 1.0 (2.2, 4.2)		
Day 7	9.3 ± 2.0 (7.2, 11.4)	2.9 ± 1.0 (1.9, 3.9)	12.62 ± 2.93# (9.55, 15.70) 42.42 ± 8.49## (33.51, 51.34)	130.6 ± 26.3* (103.0, 158.2) 282.9 ± 97.6** (180.4, 385.3)

Data represent as mean ± s.d., $n = 6$ Figures in the parentheses represent the 95% confidence interval. *: AUC(0,24 h) **: AUC (0,∞) #: calculated from t_{max} -24 h ##: calculated from 48 h to last measured point.

routine laboratory tests, suggesting that JTE-522 was well tolerated in healthy subjects.

In the single-dose studies, the concentrations of JTE-522 in blood were much higher than those in plasma. JTE-522 was almost completely distributed in blood cells. However, based on C_{max} accumulation of JTE-522 in blood cells at doses of over 100 mg JTE-522 increased to a lesser extent. These findings suggest that JTE-522 has a high affinity to blood cells, and that its distribution into blood cells is limited at the higher doses of over 100 mg.

In the multiple dose study, the pharmacokinetic parameters for JTE-522 after the fourth dose were comparable with those after the seventh dose. Thus, these findings suggest the absence of accumulation following multiple-dosing of JTE-522. Plasma concentrations of unchanged drug reached steady state after the second administration. In contrast, blood concentrations of JTE-522 reached a peak 4 h after the first dose and steady state after the third dose. These findings may be explained by the high affinity of JTE-522 and its metabolites for blood cells. After the first administration of JTE-522,

Table 5 Pharmacokinetic parameters of M-III after oral multiple administration of JTE-522.

	C_{4h} (ng mL ⁻¹)	C_{24h} (ng mL ⁻¹)	$t_{1/2}$ (h)	AUC (μg mL ⁻¹ h)
<i>Plasma</i>				
Day 1	34.5 ± 17.3 (16.4, 52.7)	128.7 ± 44.8 (81.6, 75.7)		2.2 ± 1.0* (1.1, 3.2)
Day 2	398.9 ± 50.8 (345.5, 452.2)	282.4 ± 19.5 (261.9, 302.9)		
Day 3	447.5 ± 41.4 (404.1, 491.0)	300.4 ± 18.6 (280.9, 319.9)		
Day 4	464.2 ± 19.5 (443.7, 484.7)	350.3 ± 61.9 (285.3, 415.3)		11.0 ± 2.4* (8.4, 13.5)
Day 5	662.0 ± 280.3 (367.8, 956.3)	375.4 ± 49.7 (323.3, 427.5)		
Day 6	873.7 ± 142.5 (724.2, 1023.3)	377.7 ± 50.0 (325.3, 430.1)		
Day 7	1078.3 ± 135.5 (936.1, 1220.6)	362.9 ± 48.5 (312.0, 413.7)	53.56 ± 2.30 (51.15, 55.98)	15.4 ± 2.2* (13.1, 17.7) 35.6 ± 4.6** (30.8, 40.5)
	C_{4h} (μg mL ⁻¹)	C_{24h} (μg mL ⁻¹)	$t_{1/2}$ (h)	AUC (μg mL ⁻¹ h)
<i>Blood</i>				
Day 1	2.8 ± 0.6 (2.2, 3.4)	11.3 ± 1.2 (10.0, 12.6)		157.9 ± 21.5* (135.4, 180.5)
Day 2	11.4 ± 1.3 (10.1, 12.8)	16.5 ± 1.8 (14.6, 18.5)		
Day 3	13.8 ± 1.4 (12.3, 15.3)	16.7 ± 1.8 (14.7, 18.6)		
Day 4	14.0 ± 1.9 (12.0, 16.0)	17.1 ± 2.8 (14.2, 20.0)		377.2 ± 47.2* (327.7, 426.8)
Day 5	14.2 ± 1.4 (12.7, 15.7)	16.6 ± 2.6 (13.9, 19.3)		
Day 6	14.1 ± 1.6 (12.5, 15.8)	16.4 ± 2.4 (13.9, 18.9)		
Day 7	14.1 ± 1.6 (12.4, 15.8)	14.8 ± 3.3 (11.4, 18.3)	394.68 ± 72.46 (318.64, 470.73)	360.7 ± 46.7* (311.7, 409.6) 3532.6 ± 487.6** (3020.8, 4044.3)

Data represent as mean ± s.d., $n = 6$. Figures in the parentheses represent the 95% confidence interval. Half-life ($t_{1/2}$) was calculated from the time points t_{max} to the last measured point. *: AUC(0,24 h), **: AUC (0,∞).

unchanged drug mainly distributes to blood cells, resulting in low concentration of unchanged drug in plasma. Since the blood cell-bound JTE-522 dose not generate metabolites, plasma concentrations of the latter are very low. After the next dose, unchanged drug cannot bind to blood cells because binding capacity to blood cells is saturated. Unchanged drug in plasma can be metabolized in the liver and the metabolites will also distribute to blood cells, resulting in a reduction of the unchanged drug concentration in blood. Unchanged drug and its metabolites share a similar binding capacity, which is

saturable. Thus, unchanged drug is displaced by the metabolites from blood cells back into plasma. Eventually unchanged drug concentrations in plasma and blood will reach steady state. JTE-522 and its main metabolite M-III may selectively bind to carbonic anhydrase in red blood cells (data not shown). This metabolite inhibits COX-2 activity 1/1000 times less than JTE-522.

In conclusion, the present study suggests that JTE-522 has an acceptable pharmacokinetics profile without any serious adverse effect in healthy young male volunteers.

Figure 5 a) Plasma and b) blood concentrations *vs* time of metabolite after single oral administration of JTE-522. Data represent the means $\pm$ s.d. ($n = 6$).

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