

# Establishment of a Novel In Situ Rat Model for Direct Measuring of Intestinal Drug Absorption: Confirmation of Inhibitory Effects of Daijokito on the Absorption of Ranitidine

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## ABSTRACT

**Background** Daijokito (DJKT), a classical traditional Kampo and Chinese medicine, has been used to treat acute pancreatitis in China. In our previous study, DJKT was found to reduce the area under the plasma concentration-time curve (AUC) of ranitidine in humans. Therefore, we established a novel rat model to examine the direct absorption of ranitidine after daijokito administration.

**Methods** An in situ intestinal injection with portal vein sampling (IIPS) model was created to determine the rate of intestinal drug absorption. Rats were divided into two groups: the ranitidine group (R,  $n = 6$ ) or the ranitidine and daijokito group (RD,  $n = 6$ ). Blood was collected after intestinal injection of drugs. After the experiment, the concentrations of ranitidine were measured by LC/MS/MS analysis.

**Results** The concentrations of ranitidine increased linearly with time in both groups. Compared with the R group, the concentrations of ranitidine in RD group significantly decreased throughout the experiment.

**Conclusion** Co-administration of ranitidine with DJKT resulted in significant decreases in intestinal absorption in rats. The reduction of the systemic ranitidine concentration by co-administration of DJKT may be due, at least in part, to the inhibition of intestinal absorption of ranitidine.

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Abbreviations: AUC, area under the plasma concentration-time curve; DJKT, daijokito; EGS, everted gut sac; HPLC, high-performance liquid chromatography; IPVS, intestinal perfusion with venous sampling; IIPS, intestinal injection with portal vein sampling; LC/MS/MS, liquid chromatography/mass spectrometry/mass spectrometry

**Key words** absorption; daijokito; portal vein sampling; ranitidine; rat model

Daijokito (DJKT), a classical traditional Kampo and Chinese (Da-cheng-qi-tang in Chinese) medicine, has been used as a purgative for clearing interior heat and excess stomach and intestinal content for 1800 years in China. It is composed of daio (*Radix et Rhizoma Rhei*), koboku (*Magnolia officinalis Rehd*), kijitu (*Aurantii Fructus Immaturus*) and bosho (*Natrii Sulfas*). Now, DJKT is widely used to treat acute pancreatitis in China.<sup>1</sup> On the other hand, Chinese herbal medicine is also co-administered with other Western medicines to treat acute pancreatitis such as ranitidine. Ranitidine, a H<sub>2</sub> receptor antagonist, was used to inhibit gastrointestinal and pancreatic secretion, and to protect gastric mucosa. Tang WF et al.<sup>2</sup> previously reported that the concentration of ranitidine was reduced after oral administration with DJKT in rats by high-performance liquid chromatography (HPLC). In our previous study, we found that the plasma concentration of ranitidine was reduced by DJKT in humans.<sup>3</sup> Therefore, we established a novel rat model to examine the direct absorption of ranitidine when combined with DJKT.

We modified the in situ intestinal perfusion with venous sampling (IPVS) model for simultaneous profiling of intestinal drug absorption and pre-systemic metabolism.<sup>4,5</sup> Compared with HPLC, LC/MS/MS is more accurate, sensitive, rapid and convenient.<sup>6</sup> Accordingly, we established a novel in situ intestinal injection with portal vein sampling (IIPS) rat model to evaluate the effects of DJKT on the absorption of ranitidine in rats by LC/MS/MS analysis.

## MATERIALS AND METHODS

### Drugs and preparation of perfusion solution

DJKT extract was purchased from Tsumura & Co. (Tokyo, Japan). The dosage of DJKT extract was 225 mg/kg. Ranitidine for treatment was purchased from GlaxoSmithKline (Tokyo, Japan). The dosage of

ranitidine for rats was 27 mg/kg. Ranitidine solution was prepared with saline and diluted to 1.35 mg/mL. Ranitidine + DJKT solution was prepared with saline, and diluted to 1.35 mg/mL of ranitidine and 10 mg/mL of DJKT extract. The solution was vortexed for 10 min. Ranitidine hydrochloride for LC/MS/MS analysis was purchased from Wako Pure Chemical Industries, Ltd. (No.181-01561, Osaka, Japan).

