原 著

Saturation Pharmacokinetics of Sedative Agent, JM-1232(−)((−)-3-[2-(4-methyl-1-piperazinyl)-2-oxoethyl]-2-phenyl-3,5,6,7-tetrahydrocyclopenta[f]isoindole-1(2H)-one) at High-Dose in Rats

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Abstract

JM-1232(−)((−)-3-[2-(4-methyl-1-piperazinyl)-2-oxoethyl]-2-phenyl-3,5,6,7-tetrahydrocyclopenta[f]isoindole-1(2H)-one) is a novel isoindoline chemical compound that affects benzodiazepine receptors, and is considered for application as a new sedative or intravenous anesthetic agent. To investigate the safety of JM-1232(−), preclinical studies investigating the pharmacokinetics of normal- and high-dose JM-1232(−) are needed. In this study, we used high performance liquid chromatography (HPLC) to measure the concentration of JM-1232(−) in plasma, and investigated the pharmacokinetics of low- to high-dose JM-1232(−) in rats. The effects of bolus administration of JM-1232(−) (1, 10, 25, 50, and 75 mg/kg) on the pharmacokinetic parameters were assessed in rats. We extrapolated JM-1232(−) to be a one-compartment model within 60 minutes after bolus administration. In the 50 mg/kg group, a significant increase in the elimination rate constant was observed, which is considered to be the saturation of metabolism and/or excretion. The rats were dead in the 75 mg/kg group. Thus, JM-1232(−) administered to rats at doses above 50 mg/kg is likely toxic.

Key words; JM-1232(−), sedative agent, assay, HPLC, saturation

Introduction

JM-1232(−)((−)-3-[2-(4-methyl-1-piperazinyl)-2-oxoethyl]-2-phenyl-3,5,6,7-tetrahydrocyclopenta[f]isoindole-1(2H)-one) is a novel isoindoline chemical compound that affects benzodiazepine (BZP) receptors, and is currently considered for application as a new sedative or intravenous anesthetic[1~3]. When administered intravenously to rats, a 50% hypnotic dose is observed at 0.69 mg/kg and 50% lethal dose is observed at more than 90 mg/kg[1].

In order to develop new therapeutic agents, the efficacy and safety of drugs must be investigated with preclinical studies[4]. In particular, screening of severe side effects such as ventricular fibrillation, torsades de pointes, and drug-induced liver injury should be performed to ensure the safety of such newly developed drugs. For example, we have reported the direct effects of JM-1232(−) on heart and vessels[5,6]

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in guinea pigs and rats. In addition, the saturation pharmacokinetics of newly developed drugs should also be investigated[7~9].

In the present study, we investigate the saturation pharmacokinetics of low- to high-dose (1～75 mg/kg) JM-1232(−) in rats.

Materials and Methods

A. Chemicals and Reagents

JM-1232(−) was supplied by Maruishi Pharmaceutical Co., Ltd. (Osaka, Japan). Diazepam, used as an internal standard (IS), was purchased from Sigma-Aldrich Co., Ltd. (St. Louis, MO, USA). Acetonitrile (high performance liquid chromatography (HPLC) grade) was purchased from Nacalai Tesque, Inc., (Kyoto, Japan), and all other reagents were commercially available as extra-pure grade chemicals.

B. Measurement of JM-1232(−) Concentration

1. Method of extraction (Solid phase extraction)

Bond Elut C18 column (Varian Incorporated, Santa Clara, CA, USA) was conditioned with 1 mL of acetonitrile and 1 mL of ultra pure water (H_2O) successively at 20 mL/min. Rat plasma (100 μL) and IS (5 μg/mL diazepam in methanol, 50 μL) were mixed before being absorbed to the columns at 1 mL/min. The columns were washed with 0.5 mL of H_2O and 0.5 mL of 10% acetonitrile at 20 mL/min. Next, 0.6 mL of acetonitrile was passed through the column at 1 mL/min to elute JM-1232(−) and IS. The resultant solution was evaporated to dryness and then dissolved into a 100 μL mobile phase containing 5 mM ammonium acetate and acetonitrile (1:1).

