Effect of the Absorption Enhancer Saponin on the Intrarenal Distribution of 5-Fluorouracil Following Its Kidney Surface Application in Rats

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The present study was undertaken to examine the effects of the absorption enhancer saponin on the intrarenal distribution of 5-fluorouracil (5-FU) following the kidney surface application of 5-FU in rats. We selected an experimental system utilizing a cylindrical diffusion cell attached to the kidney surface. The intrarenal concentration of 5-FU 120 min after right kidney surface application of 5-FU with saponin at concentrations of 0.25 and 1 mg/ml was modified. Among four sites in the right kidney, the concentration of 5-FU under the diffusion cell was selectively increased by saponin. These results suggest it may be possible to control the intrarenal distribution of the drug following its application with an absorption enhancer on the kidney surface.

Key words 5-fluorouracil; kidney; absorption enhancer; saponin; targeting; rat

The response rate to chemotherapy for treating renal cell carcinoma is less than 20%.[1] Renal cell carcinomas are normally located unilaterally in the kidneys, and accordingly renal arterial administration has been studied to deliver anticancer drugs to the unilateral kidney.[2] When a drug is administered systemically, however, it is distributed throughout the whole body *via* the blood stream, leading to inadequate drug delivery to the target organ. We have described the kidney- and site-selective delivery of 5-fluorouracil (5-FU) by the application of 5-FU to the kidney surface in rats.[3]

It is important to control the intrarenal distribution of 5-FU in the kidney following its application to the kidney surface for the effective chemotherapeutic treatment of a renal cell carcinoma. Many researchers have studied the effects of absorption enhancers on drug absorption across various membranes. However, few studies have applied an absorption enhancer to the kidney surface membrane. In the present study, we examined the effect of an absorption enhancer saponin on the intrarenal distribution of 5-FU following the kidney surface application of 5-FU in rats. Saponin is a surface active agent and has been widely used as an absorption enhancer in the study of biopharmaceutics.

MATERIALS AND METHODS

Chemicals 5-FU and saponin were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). All other chemicals were of reagent grade.

Animal Experiments All experiments were carried out in accordance with the Guidelines for Animal Experimentation of Nagasaki University. Male Wistar rats (260—310 g) were anesthetized with sodium pentobarbital (50 mg/kg i.p.). After the right peritoneum was dissected about 3 cm, a cylindrical diffusion cell (i.d. 9 mm, effective area 0.64 cm^2) was attached to the right kidney surface with adhesive (Aron Alpha, Sankyo Co., Ltd., Tokyo) (Chart 1). Saponin was dissolved with 5-FU (10 mg/ml×0.5 ml, isotonic phosphate-buffered saline, pH 7.4), and added to the diffusion cell directly. The body temperature of the rats was maintained at 37 °C with a heat lamp during the experiment. The left femoral artery was cannulated with a polyethylene tube (i.d. 0.5 mm, o.d. 0.8 mm, Dural Plastics, Dural, Australia), and blood was collected at the predetermined periods. For systemic and intrarenal distribution experiments, the rats were sacrificed at the predetermined periods, and the left kidney, right kidney, liver, spleen, heart, and lung were removed. To evaluate the intrarenal distribution of 5-FU, the right kidney was divided from the reniportal structure, and then further separated into sites under and not under the diffusion cell (Chart 1). The tissues were weighed and homogenized in a two-fold volume of isotonic phosphate-buffered saline, pH 7.4.

5-FU Assay Procedures in Blood and Tissues The concentration of 5-FU in each tissue homogenate or blood was determined by modifying previously described methods.[4,5] Briefly, the tissue homogenates (300 μl) and blood samples (300 μl) were added to a solution of 5-bromouracil (20 μg/ml, 150 μl) dissolved in isotonic phosphate buffer (pH