## Animals and surgical procedures

### Animal care and anesthesia

The experimental protocol was approved by the ethics committee on animal experiments at Tottori University (#15-Y-15), and the experiments were carried out in accordance with the guidelines for animal experiments at the same facility.

Twelve 10-week-old male Wistar rats (purchased from Shimizu Laboratory Supplies Co., Ltd., Kyoto) were acclimated in an air-conditioned room at 25 °C with 55% humidity, and given standard chow for 4 days for adaptation and ad libitum access to water. At the end of the fourth day, the rats were randomly divided into two groups: the ranitidine group (R,  $n = 6$ ) or the ranitidine and DJKT group (RD,  $n = 6$ ) (body weight,  $272.53 \pm 6.79$  g and  $274.32 \pm 6.29$  g, respectively). The rats were fasted 12 hours before the experiment.

Rats were anesthetized with pentobarbital sodium (50 mg/kg) by peritoneal injection, and then placed in a warm jacket circulated with warm (38 °C) water and under a heating lamp to maintain body temperature during the experiment. O<sub>2</sub> was also supplied over the head of the rat during the experiment. To maintain anesthesia during the course, one-third of the initial dose of pentobarbital sodium was administered throughout the remainder of the experiment.

### Surgical procedures of in situ intestinal injection with portal vein sampling (IIPS)

The surgical procedures were adapted from previous reports with some modifications to prepare the perfused rat intestine for venous sampling.<sup>4, 5, 7-9</sup> First, the rat was intraperitoneally injected with 1 mL of heparin. The abdomen of the anesthetized animal was shaved, and a longitudinal midline incision of 5 cm was carefully made to expose the small intestine and the portal vein. A 22-G catheter (No.SR-OT2232C, Terumo Corporation, Tokyo, Japan) was used without the catheter hub to reduce the dead space for blood sampling. A catheter filled with heparinized saline was inserted into the portal vein and secured with instant glue to avoid obstruction of portal venous flow into the liver. The original inner cylinder was replaced with a plugged one inside the catheter

to prevent blood overflow. For intestinal perfusion, an intestinal segment 5 cm under the pylorus was selected and ligated with 1-0 sutures. Next, the drug solution was injected into the intestine using a 10-mL syringe from 6 cm under the pylorus. Rats in group R were injected with ranitidine solution and those in group RD were injected with ranitidine + DJKT solution. The injection flow rate was 1 mL/min, and the start time of injection was recorded. The inner plugged cylinder of the catheter was removed to collect blood 10 min later, and then reinserted to stop blood flow after 0.3–0.4 mL of blood was collected. Subsequently, blood was collected another 3 times at 20, 30 and 40 min. During the experiment, the exposed intestinal segment was kept moist by covering it with a piece of sterilized gauze wetted with saline, and warm isotonic saline was sprinkled on the gauze many times by a syringe. At the termination of the experiment, systemic blood was collected from the heart, and rats were sacrificed by aortic bleeding.

## LC/MS/MS Analysis

### Sample preparation

The blood sample was centrifuged (2,500 rpm, 15 min, 4 °C), and the supernatant of plasma was collected and stored at –80 °C. Subsequently, 100 µL of plasma was mixed with 300 µL of 100% methanol. The solution was vortexed at 4 °C, and then collected by centrifuging (15,000 rpm, 17 min, 4 °C). Next, 100 µL of supernatant was placed into a tube for analysis.

