Further Studies on the Kidney- and Site-Selective Delivery of 5-Fluorouracil Following Kidney Surface Application in Rats

Junzo NAKAMURA,*[,a] Tomomi HORIMOTO,[a] Ryu HIRAYAMA,[a] Takahiro MUKAI,[a] Mikiro NAKASHIMA,[b] Hitoshi SASAKI,[b] and Koyo NISHIDA[a]

[a] *Graduate School of Biomedical Sciences, Nagasaki University; 1–14 Bunkyo-machi, Nagasaki 852–8521, Japan: and* [b] *Department of Hospital Pharmacy, Nagasaki University School of Medicine; 1–7–1 Sakamoto, Nagasaki 852–8501, Japan.* Received June 13, 2003; accepted September 2, 2003

The present study was undertaken to elucidate the kidney- and site-selective delivery of 5-fluorouracil (5-FU) following kidney surface application in rats. We selected an experimental system utilizing a cylindrical diffusion cell attached to the right kidney surface. After 5-FU was applied to this surface, approximately 60% was absorbed in 180 min. A semi-log plot of the remaining amount of 5-FU in the diffusion cell gave a straight line. The cumulative amount of urinary excretion of 5-FU for up to 180 min from the right ureter was significantly higher than that from the left ureter. On the other hand, the cumulative amount of urinary excretion of 5-FU from the right and left ureters after intravenous administration of the drug was similar. The 5-FU concentration at four sites in the right kidney after intravenous administration was also similar, while the drug was site-selectively delivered in the kidney after its surface application. 5-FU accumulated at the site under the diffusion cell was rapidly eliminated after its removal from the diffusion cell. From these results, we demonstrated that the absorption of 5-FU on the kidney surface in rats is explained mostly by passive diffusion. It was further elucidated that kidney surface application of this drug in rats results in its the kidney- and site-selective delivery.

Key words 5-fluorouracil; kidney; targeting; drug delivery system; rat; anticancer drug

5-Fluorouracil (5-FU) is commonly used in clinical oncology practice and is among the active drugs against renal cell carcinoma in the extensive list of anticancer drugs reviewed by Yagoda *et al.*[1] A renal cell carcinoma is normally located in a part of the unilateral kidney. Although the anticancer drugs are administered orally,[2] intravenously[3] and intraarterially[4,5] in the chemotherapy of renal cell carcinoma, they are distributed throughout the whole body *via* the bloodstream following the administration, leading to inadequate kidney- and site-selective drug delivery.

We previously elucidated that phenolsulphonphthalein as a model drug was adequately absorbed on the liver and gastric serosal surface, and accumulated site-selectively in the liver and stomach in rats.[6—8] In the previous report,[9] we also demonstrated the kidney- and site-selective delivery of 5-FU utilizing its absorption on the kidney surface in rats.

The present investigation was undertaken to gain further insight into the absorption mechanism, the urinary excretion and the intrarenal distribution following the application of 5-FU on the rat kidney surface. In the present study, the metabolite of 5-FU was not determined.

MATERIALS AND METHODS

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

Absorption and Intrarenal Distribution of 5-FU All experiments in the present study were carried out in accordance with the Guidelines for Animal Experimentation in 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 Aron Alpha (Sankyo Co., Ltd., Tokyo, Japan) (Chart 1). 5-FU (0.2, 1, 2 and 10 mg/ml×0.5 ml, isotonic phosphate-buffered saline, pH 7.4) was added to the diffusion cell directly. The body temperature of the rats was maintained at 37 °C with a heat lamp during the experiment. As a control experiment, 5-FU (10 mg/ml×0.5 ml) was injected into the jugular vein. After application to the kidney surface, the solution in the diffusion cell was sampled at appropriate intervals. For intrarenal distribution experiments, the rats were sacrificed at predetermined periods, and the right and left kidneys were removed. To evaluate the intrarenal distribution of 5-FU, the right kidney was divided from the reniportal structure, and was further separated into the site under the diffusion cell and the site not under this cell (Chart 1). The tissues were weighed and homogenized in a two-fold volume of isotonic phosphate-buffered saline, pH 7.4.

Urinary Excretion of 5-FU from Ureter The rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and the middle abdomen was dissected. The right and left ureters were cannulated with polyethylene tubes (i.d. 0.28 mm, Becton Dickinson & Co., NJ, U.S.A.). The cylindrical diffusion cell was attached to the right kidney surface with Aron Alpha, and 5-FU (10 mg/ml×0.5 ml) was added to the cell directly. As a control experiment, 5-FU (10 mg/ml×0.5 ml) was injected to the jugular vein, and at predetermined periods, the urine from the right and left ureters was collected.