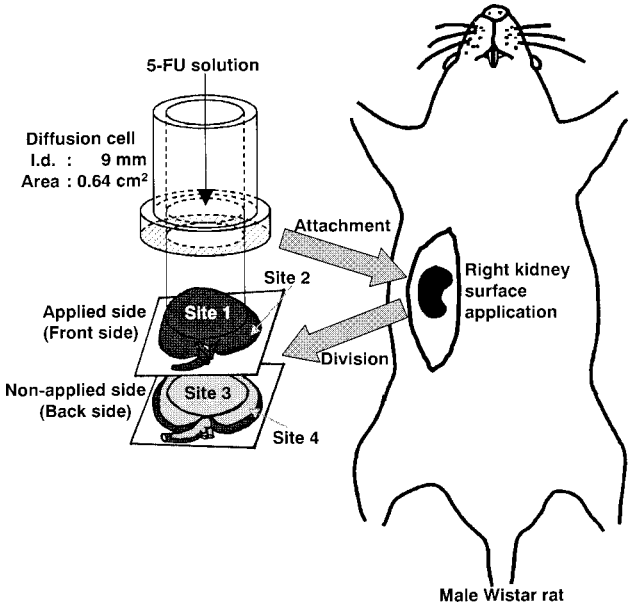

Chart 1. Experimental Procedures and Division of Kidney

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7.4) as an internal standard, 1 M sodium acetate buffer (pH 4.8, 100 μl), and 20% anhydrous sodium sulfate (500 μl). The mixtures were shaken with ethyl acetate (4 ml) for 10 min and centrifuged at 900×g for 10 min. The organic layers (3 ml) were collected. Ethyl acetate (4 ml) was then added to the residue and the mixtures were shaken for 10 min, and thereafter centrifuged at 900×g for 10 min. The organic layers (4 ml) were collected and the mixed organic layers (7 ml) were evaporated. The extraction residues were dissolved in 500 μl of distilled water and washed twice with 1.0 ml of hexane. Samples (100 μl) were injected onto the HPLC column. The recovery rates for extraction of 5-FU from blood and each tissue homogenate were approximately 75%. The detection limits of 5-FU in the blood and each tissue homogenate were 0.2 μg/ml. An HPLC system (LC-6A, Shimadzu Co., Ltd., Kyoto, Japan) with a variable-wavelength UV detector (SPD-10A, Shimadzu) was used in reverse-phase mode. The detector wavelength, flow rate, and column temperature were set at 266 nm, 0.7 ml/min, and 25 °C, respectively. The mobile phase consisted of 10 mM sodium acetate buffer (pH 4.0). The stationary phase used was a YMC-Pack ODS-A A-302 column (150 mm length× 4.6 mm i.d., YMC Inc., Kyoto).

Statistical Analysis Statistical comparisons were performed by analysis of variance with subsequent Dunnett's test. A $p<0.05$ was considered to be indicative of statistical significance.

RESULTS AND DISCUSSION

5-FU is commonly used in clinical oncology and is among the drugs active against renal cell carcinoma in the extensive list of drugs reviewed by Yagoda *et al.*[6] First, we selected an experimental system utilizing a cylindrical diffusion cell attached to the kidney surface to examine the effect of saponin on the intrarenal distribution of 5-FU following the kidney surface application of 5-FU in rats (Chart 1).

Figure 1 shows the intrarenal concentration of 5-FU 120 min after the surface application of 5-FU (5 mg) to the right kidney with saponin at concentrations of 0.125, 0.25 and 1 mg/ml in rats. Among the four sites in the right kidney, the concentration of 5-FU at site 1 was selectively increased by saponin, and was 1.4, 1.8, and 1.6-fold higher than control at saponin concentrations of 0.125, 0.25, and 1 mg/ml, respectively. Dose-dependency of saponin on the concentration of 5-FU at site 1 was not observed. In these experiments, the application of 5-FU with saponin at a concentration of 0.25 mg/ml showed the highest 5-FU concentration at site 1, and accordingly, a saponin concentration of 0.25 mg/ml was selected for the systemic distribution experiments.

Figure 2 shows the blood concentration of 5-FU until 120 min after right kidney surface application of 5-FU (5 mg) with saponin at a concentration of 0.25 mg/ml or without saponin. After right kidney surface application of 5-FU with or without saponin, the blood concentration was similar, and a low blood concentration was observed until 120 min.