2. HPLC conditions and mobile phase

The HPLC system was equipped with a pump (LC 10AD, Shimadzu Corporation, Kyoto, Japan) and an ultraviolet-visible detector (SPD-10A VP, Shimadzu Corporation). The detection wavelength was 280 nm. The column was STR ODS-II (4.6×250 mm, Shimadzu Corporation), and the temperature was set at 40℃. The mobile phase contained 5 mM ammonium acetate and acetonitrile (1:1), and the flow rate was set at 1.0 mL/min.

3. Calibration curves

JM-1232(−) was weighed and dissolved in methanol, after which a 10 mg/mL solution was prepared. JM-1232(−) standard solution was then diluted to different concentrations and evaporated to dryness, to which 100 μL of blank rat plasma was added to prepare standard solutions with concentrations of 0.5, 1.0, 5.0, 10, 50, and 100 μg/mL. Samples for calibration curves were prepared by adding 50 μL of IS that had been mixed for 1 minute. The eluate was obtained by the extraction method described above, and 80 μL of the eluate was injected onto the HPLC column. Calibration curves were obtained by comparing the peak area of ligand detected in each standard solution to the peak area of IS.

4. Reproducibility

Standard solutions of JM-1232(−) at concentrations of 1.0, 5.0, and 50 μg/mL were used. These were extracted in the manner described above, and 80 μL of the eluate was injected onto the HPLC column. Measurement was performed five times to examine within-day variations, and for five days to examine between-day variations, after which coefficients of variation (CV) were calculated.

C. Measurement of JM-1232(−) Concentration in Rat Plasma

1. Animals

Nine- to 10-week-old Wistar ST strain male rats (Japan SLC Co, Inc., Shizuoka, Japan) were used with five rats per group. The animals were housed in a room maintained at a temperature of 24±1℃, humidity of 55±10%, lighting from 6:00 to 18:00, and allowed free access to tap water and solid diet (NMF, Oriental yeast Co., Ltd., Tokyo, Japan) *ad libitum*. The animals were acclimatized to the environment for at least one week prior to the experiments. All experimental procedures were conducted according to the guidelines for the use of experimental animals and animal facilities established by Osaka University of Pharmaceutical Sciences.

2. Preparation of test solutions

JM-1232(−) solution (50 mg/kg) was prepared in 0.2 M hydrochloric acid and physiological saline.

This solution was further diluted with physiological saline to obtain 1, 10, and 25 mg/mL of JM-1232(−). The rats were divided into the following groups: 1 mg/kg group receiving 1 mg/kg of JM-1232(−) intravenously; 10 mg/kg group receiving 10 mg/kg of JM-1232(-) intravenously; 25 mg/kg group receiving 25 mg/kg of JM-1232(−) intravenously; 50 mg/kg group receiving 50 mg/kg of JM-1232(−) intravenously; and a 75 mg/kg group receiving 75 mg/kg of JM-1232(−) intravenously. The 50 mg/mL JM-1232(−) solution was administered at a dose of 1.5 mL/kg in the 75 mg/kg group. The 1, 10, 25, and 50 mg/mL JM-1232(−) solutions were administered at a dose of 1 mL/kg in the 1, 10, 25, and 50 mg/kg group, respectively.

3. Method

Immediately before the experiment, urethane (Sigma-Aldrich Co., Ltd.) was dissolved in a physiological saline solution (1.5 g/mL) and administered (2 mL/kg) to the rats intraperitoneally. Cannulation (PE10/PE50, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) was performed on the right femoral artery and vein. After 30 minutes of stabilization, bolus administration of either 1, 10, 25, or 50 mg/mL/kg of JM-1232(−) hydrochloride was administered. The 75 mg/kg JM-1232(−) hydrochloride was administered at 1.5 mL/kg with bolus infusion. Blood samples (0.3 mL) were taken from the right femoral artery at 5, 10, 30, and 60 minutes after administration. The obtained blood samples were put into microtubes, kept on ice for approximately 30 minutes, and then 50 μL of IS was added and mixed for 1 minute. The obtained solution was extracted using the method described above, and JM-1232(−) concentration in the plasma was determined by HPLC.

D. Pharmacokinetics Analysis of JM-1232(−)

Based on changes in plasma concentrations of JM-1232(−), the area under plasma concentration curve (AUC) was determined by trapezoidal approximation and the elimination rate constant (K_{el}) was calculated. Clearance was also calculated from the applied dose and AUC.