Elimination of 5-FU from Kidney The rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.). After the right peritoneum was dissected about 3 cm, a cylindrical diffusion cell was attached to the right kidney surface with Aron Alpha, and 5-FU (10 mg/ml×0.5 ml) was added to the cell directly. After 30 min, the drug was removed from the diffusion cell and the kidney surface in the cell was washed with saline (0.5 ml) 3 times. At 2 and 5 min after the removal of 5-FU, the rats were sacrificed and the right kidney was removed. The right kidney was divided from the reniportal structure, and was further separated into the site under the

* To whom correspondence should be addressed. e-mail: junzo@net.nagasaki-u.ac.jp

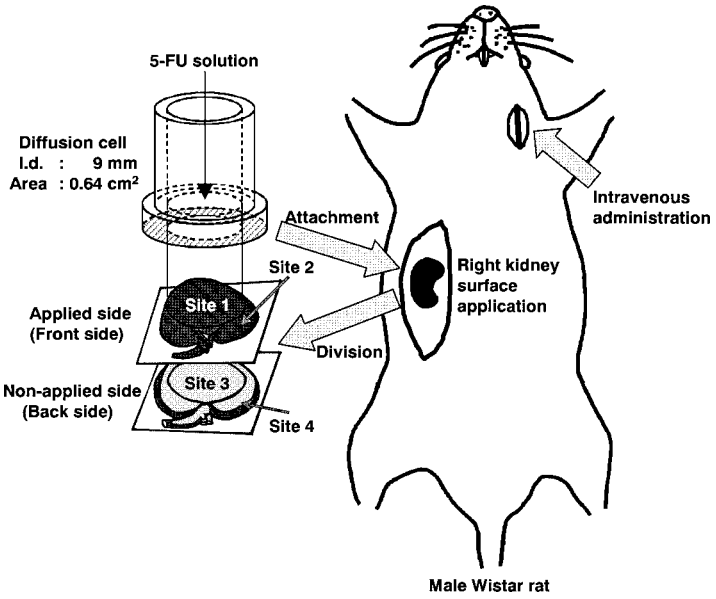

Chart 1. Experimental Procedures and Division of Kidney

diffusion cell and the site not under this cell. The tissues were weighed and homogenized in a two-fold volume of isotonic phosphate-buffered saline, pH 7.4.

5-FU Assay Procedures The concentration of 5-FU in each tissue homogenate, urine and solution in the diffusion cell was determined by modifying the reported methods.[10,11] Briefly, the tissue homogenates (300 μl), urine (300 μl) or solution in the diffusion cell (300 μl) was added to a solution of 5-bromouracil (20 μg/ml, 150 μl) dissolved in isotonic phosphate-buffered saline (pH 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. Then, ethyl acetate (4 ml) was 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 were washed twice with 1.0 ml of hexane. Samples (100 μl) were injected onto the HPLC column. The recovery rate for extraction of 5-FU from each tissue homogenate was approximately 75%. An HPLC system (LC-6A, Shimadzu Co., Ltd., Kyoto) 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).

RESULTS AND DISCUSSION

Chemotherapy against renal cell carcinoma has shown approximately less than a 20% response rate.[12] In the present study, we investigated the kidney- and site-selective delivery of 5-FU following its kidney surface application in rats. We selected an experimental system utilizing a cylindrical diffusion cell attached to the right kidney surface. This system enabled us to examine drug absorption from the kidney surface without interference by absorption from the other organs.

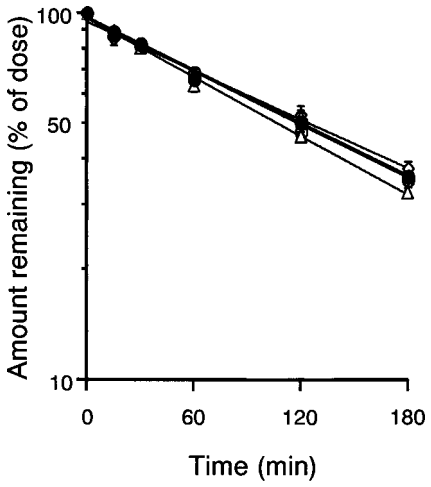

Fig. 1. Semi-log Plot of Time Course of Amount of 5-FU Remaining in the Diffusion Cell for 180 min Following the Right Kidney Surface Application of the Drug at Doses of 0.1 (◇), 0.5 (△), 1 (○) and 5 (●) mg in Rats