### Preparation of standard solution

Ranitidine was weighed and dissolved in 7.5% methanol. The concentration of ranitidine was 1 mg/mL. The solution was then diluted to 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50, 100, 500 or 1,000 ng/mL, and placed into tubes for analysis.

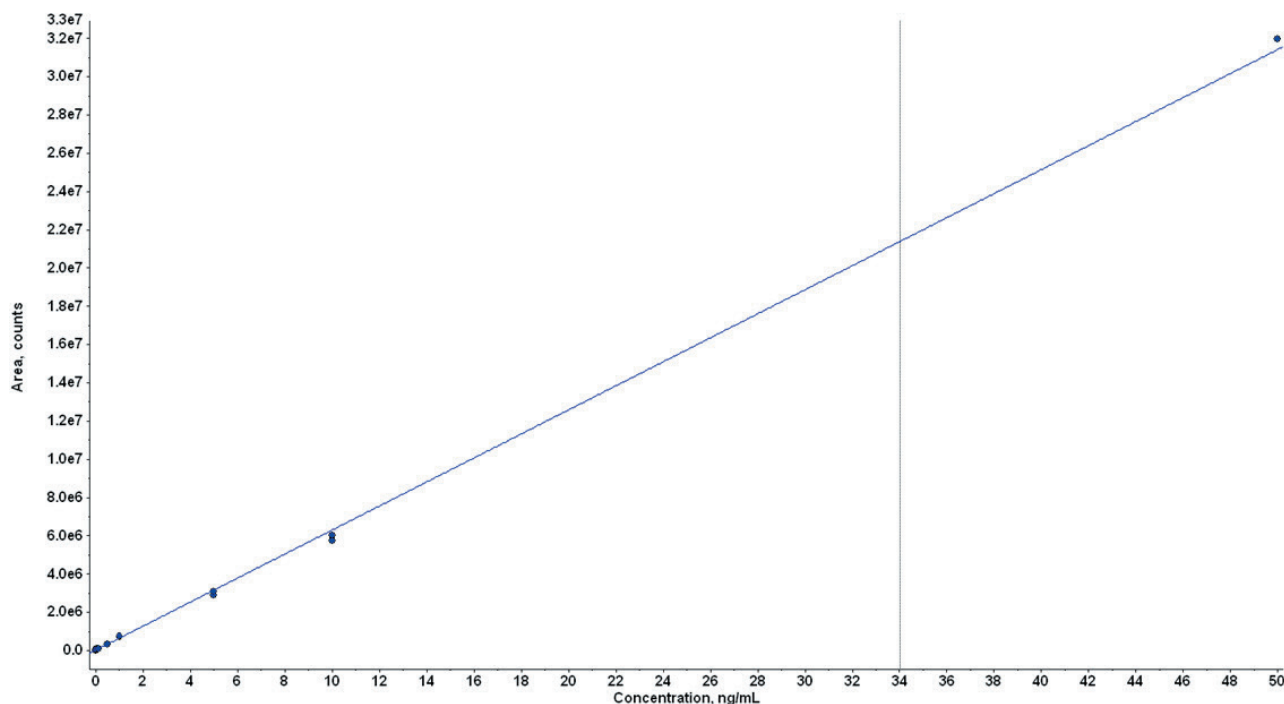
### Sample analysis

The concentration of ranitidine in the plasma was analyzed using Prominence ultra fast liquid chromatography (Shimadzu, Kyoto) coupled with tandem mass spectrometry (LC/MS/MS) method (QTRAP 5500, SCIEX, Tokyo). Ten µL of each sample was injected. Data acquisition and analysis were performed by Analyst 1.5 software (SCIEX, MA).

