Absorption and Distribution Characteristics of 5-Fluorouracil (5-FU) after an Application to the Liver Surface in Rats in Order to Reduce Systemic Side Effects

Yukinobu KODAMA,[a] Shintaro FUMOTO,[a] Junya NISHI,[a] Mikiro NAKASHIMA,[a] Hitoshi SASAKI,[b] Junzo NAKAMURA,[a] and Koyo NISHIDA*,[a]

[a] *Department of Clinical Pharmacy, 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 September 2, 2007; accepted February 25, 2008; published online February 28, 2008

The present study was undertaken to elucidate the absorption and distribution characteristics of 5-fluorouracil (5-FU) after its application to the liver surface in rats to examine the possibility of reducing the systemic side effects of this agent. 5-FU was applied to the surface of the liver by employing a cylindrical diffusion cell. Approximately 69% of the dose was absorbed in 360 min. The time course of the change in the amount of 5-FU remaining in the diffusion cell obeyed first-order kinetics. Also, a linear relationship was observed between the apparent permeability coefficient, P_{app}, and the reciprocal of the square root of the molecular weight of several compounds including 5-FU. The estimated P_{app} value of 5-FU was in good agreement with the experimental value. The plasma concentration of 5-FU was low ($<$1.2 μg/ml) until 360 min after the application. Following i.v. administration, 5-FU was rapidly eliminated from the plasma and could not be detected at 120 min. In the analysis of tissue distribution, the liver was divided into three sites; the region under the diffusion cell attachment site (site 1), the treated lobe excluding site 1 (site 2), and untreated lobes (site 3). After being administered i.v., 5-FU mainly distributed in the kidney, and the concentration in the liver was significantly lower than that in kidney, spleen, or heart. After its application to the liver surface, however, 5-FU preferentially distributed at site 1, and was not detected at the other sites or in other tissues. Thus, these results suggested the possibility of a reduction in the systemic side effect of 5-FU on its application to the liver surface.

Key words chemotherapy; 5-fluorouracil; liver surface; pharmacokinetics

Advanced or recurrence hepatocellular carcinoma is usually unresectable. Local treatments, such as percutaneous ethanol injection, subsegmental transcatheter arterial embolization, microwave coagulation therapy, and radiofrequency ablation, have been reported to be useful for treating patients with unresectable disease.[1)] In most patients with hepatocellular carcinoma, however, the disease progresses to an advanced stage for which effective local treatment is not available. For this stage, chemotherapy is only a palliative treatment, but the response rate of anticancer drugs against hepatocellular carcinoma is very low.[2)] Also, the efficacy of cancer chemotherapy is limited by side effects from anticancer drugs. In general, side effects might be caused by high blood concentrations and a non-specific systemic distribution of anticancer drugs.

Consequently, regional chemotherapy, including intra-arterial infusion,[3)] intrahepatic perfusion,[4,5)] and intratumoral injection,[6—8)] has been performed to improve the therapeutic effect of anticancer drugs on hepatocellular carcinoma. However, these routes of administration could not achieve an adequate delivery to target sites in the liver because of a distribution to the entire liver or rapid drainage into the systemic circulation from the injection site.

We originally proposed the direct application of a drug to the liver surface and elucidated the absorption mechanism of model compounds after their application to rat liver surface.[9—14)] In addition, we reported that application to the liver surface could achieve a site-selective delivery of drugs, including 5-fluorouracil (5-FU), to the liver.[15)]

In the present study, we investigated the absorption and distribution characteristics of 5-FU after its application to the liver surface in rats to examine the possibility of reducing its systemic side effects.

MATERIALS AND METHODS

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

Animal Experiments All animal experiments in the present study conformed to the Guidelines for Animal Experimentation in Nagasaki University. Male Wistar rats (260—310 g) were anesthetized with sodium pentobarbital (50 mg/kg i.p.). The left femoral artery was cannulated with polyethylene tubes. The body temperature of the rats was maintained at 37 °C with a heat lamp during the experiment. A 5-FU solution was prepared in an isotonic phosphate buffer (pH 7.4), and administered as follows.