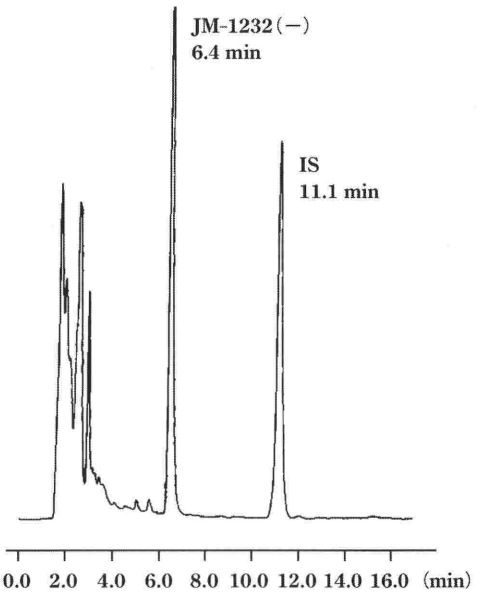

Figure 1 Chromatogram of rat plasma
Two peaks at 6.4 and 11.1 minutes represent JM-1232(−) and the internal standard (IS), respectively.

E. Statistical Analysis

The standard curves were obtained by simple-regression analysis. The Tukey test was used for multi-group examinations (StatMate III, Atms Co., Ltd., Tokyo, Japan), where $p<0.05$ was considered statistically significant.

Results

A. Determination of JM-1232(−) Concentration in Plasma using HPLC

1. Chromatogram

Retention times for JM-1232(−) and IS were 6.4 min and 11.1 min, respectively; the chromatogram is shown in **Fig. 1**. The assay of each specimen was completed within 15 minutes.

2. Calibration curve

The calibration curve of a JM-1232(−) standard solution prepared with the blank plasma of untreated rats is shown in **Fig. 2**. As indicated by the graph, a straight line starting roughly at the origin of the coordinate axes was obtained, with the regression line at $y=0.887x-0.600$ and correlation co-efficient at $r=0.999$ ($p<0.01$).

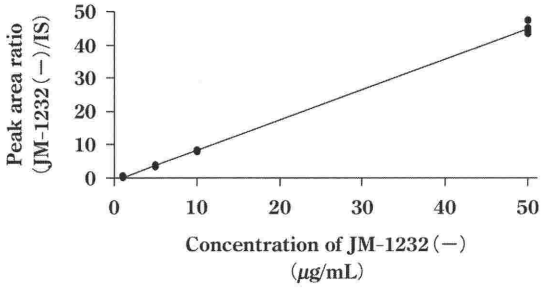

Figure 2 Standard curve for JM-1232(−) at concentrations of 1, 5, and 50 μg/mL

The regression equation determined by the least-squares method is y=0.887x−0.600 and the co-efficient of determination is r=0.999 ($p<0.01$).

Table 1 Co-efficient of variation for within-, between-day JM-1232(−) assay

Concentration of JM-1232(−) (μg/mL)	Co-efficient of variation (%)
Within-day assay	
1	4.62
5	3.82
50	4.52
Between-day assay	
1	5.78
5	4.70
50	0.98

Data are presented as mean (n=3).

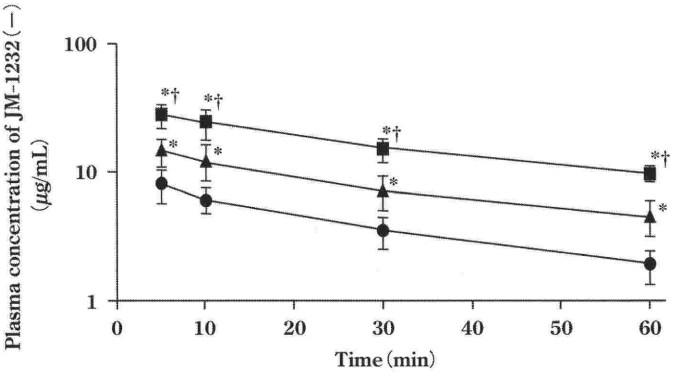

Figure 3 Plasma concentration of JM-1232(−) after intravenous administration

Data are presented as mean±SD (n=5). *$p<0.05$, compared with control. †$p<0.05$, compared with 25 mg/kg of JM-1232(−). ●, 10 mg/kg; ▲, 25 mg/kg; ■, 50 mg/kg

3. Reproducibility

Within-day variations: CV values were measured five times (**Table 1**). CV values for within-day variations were 6% or below with solutions of 1～50 μg/mL, and 10% or above with solutions of 0.5 and 100 μg/mL.