## RESULTS

### Calibration curves for ranitidine

The linear range of ranitidine obtained using the selected internal standards is shown in Fig. 1. The regression equation was established by the ratio of analyte peak area (counts) to analyte concentration. The “linear” re-



**Fig. 1.** Relationship between ranitidine concentration and peak area. The linear range of ranitidine is shown. Concentrations of 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50, 100, 500 and 1000 ng/mL of ranitidine were measured using the LC/MS/MS approach as internal standards. LC/MS/MS, liquid chromatography/mass spectrometry/mass spectrometry.

gression equation (“1/X” weighted) of  $y = 6.28e + 005x + 2.26e + 004$  ( $r = 0.9990$ , where  $x$  is the concentration of analyte and  $y$  is the peak area) was obtained.

### Concentration of ranitidine

The concentration of ranitidine is described in Fig. 2 and Fig. 3. The plasma concentrations of ranitidine increased linearly with time in both groups (Fig. 3). In group R, the ranitidine concentrations at 10, 20, 30 and 40 min after intestinal injection were  $0.17 \pm 0.06$ ,  $0.26 \pm 0.03$ ,  $0.42 \pm 0.06$  and  $0.65 \pm 0.08$  ng/mL, respectively, whereas in group RD, the ranitidine concentrations at 10, 20, 30 and 40 min were  $0.11 \pm 0.02$ ,  $0.15 \pm 0.04$ ,  $0.20 \pm 0.05$  and  $0.33 \pm 0.05$  ng/mL, respectively. Compared with ranitidine alone (group R), the concentrations of ranitidine after co-administration with DJKT (group RD) were significantly lower ( $P < 0.05$ ).

## DISCUSSION

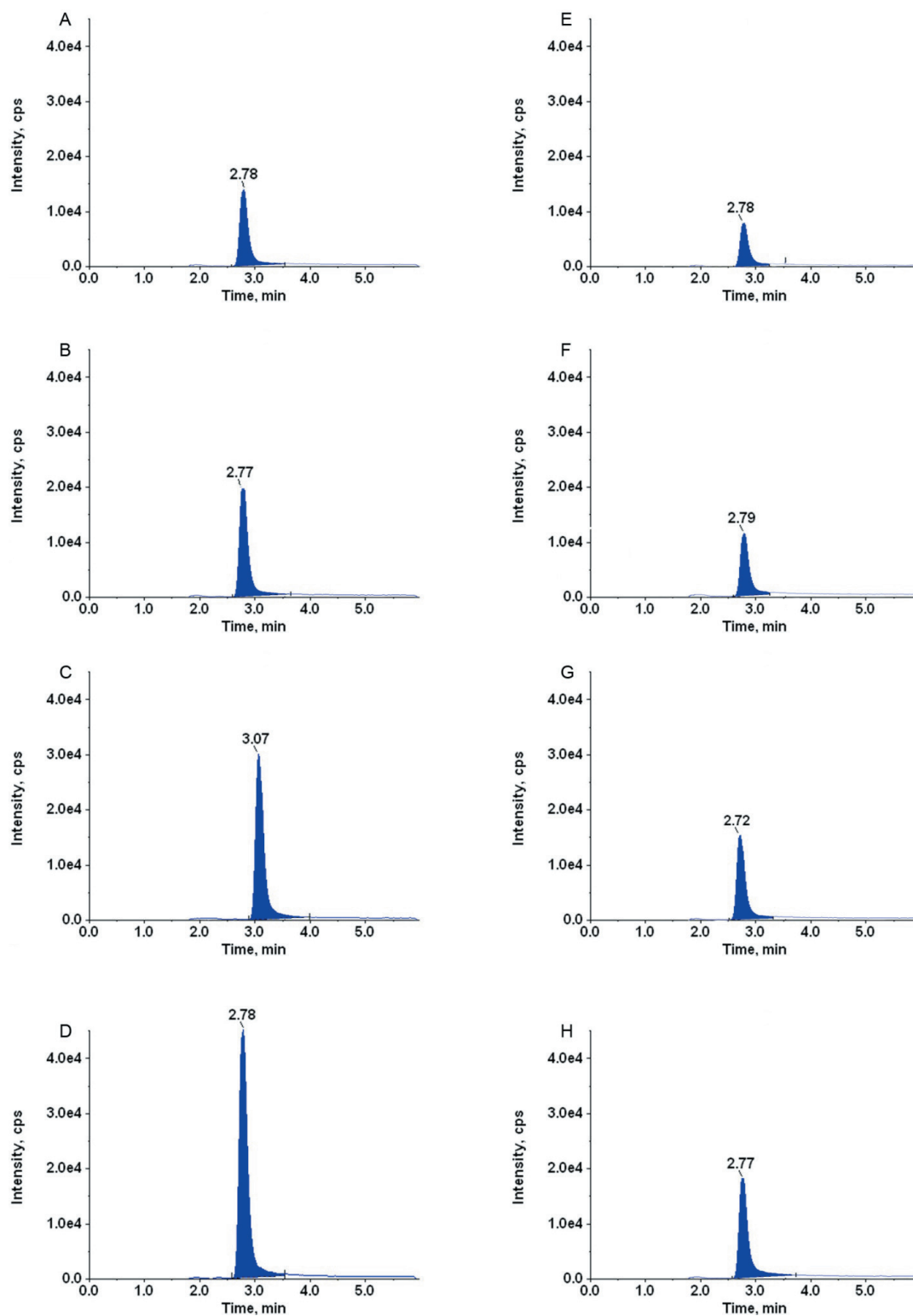
In this study, the ranitidine concentration in the portal vein in the IIPS rat model was successfully measured after intestinal injection. Compared with the conventional rat model by oral administration, the IIPS model demonstrated more direct absorption of the medicine. The intestines as the main organ of absorption play a key role in pharmacokinetics. Therefore, intestinal injection is a