Application to Rat Liver Surface: After the middle abdomen was cut open about 3 cm, a cylindrical diffusion cell (i.d. 9 mm, area 0.64 cm^2) was attached to the liver surface with Aron Alpha (Daiichisankyo Co. Ltd., Tokyo, Japan), and 5-FU (10 mg/ml$\times$0.5 ml) was added to the cell directly. The top of the diffusion cell was sealed with a piece of aluminum foil to prevent evaporation of the applied solution. Then, blood samples were collected at selected times (5, 15, 30, 45, 60, 120, 240, 360 min), and centrifuged. Also, the solution in the diffusion cell was withdrawn, followed by excision of the liver, kidney, spleen, lung, and heart. The excised liver was divided into three sites; the region under the diffusion cell attachment site (site 1), the treated lobe excluding the site 1 (site 2), and the untreated lobes (site 3). The tissues

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

were weighed, and then liver or other tissues were homogenized in three- or two-fold volumes of their weight of pH 7.4 isotonic phosphate buffer, respectively.

i.v. administration: 5-FU (10 mg/ml×0.5 ml) was injected into the jugular vein using a syringe with a needle (26 G×1/2″). Then, blood samples were collected at selected times (2, 5, 10, 15, 30, 45, 60, 90, 120 min), and centrifuged. In addition, the liver, kidney, spleen, lung, and heart were excised at predetermined times (15, 30, 60, 120, 180 min). In the case of application to the liver surface, the excised liver was divided into three sites and the tissues were weighed and homogenized.

5-FU Assay Procedures The concentration of 5-FU in each tissue homogenate, plasma sample, or the solution remaining in the diffusion cell was determined by modifying reported methods.[16,17] Briefly, the tissue homogenate, plasma sample, or solution remaining in the diffusion cell (300 μl) was added to a solution of 5-bromouracil (20 μg/ml, 150 μl) dissolved in isotonic phosphate buffer (pH 7.4) as an internal standard, 1 M sodium acetate buffer (pH 4.8, 100 μl), and 20% anhydrous sodium sulfate solution (500 μl). The mixtures were shaken with ethyl acetate (4 ml) for 10 min, and thereafter 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 extracted residues were dissolved in 500 μl of distilled water and washed twice with 1 ml of hexane. Samples (100 μl) were injected into the HPLC column. An HPLC system (LC-6A, Shimadzu Co., Ltd., Kyoto, Japan) with a variable-wavelength UV detector (SPD-10A, Shimadzu) was used in the 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 Cosmosil $5C_{18}$-MS-II packed column (150 mm length×4.6 mm i.d. connected with 150 mm length×4.6 mm i.d., Nacalai Tesque, Inc.).

Calculation of Moment Parameters The plasma concentration–time profile of 5-FU until 360 min after the i.v. administration or application to the liver surface was analyzed based on the statistical moment theory.[18] Moment parameters for the plasma concentration-time profile up to 360 min ($AUC_{\text{plasma, 0—360}}$) were calculated by numeral integration using a linear trapezoidal formula.

Statistical Analysis Statistical comparisons were performed with the unpaired Student's *t*-test. $p<0.05$ was considered to be statistically significant. All values were expressed as the mean value±standard error (S.E.) of at least three experiments.

RESULTS AND DISCUSSION

Absorption Characteristics of 5-FU after Its Application to the Rat Liver Surface The extent to which 5-FU was absorbed in 360 min after its application to the liver surface was calculated as 69.1% of the dose based on the amount recovered from the diffusion cell. To examine the absorption characteristics of 5-FU after its application, we studied the time course of the change in the amount of 5-FU in the diffusion cell. As shown in Fig. 1, a semi-log plot of the amount remaining in the diffusion cell gave a straight line (correlation coefficient: $r^2=0.996$), indicating that the absorption of 5-FU from the liver surface proceeds *via* a first-order process as with the compounds in previous reports.[9—11] The absorption rate constant k_a of 5-FU from the liver surface was calculated to be 0.0033 min^{-1}.

