Pharmacokinetics of Oral and Intravenous Administration of Digoxin after Intestinal Ischemia-Reperfusion

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Intestinal ischemia-reperfusion (I/R) is a common clinical problem in the settings of small bowel transplantation, circulatory shock and strangulation ileus. Intestinal I/R causes gut dysfunction characterized by decreased basement membrane integrity and decreased barrier function. It has also been shown that blood urea nitrogen (BUN) increases and that inulin clearance decreases after intestinal I/R injury. Plasma digoxin concentration in I/R rats was not significantly altered at any time compared with that in sham-operated rats. Plasma digoxin concentration in rats reperfused for 1 h after intravenous administration was significantly higher than that in sham-operated rats. Plasma digoxin concentrations in rats reperfused for 6 and 24 h were the same as those in sham-operated rats. The area under the concentration–time curve after intravenous administration (AUC, i.v.) and total clearance (CL, i.v.) in rats reperfused for 1 h was 1.89- and 0.57-fold higher than that in sham-operated rats. However, elimination rate (k) and half-life (t,1/2) in rats reperfused for 1 h were not altered. Distribution volume (V) in rats reperfused for 1 h was decreased than that in sham-operated rats, but there was not statistical difference. These results suggest that intestinal I/R affected the V of digoxin, and plasma concentration of digoxin was increased. The present study suggests that understanding pharmacokinetics of drug after intravenous administration in intestinal I/R injury is important to provide valuable information for safe drug therapy for intestinal I/R patients.

Key words intestinal ischemia-reperfusion; pharmacokinetics; intravenous administration; digoxin; multi-organ failure

MATERIALS AND METHODS

Chemicals All chemicals and reagents used were of analytical grade. d-[3H]-Mannitol was obtained from Perkin Elmer (Waltham, MA, U.S.A.). Digoxin was obtained from Sigma (St. Louis, MO, U.S.A.).

Animals Male Wistar rats, aged 6 weeks, were obtained from Jia (Tokyo, Japan). The rats were housed for at least 1 week (until reaching 250—350 g in weight). The housing conditions were the same as those described previously.13) The experimental protocols were reviewed and approved by the Hokkaido University Animal Care Committee in accordance with the “Guide for the Care and Use of Laboratory Animals.”

Intestinal I/R Model Surgical procedures were carried out as described in a previous report with some modification.14) Rats were not fed for 16 h prior to the experiments but were allowed free access to water. The animals were anesthetized with sodium pentobarbital (30 mg/kg body weight, intraperitoneal i.p. injection). Through a midline laparotomy, each rat was subjected to 30 min of ischemia by ligating small anastomosing vessels and occluding the superior mesenteric artery (SMA), and reperfusion was induced by removing the clamp. The abdomen was then covered with a sterile plastic wrap.

Transport Experiments The intestine was quickly removed and the longitudinal muscle layer was carefully stripped off with scissors. Prepared intestinal sheets were filled with Hanks’ balanced salt solution (HBSS) buffer (137 mM NaCl, 5.4 mM KCl, 1.0 mM CaCl2, 0.8 mM MgCl2, 0.35 mM NaHCO3, 1.19 mM KH2PO4, 0.58 mM Na2HPO4, 4.5 mM Glucose) at a pH of 7.2.
0.4 mM KH₂PO₄, 0.3 mM NaH₂PO₄ and 25 mM D-glucose). The pH of the buffer was adjusted to 6.0 or 7.4 with 1 N HCl or NaOH. The prepared intestinal sheets were mounted between two Ussing-type diffusion chambers (Corning Costar Corporation, Cambridge, MA, U.S.A.) that provided an exposed area of 0.64 cm². HBSS buffer was added to the chambers. Samples of 0.5 ml were taken from the receptor side at 30, 60, 90 and 120 min after incubation. A scintillation spectrometer (1600TR, Packard Instruments, Meriden, CT, U.S.A.) was used to measure D-[³H]-mannitol.

The permeation rate of D-[³H]-mannitol was expressed as an apparent permeability coefficient (P_app) according to the following equation: P_app = dQ/dt/S, where dQ/dt is the linear appearance rate of mass in the receiver solution, S is the exposed area (0.64 cm²), and C₀ is the initial concentration of D-[³H]-mannitol (500 μM). P_app is expressed in centimeters per minute.

Measurement of Transepithelial Electrical Resistance (TEER) The TEER values of the intestinal membranes were measured by an in vitro diffusion chamber method using stripped rat intestinal membranes. After surgical operation, the small intestine was isolated and the intestinal segments were mounted in a diffusion chamber in which a surface area of 0.64 cm². Two pairs of electrodes connected to a Millicell-ERS (Nihon Millipore K.K., Tokyo, Japan) were inserted into each side of the diffusion chamber, respectively. Phosphate buffer saline (PBS) 2.25 ml was added to each side. TEER values were calculated by the following equation: TEER = resistance value (Ω) × surface area (cm²).