Figure 3 shows the tissue concentrations of 5-FU 120 min after right kidney surface application of 5-FU (5 mg) with saponin at a concentration of 0.25 mg/ml or without saponin. After right kidney surface application of 5-FU without saponin, 5-FU was specifically detected in the right kidney, and was not detected in the left kidney or other tissues. After right kidney surface application of 5-FU with saponin, on the other hand, 5-FU was mainly detected in the right and left kidney, but was still not detected in the other tissues. After

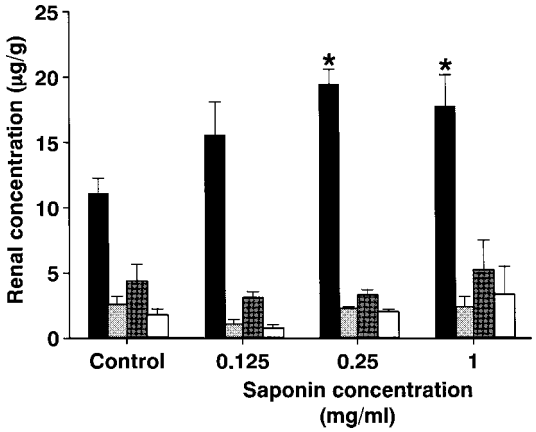

Fig. 1. Intrarenal Concentration of 5-FU 120 min after Right Kidney Surface Application of 5-FU (5 mg) with Saponin at Concentrations of 0.125, 0.25 and 1 mg/ml in Rats

5-FU was determined at site 1 (■), site 2 (▨), site 3 (▩), and site 4 (□). Statistical comparisons were performed by analysis of variance with subsequent Dunnett's test. (*$p<0.05$, significantly different from the control). Each value represents the mean±S.E. of at least four experiments.

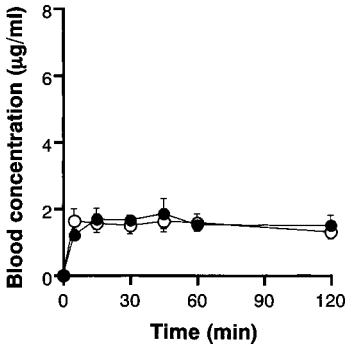

Fig. 2. Blood Concentration of 5-FU until 120 min after Right Kidney Surface Application of 5-FU (5 mg) with Saponin at a Concentration of 0.25 mg/ml (●) or without Saponin (○) in Rats

Each value represents the mean±S.E. of at least five experiments.

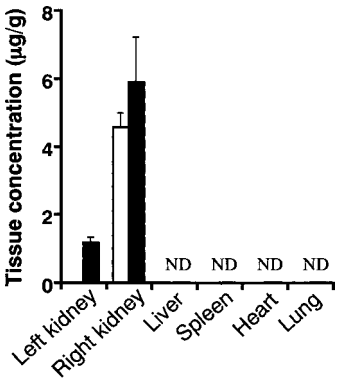

Fig. 3. Tissue Concentration of 5-FU 120 min after Right Kidney Surface Application of 5-FU (5 mg) with Saponin at a Concentration of 0.25 mg/ml (■) or without Saponin (□) in Rats

5-FU was determined in the left kidney, right kidney, liver, spleen, heart, and lung. ND: not detected. Each value represents the mean±S.E. of at least five experiments.

intravenous administration of 5-FU, 5-FU was mainly distributed to the right and left kidneys,[3] suggesting that the absorbed 5-FU, which was enhanced by saponin, might be distributed to the right and left kidneys. In Fig. 2, the blood concentration of 5-FU after right kidney surface application of 5-FU with saponin was not increased. These results may be due to the rapid transfer of 5-FU to the right and left kidneys from the blood.

With respect to strategies for drug administration methods on the kidney surface, we reported that liver site-selective drug accumulation was enhanced by gradually and continuously instilling a small amount of drug solution on the liver surface.[7] Recently, implantable infusion pumps have been developed for the treatment of several diseases,[8] and remarkable progress has been made in endoscopic and laparoscopic operation techniques.[9,10] Furthermore, continuous ambulatory peritoneal dialysis is an extremely common treatment modality for end stage renal failure[11]; consequently, the skill needed for inserting the catheter on the intraperitoneal organs should be improved in the future. Scheyer and Zimmermann[10] reported that a collagen fleece coated with fibrin glue could be inserted on liver and stomach by endoscopic surgery, suggesting that a dosage form, which could achieve the controlled drug release, could be applied to the kidney surface by endoscopic surgery. Taking these findings into consideration, the suitable medical skill could make possible the clinical application of 5-FU onto the kidney surface for the sustained and unilateral kidney site-selective delivery of 5-FU.

In summary, we have demonstrated that the intrarenal distribution of 5-FU following the kidney surface application of 5-FU in rats was modified by the absorption enhancer saponin. These results suggest the possibility of controlling the intrarenal distribution of the drug following its application together with an absorption enhancer on the kidney surface.

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