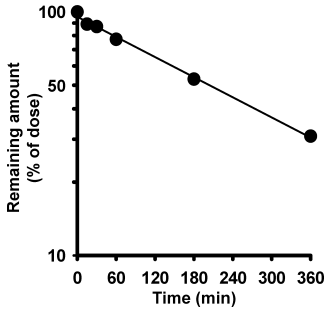

Fig. 1. Semi-Log Plot of the Amount of 5-FU Remaining in the Diffusion Cell at a Dose of 5 mg after Application to the Liver Surface in Rats

Each point represents the mean±S.E. of at least four experiments.

Previously, we have clarified that the rate of absorption from the liver surface correlated with the molecular weight of the compound.[9,11] We compared the absorption rate of 5-FU with that of several compounds examined previously. We calculated the apparent permeability coefficient, P_{app} (μm/min), of several compounds after their application to the liver surface, according to the following Eq. 1.

$$P_{app}=\frac{k_a \cdot V_a}{A_{cell}} \tag{1}$$

Where V_a is the application volume of drug solution, and A_{cell} is the application area of the diffusion cell.

Also, the following equation has been proposed with respect to drug absorption from the gastrointestinal mucosa *via* passive diffusion[19,20]:

$$\frac{1}{\sqrt{\text{MW}}\cdot P_{app}}=A+\frac{B}{P_a} \tag{2}$$

P_a represents the partition coefficient and constants A and B are the correction factor to P_a and constant for diffusion, respectively.

Because each compound is highly hydrophilic, the right-hand side of Eq. 2 can be transformed as a fixed number. Then, based on Eq. 2, we plotted P_{app} against the reciprocal of the square root of the molecular weight ($1/\sqrt{\text{MW}}$) of compounds with different molecular weights and 5-FU (Fig. 2). As shown in Fig. 2, a linear relationship was observed between the P_{app} and $1/\sqrt{\text{MW}}$ of several compounds including 5-FU (correlation coefficient: $r^2=0.949$) although there is a little difference. The estimated P_{app} value of 5-FU (22.9 μm/min) was in good agreement with the experimental value (25.8 μm/min).

Systemic Distribution of 5-FU after Application to the Rat Liver Surface It was reported that the systemic distribution and toxicity of 5-FU varied with the administration route.[21—24] In the case of hepatic arterial infusion, hepatic extraction of anticancer drugs resulted in minimal systemic exposure, potentially minimizing systemic side effect.[25] Then, we examined the systemic distribution of 5-FU after

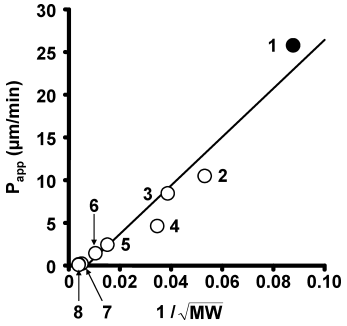

Fig. 2. Relationship between the Reciprocal of the Square Root of Molecular Weight ($1/\sqrt{MW}$) and P_{app} of the Compounds after Application to the Liver Surface in Rats

● represents the value obtained in this study. ○ represents the value obtained previously.[9,11] Key: 1, 5-FU; 2, phenolsulfonphthalein; 3, bromphenol blue; 4, bromosulfonphthalein; 5, fluorescein isothiocyanate-dextrans (FD-4, MW 4400); 6, FD-10 (MW 9300); 7, FD-40 (MW 40500); 8, FD-70 (MW 69000).[9,11]

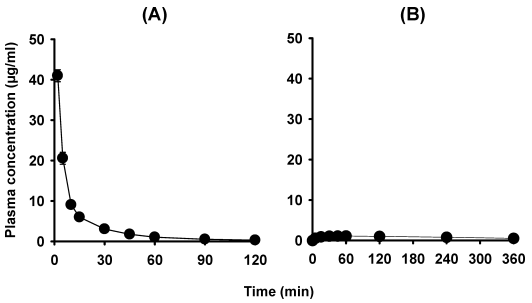

Fig. 3. Plasma Concentration Profile of 5-FU after (A) i.v. Administration or (B) Application to Liver Surface at a Dose of 5 mg in Rats

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

its application to the rat liver surface.