more direct, reliable and effective way to examine the pharmacokinetics of medicine than oral administration in animal studies.

Experimental techniques for evaluating the absorption of drugs include the use of isolated cells, everted gut sac (EGS) and in situ single-pass intestinal perfusion (SPIP).<sup>4</sup> However, morphological damage to intestinal tissue due to everting and the lack of intact vascular supply are disadvantages of the EGS model.<sup>10</sup> Recently, Li et al.<sup>4</sup> reported the superiority of intestinal perfusion with venous sampling (IPVS) to EGS and SPIP as methods to study drug absorption. Cummins et al.<sup>9</sup> examined the modulation of P-glycoprotein in the intestinal drug metabolism by cytochrome P450 3A4 using IPVS, and reported the advantages of IPVS for studies on intestinal drug absorption.

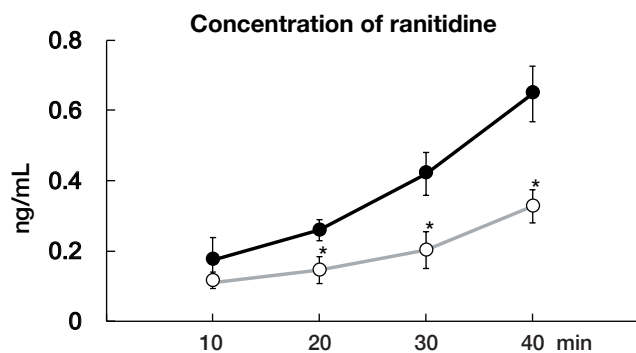
However, for IPVS, a total of 50–70 mL of blood drawn from some donor rats needs to be prepared to be transfused into the recipient rat through the jugular vein to replace the blood lost via the mesenteric or portal vein.<sup>7, 9, 10</sup> On the other hand, with our IIPS method, no donor rats are needed due to the minimal blood loss. This is an advantage of IIPS over IPVS to reduce the number of sacrificed animals.