Between-day variations: Coefficients of variation were measured for five days (**Table 1**). CV values for between-day variations were 6% or below with solutions of 1～50 μg/mL, and 10% or above with solutions of 0.5 and 100 μg/mL.

B. Pharmacokinetics of JM-1232(−) in Rats

The plasma concentration of JM-1232(−) in rats is shown in **Fig. 3**, and the pharmacokinetic parameters

Table 2 Pharmacokinetic parameters of JM-1232(−)

Dose of JM-1232(−) (mg/kg)	AUC (μg・min/mL)	K_{el} (/min)	CL (mL/min)
10	212.2±49.1	0.026±0.006	0.014±0.003
25	447.2±130.6	0.021±0.002	0.017±0.003
50	814.7±159.4	0.018±0.003*	0.019±0.004
75	—	—	—

AUC, area under the blood concentration-time curve; K_{el}, elimination rate constant; CL, clearance.
Data are presented as mean±SD(n=5). *$p<0.05$, compared with 10 mg/kg.

are shown in **Table 2**. Compared with 10 mg/kg of JM-1232(−), the plasma concentration of JM-1232(−) increased significantly in both the 25 and 50 mg/kg groups, respectively. JM-1232(−) concentrations in plasma were eliminated in a mono-exponential manner within 60 minutes after bolus administration. Among the JM-1232(−) administered groups, K_{el} showed a significant difference in the 50 mg/kg group compared with the 10 mg/kg group ($p<0.05$). In the 1 mg/kg group, the concentration data were below the limit of detection; while in the 75 mg/kg group, all the subjects were confirmed dead immediately after the administration (n=2).

Discussion

The development of new drugs should be investigated for efficacy and safety[7~9]. When the safety of possible anesthetics and sedatives is investigated, it is very important to research the influence of these drugs on the circulatory system[10]. There have been many studies reporting that cardiac arrest or vessel relaxation is caused not only by central depressants but also by direct effects[11~15]. We previously reported that JM-1232(−) has a direct effect on heart and vessels in guinea pigs and rats[5,6]. These studies were performed to screen the severe side effects of torsades de pointes and/or cardiac arrest, and severe hypotension.

On the other hand, some severe side effects can be predicted by saturation pharmacokinetics. Generally, the plasma concentration of drugs increases linearly according to the dose. However, with some drugs, the plasma concentration increases non-linearly. This non-linearity is caused by the saturation of metabolism and/or the excretion processes[16]. To clarify the dosage of the drug that leads to saturation, pharmacokinetics not only at normal doses but also at high doses should be investigated with preclinical studies. In this study, a simple and rapid determination of the plasma concentrations of JM-1232(−) was performed using HPLC. We investigated the pharmacokinetics of JM-1232(−) with low to high doses (2~100 times HD_{50}; 1~75 mg/kg). From the profile of JM-1232(−) concentrations in plasma, it appears that the elimination follows a mono-exponential manner within 60 minutes after bolus administration (**Fig. 3**). The elimination rate constant, K_{el} in the 50 mg/kg group decreased significantly compared with the 10 mg/kg group (**Table 2**). It is reported that benzodiazepines show saturation pharmacokinetics and significantly decreased K_{el} at higher concentrations[17]. For JM-1232(−), the saturation of metabolism and/or excretion might exist in the 50 mg/kg group. The significant decrease of K_{el} in the 50 mg/kg group suggests that accumulation or toxicity might be linked to the death observed in the 75 mg/kg group (**Table 2**). Thus, it is likely that JM-1232(−) at doses above 50 mg/kg is toxic to rats.

Conclusion

At doses above 50 mg/kg, the saturation of metabolism and/or excretion of JM-1232(−) in rats might occur, and severe side effects might easily appear in rats.

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