Figures 3A and B show the plasma concentration profiles of 5-FU after an i.v. administration or liver surface application, respectively. After the i.v. administration, 5-FU was rapidly eliminated from the plasma and could not be detected at 120 min (Fig. 3A). After the application of 5-FU to the liver surface, on the other hand, low plasma concentrations (<1.2 μg/ml) were observed until 360 min (Fig. 3B). The AUC_{plasma} of 5-FU was calculated as representative of systemic drug exposure. The AUC_{plasma} after application to the liver surface (306.6±24.9 μg·min/ml) was significantly smaller than that after i.v. administration (406.3±15.2 μg·min/ml). 5-FU is catabolised by dihydropyrimidine dehydrogenase, the rate-limiting enzyme that bring about first pass effect of 5-FU, in the liver. Also, the hepatic extraction ratio of 5-FU was reported to be higher than about 90%.[26] We considered that the clearance from the liver of 5-FU after application to liver surface is the metabolism in the liver. It would appear that the systemic drainage of 5-FU was suppressed by the hepatic extraction after the application to the liver surface.

Figures 4A and B illustrate the concentration profiles of 5-FU in the liver, kidney, spleen, lung, and heart until 180 min after i.v. administration or until 360 min after application to the liver surface of 5-FU, respectively. After i.v. administration, 5-FU mainly distributed in the kidney, and the concentration in the liver was significantly lower than that in kidney, spleen or heart at 15 min and could not be detected thereafter (Fig. 4A). Also, the concentrations of 5-FU at the three sites of the liver were almost the same at 15 min after i.v. administration (data not shown). After its application to the liver surface, on the other hand, 5-FU was preferentially distributed at site 1, and was not detected at the other sites or in other tissues (Fig. 4B). Therefore, these results suggest that the application of 5-FU to the liver surface suppressed drainage into the systemic circulation and other tissues, potentially minimizing the systemic side effect. However, further studies are necessary for us to investigate the possibility of accumulation to the liver tumor cells in tumor-bearing rats because distribution of 5-FU to tumor should be the most important issue for clinical application.

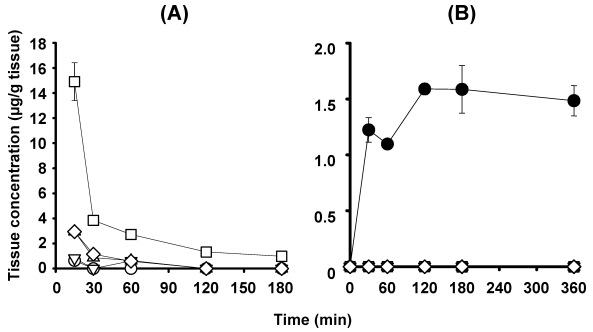

Fig. 4. Tissue Concentration Profiles of 5-FU after (A) i.v. Administration or (B) Application to the Liver Surface at a Dose of 5 mg in Rats

Key: (A) liver (○), kidney (□), spleen (△), lung (▽), heart (◇). (B) site 1 (●), kidney (□), spleen (△), lung (▽), heart (◇). Each point represents the mean±S.E. of at least four experiments.

In conclusion, we have demonstrated that 5-FU was absorbed from the surface of the liver in rats according to a first-order process and the absorption rate could be estimated from the molecular weight. Also, the 5-FU applied to the liver surface was preferentially distributed at site 1 and the plasma concentration of 5-FU was low until 360 min. These results suggested the possibility of reducing the systemic side effects of 5-FU by applying it to the liver surface.

Acknowledgments We wish to thank Masayo Shimomura and Miyuki Horishita for their skilled technical assistance. This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan, by a Grant-in-Aid from the Uehara Memorial Foundation, and by a Grant-in-Aid for Scientific Research from the President of Nagasaki University.