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

We examined the absorption characteristics of 5-FU following the application to the right kidney surface for 180 min at doses of 0.1, 0.5, 1 and 5 mg (Fig. 1), and found that approximately 60% of the drug was absorbed in 180 min at each dose. A semi-log plot of the remaining amount of 5-FU in the diffusion cell at each dose gave a straight line, indicating that its absorption on the kidney surface proceeds *via* a first-order process. The absorption rate constant of 5-FU at doses of 0.1, 0.5, 1 and 5 mg was calculated to be 0.0118± 0.0004, 0.0142±0.0004, 0.0127±0.0008 and 0.0130± 0.0011 min^{-1}, respectively. From these results, it is suggested that the absorption of the drug on the kidney surface is explained mostly by passive diffusion.

We then examined the urinary excretion following the ap-

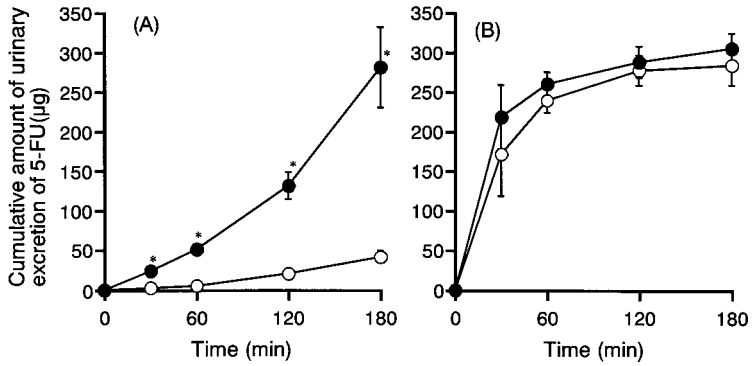

Fig. 2. Cumulative Amount of Urinary Excretion of 5-FU for 180 min Following the Drug's Right Kidney Surface Application (A) or Intravenous Administration (B) at a Dose of 5 mg in Rats

5-FU in the urine from right (●) and left (○) ureters was determined. Statistical comparisons were performed by Student's *t*-test (∗$p<0.001$, significantly different from the urine from the left kidney). Each value represents the mean±S.E. of at least six experiments.

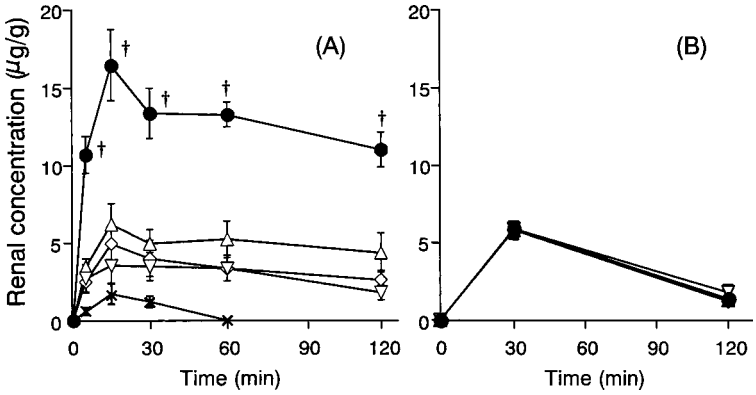

Fig. 3. Intrarenal Concentration of 5-FU for 120 min Following the Right Kidney Surface Application (A) or Intravenous Administration (B) of 5-FU at a Dose of 5 mg in Rats

5-FU at the site 1 (●), site 2 (◇), site 3 (△), site 4 (▽) and left kidney (×) was determined. Statistical comparisons were performed by analysis of variance with subsequent Dunnett's test (†$p<0.01$, significantly different from the other sites and left kidney). Each value represents the mean±S.E. of at least four experiments.

plication of 5-FU on the right kidney surface. As shown in Fig. 2, the cumulative amount of urinary excretion of 5-FU for 180 min in the urine from the right ureter was significantly higher than that from the left ureter. In contrast, the cumulative amount of urinary excretion of 5-FU from the right and left ureters after the drug's intravenous administration was similar. As shown in Fig. 2A, however, the underlying mechanism by which the rate of urinary excretion of 5-FU increased with time remains to be clarified in the future. From these results, 5-FU absorbed on the right kidney surface is thought to be excreted in the urine from the right ureter prior to its distribution into the systemic circulation, indicating a decrease of the systemic side effects of 5-FU.