Administration of Digoxin Experiment Digoxin suspension (0.25 mg/ml) was orally administered at 0.25 mg/kg body weight. The volume of digoxin suspension was 1 ml/kg body weight. Blood samples (each 0.4 ml) were collected into tubes from a cervical vein at 20, 40, 60, 90, 120 and 180 min after oral administration of digoxin. Digoxin solution (10 μg/ml) was intravenously administered at 10 μg/kg body weight. The volume of digoxin solution was 1 ml/kg body weight. Blood samples (each 0.4 ml) were collected into tubes from a cervical vein at 5, 10, 30, 60 and 120 min after intravenous administration of digoxin. Following centrifugation (850×g for 15 min at 4 °C), the plasma was diluted to a concentration in the range of 0.5 to 4.5 ng/ml. A TDxFLX analyzer (Abbott Japan CO., LTD., Tokyo, Japan) was used to measure dilute plasma concentration of digoxin. The standard curve was established by digoxin calibrators (Abbott Japan CO., LTD., Tokyo, Japan). The concentration range of the standard curve is 0—5.0 ng/ml. The correlation coefficient for the standard curves is 0.995 ± 0.003. The area under the concentration-time curve (AUC) of digoxin was calculated using the trapezoidal rule from the concentration-time curve.

Total clearance (C_Ltot) was calculated by the following equation: C_Ltot = D/AUC, where D is the dosage amount. The elimination rate constant (k) was calculated by the following equation: ln C₀ = k t + ln C₀, where C is plasma concentration. The half-life (t½) was calculated by the following equation: t½ = ln 2/k. The distribution volume (Vₐ) was calculated by the following equation: Vₐ = C_Ltot/k.

Data Analysis Statistical significance was evaluated using unpaired Student’s t-test. A value of p<0.05 was considered significant.

RESULTS AND DISCUSSION

Decreasing basement integrity is often assessed by the amount of mannitol or dextran permeation through the intestinal membrane. Mucosal to serosal flux for mannitol was calculated from the linear serosal radioisotope appearance curves. P_app of mannitol through intestinal membranes of rats reperfused for 1 h was significantly higher than that of mannitol through intestinal membranes of sham-operated rats (Fig. 1A). P_app of mannitol through intestinal membranes of rats reperfused for 6 h and 24 h were almost the same as those in sham-operated rats. The viability of the intestinal membrane was maintained during the experiments (data not shown). The TEER value of intestinal membrane of rats reperfused for 1 h was significantly decreased (Fig. 1B). The TEER values of intestinal membrane of rats reperfused for 6 h and 24 h were almost the same as those of sham-operated rats. These results indicated that barrier function of the intestinal membrane and tight junction in the intestine were damaged at 1 h after I/R and were recovered at 24 h after I/R. These results are associated with results of histological features of the intestine. Next, we performed an in vivo oral and intravenous administration study using digoxin. Digoxin is a typical substrate of P-glycoprotein (P-gp) as is tacrolimus. Omae et al. reported that plasma concentration of tacrolimus after oral administration was affected by enhanced P-gp function in the ileum after intestinal I/R. Figure 2 shows the time courses of plasma digoxin concentration after oral administration at a dose of 0.25 mg/kg. Plasma digoxin concentration in I/R rats was not significantly altered at any time compared with that in sham-operated rats. At 1 h after intestinal I/R, the absorption of digoxin was not affected by intestinal I/R, although the permeability of mannitol through the intestinal membrane was increased. Digoxin (log P=1.26) where P is octanol/water partition coefficient is more hydrophilic compound than mannitol (log P=−3.10). This hydrophilic/hydrophobic difference between these compounds may affect the permeability through the intestinal membrane. The paracellular pathway to the lateral intercellular space via tight junctions between epithelial cells is a passive transport for many water-soluble and poorly lipid-soluble compounds. As shown in Fig. 1B,
tight junction in the intestine after intestinal I/R was maintained about 80%, although tight junction in intestinal I/R rats was significantly weak compared with that in sham-operated rats. Therefore, we suggest that the permeability of digoxin, which is more hydrophobic compound, through the intestinal membrane was not affected by intestinal I/R. At 24 h after intestinal I/R, plasma concentration of tacrolimus after oral administration was significantly decreased. However, plasma concentration of digoxin was not altered. The reason of the difference between digoxin and tacrolimus is not clear. Intestinal I/R is known to affect the kidney and remote organs, including the kidney and liver.

In summary, we investigated pharmacokinetics of digoxin after intestinal I/R. Although plasma concentration of digoxin after oral administration was not altered by intestinal I/R, that after intravenous administration was significantly increased at 1 h after intestinal I/R. The increasing of plasma concentration of digoxin was caused by the decreasing $V_d$. These results imply that understanding pharmacokinetics of drugs after intravenous administration, as well as that after oral administration is important for safe drug therapy for intestinal I/R patients.

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REFERENCES