REFERENCES

1) Ikeda M., Okusaka T., Ueno H., Takezako Y., Morizane C., *Cancer*, **103**, 756—762 (2005).
2) Yamasaki T., Kurokawa F., Shirahashi H., Kusano N., Hironaka K., Masuhara M., Okita K., *Hepatol. Res.*, **23**, 7—17 (2002).
3) Ando E., Tanaka M., Yamashita F., Kuromatsu R., Yutani S., Fukumori K., Sumie S., Yano Y., Okuda K., Sata M., *Cancer*, **95**, 588—595 (2002).
4) Alexander H. R., Jr., Bartlett D. L., Libutti S. K., *Oncologist*, **5**, 416—424 (2000).
5) Nakamoto T., Inagawa H., Takagi K., Soma G., *Anticancer Res.*, **20**, 4087—4096 (2000).
6) Averbach A., Stuart O. A., Chang D., Sugarbaker P. H., *Eur. J. Surg. Oncol.*, **26**, 73—79 (2000).
7) Mok T. S. K., Kanekal S., Lin X. R., Leung T. W. T., Chan A. T. C., Yeo W., Yu S., Chak K., Leavitt R., Johnson P., *Cancer*, **91**, 2369—2377 (2001).

8) Vogl T. J., Engelmann K., Mack M. G., Straub R., Zangos S., Eichler K., Hochmuth K., Orenberg E., *Br. J. Cancer*, **86**, 524—529 (2002).
9) Nishida K., Sato N., Sasaki H., Nakamura J., *J. Pharm. Pharmacol.*, **46**, 867—870 (1994).
10) Nishida K., Sato N., Sasaki H., Nakamura J., *J. Pharm. Pharmacol.*, **47**, 227—231 (1995).
11) Nishida K., Sato N., Sasaki H., Nakamura J., *J. Drug Target.*, **4**, 141—150 (1996).
12) Nishida K., Sato N., Nakakoga Y., Mukai T., Sasaki H., Nakamura J., *J. Pharm. Pharmacol.*, **49**, 976—980 (1997).
13) Nakamura J., Yoshida Y., Mera K., Mukai T., Nishida K., Sasaki H., *Biol. Pharm. Bull.*, **22**, 713—715 (1999).
14) Nishida K., Yoshida Y., Mukai T., Kawakami S., Sakaeda T., Nakashima M., Sasaki H., Nakamura J., *J. Pharm. Pharmacol.*, **53**, 1341—1346 (2001).
15) Nishida K., Fujiwara R., Kodama Y., Fumoto S., Mukai T., Nakashima M., Sasaki H., Nakamura J., *Pharm. Res.*, **22**, 1331—1337 (2005).
16) Watanabe J., Hayashi Y., Iwamoto K., Ozeki S., *Chem. Pharm. Bull.*, **33**, 1187—1194 (1985).
17) Sawai Y., Yamaoka K., Ito T., Nakagawa T., *Biol. Pharm. Bull.*, **20**, 1313—1316 (1997).
18) Yamaoka K., Nakagawa T., Uno T., *J. Pharmacokinet. Biopharm.*, **6**, 547—558 (1978).
19) Koizumi T., Arita T., Kakemi K., *Chem. Pharm. Bull.*, **12**, 413—420 (1964).
20) Koizumi T., Arita T., Kakemi K., *Chem. Pharm. Bull.*, **12**, 421—427 (1964).
21) Mossa M. A., Osman A. M., El-Sayed M., El-Aaser A. A., Ismael N., El-Merzabani, *J. Pharm. Belg.*, **47**, 129—134 (1992).
22) Gianola F. J., Sugarbaker P. H., Barofsky I., White D. E., Meyers C. E., *Am. J. Clin. Oncol.*, **9**, 403—410 (1986).
23) Visser G. W., Gorree G. C., Peters G. J., Herscheid J. D., *Cancer Chemother. Pharmacol.*, **26**, 205—209 (1990).
24) Thrall M. M., Wood P., King V., Rivera W., Hrushesky W., *Int. J. Radiat. Oncol. Biol. Phys.*, **46**, 873—881 (2000).
25) Wagner J. G., Gyves J. W., Stetson P. L., Walker-Andrews S. C., Wollner I. S., Cochran M. K., Ensminger W. D., *Cancer Res.*, **46**, 1499—1506 (1986).
26) Warren B. S., LaCreta F. P., Kornhauser D. M., Williams W. M., *Cancer Res.*, **47**, 5261—5265 (1987).