Now, DJKT is widely used to treat acute pancreatitis in China.<sup>1</sup> Moreover, Chinese herbal medicines are



**Fig. 2.** LC/MS/MS analysis of ranitidine. Representative raw data for LC/MS/MS analysis are shown. **A) – D):** group R, **E) – H):** group RD. **A)** at 10 min. Area:  $1.47e + 005$  counts, Height:  $1.42e + 004$  cps RT: 2.78 min; **B)** at 20 min. Area:  $2.01e + 005$  counts, Height:  $1.98e + 004$  cps RT: 2.77 min; **C)** at 30 min. Area:  $3.31e + 005$  counts, Height:  $3.35e + 004$  cps RT: 3.07 min; **D)** at 40 min. Area:  $4.60e + 005$  counts, Height:  $4.49e + 004$  cps RT: 2.78 min; **E)** at 10 min. Area:  $8.11e + 004$  counts, Height:  $7.89e + 003$  cps RT: 2.78 min; **F)** at 20 min. Area:  $1.21e + 005$  counts, Height:  $1.17e + 004$  cps RT: 2.79 min; **G)** at 30 min. Area:  $1.60e + 005$  counts, Height:  $1.56e + 004$  cps RT: 2.72 min; **H)** at 40 min. Area:  $1.97e + 005$  counts, Height:  $1.82e + 004$  cps RT: 2.77 min. LC/MS/MS, liquid chromatography/mass spectrometry/mass spectrometry. Area indicates the area counts of peaks on the ion chromatogram, and height indicates the peak height (cps). RT indicates the retention time. The area was converted into the concentration of ranitidine by calculation. Group R received ranitidine alone. Group RD received ranitidine with Daijokito.





**Fig. 3.** Plasma concentration-time curves for ranitidine. Filled circles with black lines represent the concentration-time curve for ranitidine in portal venous plasma in group R, and open circles with gray lines represent the curve in group RD. The vertical line indicates the concentration of ranitidine and the horizontal line indicates the time after injection of drugs into the intestine. \* $P < 0.05$  vs. group R. Group R received ranitidine alone (R). Group RD received ranitidine with Daijokito (RD).

usually co-administered with other Western medicines during the treatment of many diseases. Ranitidine, a  $H_2$  receptor antagonist, is used to inhibit gastrointestinal and pancreatic secretion, and to protect the gastric mucosa. We previously reported that the area under the plasma concentration-time curve (AUC) of ranitidine was reduced by DJKT in humans.<sup>3</sup> In the present study, although the dose of ranitidine in both groups was 27 mg/kg, the rate of increase in the concentration of ranitidine greatly differed between the groups. This difference in concentration in the portal vein demonstrates the direct effects of DJKT on intestinal absorption of ranitidine. These results suggest that the reduction of the systemic concentration of ranitidine by co-administration of DJKT is due, at least in part, to the inhibition of intestinal absorption of ranitidine.

In our previous report, we speculated the mechanism underlying the inhibitory effect of DJKT on ranitidine AUC as a reduction of absorption, and discussed about some elements concerning to drug absorption such as changes in gastric emptying rate, intestinal motility, activities of P-glycoprotein and CYP3A4. The direct intestinal injection of drugs applied in this study may exclude a gastric role in reduced absorption. Ranitidine and DJKT were injected simultaneously into intestine, so P-glycoprotein activation and/or increased motility of intestine by DJKT are quite unlikely to be simple mechanisms, because onset of reduction of ranitidine concentration was so rapid. The precise mechanism underlying the inhibitory effects on absorption are still unclear and further studies should be performed.

In conclusion, the novel IIPS rat model with LC/

MS/MS analysis is a direct, reliable and effective way to study the pharmacokinetics of drugs. Co-administration of ranitidine with DJKT resulted in significantly decreased absorption of ranitidine in rats. Therefore, we speculate that DJKT reduces the systemic ranitidine levels, at least in part, by inhibiting the intestinal absorption of ranitidine. Thus, the co-administration of ranitidine and DJKT should be avoided in clinical settings.

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*The authors declare no conflicts of interest.*

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