Figure 3 shows the intrarenal distribution of 5-FU for 120 min after its right kidney surface application or intravenous administration. To evaluate the intrarenal distribution, the right kidney was divided from the reniportal structure, and further separated into the site under the diffusion cell and the site not under the diffusion cell. 5-FU concentration at four sites of the right kidney after intravenous administration was similar at 30 and 120 min. In contrast, the concentration at site 1 after right kidney surface application was significantly higher than those at sites 2, 3 and 4. Thus, the kidney surface application of 5-FU is useful for kidney- and site-selective delivery.

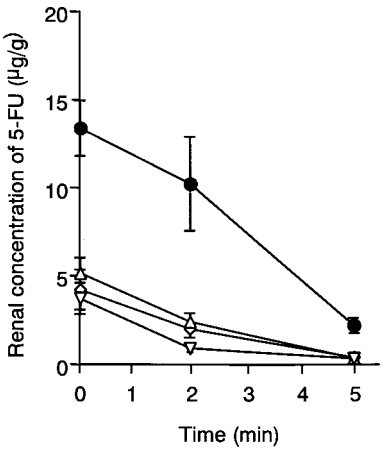

Fig. 4. Amount of 5-FU Remaining for 5 min in the Kidney Following the Right Kidney Surface Application at a Dose of 5 mg for 30 min in Rats

5-FU (10 mg/ml×0.5 ml) was added to the diffusion cell directly, and after 30 min it was removed from the cell. At 2 and 5 min after the removal, the right kidney was removed. 5-FU at site 1 (●), site 2 (◇), site 3 (△) and site 4 (▽) was determined. Each value represents the mean±S.E. of at least four experiments.

It is important to examine the retention of 5-FU after its kidney- and site-selective delivery. Figure 4 shows the amount of 5-FU remaining for 5 min in the kidney following

its right kidney surface application at a dose of 5 mg for 30 min. 5-FU accumulated at site 1 was rapidly eliminated after its removal from the diffusion cell.

In summary, we demonstrated that the absorption of 5-FU on the kidney surface in rats is explained mostly by passive diffusion. Furthermore, the kidney- and site-selective delivery of 5-FU following its application on the kidney surface was elucidated. We previously reported the continuous microinstillation of phenolsulphonphthalein on the liver surface for liver site-selective delivery in rats.[6] Consequently, the medical technologies and dosage forms in the future should make possible the clinical application of anticancer drugs on the kidney surface for the treatment of renal cell carcinoma.

Acknowledgments This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan. The authors thank Hiromi Shibutani for technical assistance.

REFERENCES

1) Yagoda A., Abi-Rached B., Petrylak D., *Semin. Oncol.*, **22**, 42—60 (1995).
2) Stebbing J., Benson C., Eisen T., Pyle L., Smalley K., Bridle H., Mak I., Sapunar F., Ahern R., Gore M. E., *Br. J. Cancer*, **85**, 953—958 (2001).
3) Ellerhorst J. A., Sella A., Amato R. J., Tu S. M., Millikan R. E., Finn L. D., Banks M., Logothetis C. J., *Cancer*, **80**, 2128—2132 (1997).
4) Noda S., Konno S., Tanaka J., Yamada M., Yoshitake N., *Anticancer Res.*, **10**, 709—716 (1990).
5) Kato T., Sato K., Sasaki R., Kakinuma H., Moriyama M., *Cancer Chemother. Pharmacol.*, **37**, 289—296 (1996).
6) Nakamura J., Yoshida Y., Mera K., Mukai T., Nishida K., Sasaki H., *Biol. Pharm. Bull.*, **22**, 713—715 (1999).
7) Nakamura J., Tsurumaru A., Mera K., Mukai T., Nishida K., Sasaki H., *Pharm. Pharmacol. Commun.*, **5**, 519—522 (1999).
8) Mukai T., Tsurumaru A., Mera K., Nishida K., Nakamura J., Sasaki H., Sakaeda T., *Pharm. Pharmacol. Commun.*, **5**, 609—614 (1999).
9) Kawakami S., Horimoto T., Nishida K., Hirayama R., Mukai T., Nakashima M., Sasaki H., Sakaeda T., Nakamura J., *Biol. Pharm. Bull.*, **25**, 928—930 (2002).
10) Watanabe J., Hayashi Y., Iwamoto K., Ozeki S., *Chem. Pharm. Bull.*, **33**, 1187—1194 (1985).
11) Sawai Y., Yamaoka K., Ito T., Nakagawa T., *Biol. Pharm. Bull.*, **20**, 1313—1316 (1997).
12) Motzer R. J., Russo P., *J. Urol.*, **163**, 408—417 (2000).