Evaluation of Intestinal Absorption into the Portal System in Enterohepatic Circulation by Measuring the Difference in Portal–Venous Blood Concentrations of Diclofenac

Kenji Tabata,[1] Kiyoshi Yamaoka,[1,2] Takako Fukuyama,[1] and Terumichi Nakagawa[1]

Received July 27, 1994; accepted January 18, 1995

Purpose. We evaluated the first-pass effects in vivo by the intestine and liver during enterohepatic circulation (EHC) by simultaneously measuring the portal and venous plasma concentrations of the rat. ***Methods.*** The venous and upper portal blood vessels were cannulated through the jugular and the pyloric veins, respectively, to obtain simultaneously blood samples from both sites. After diclofenac was injected as a bolus through the jugular vein, the concentrations of diclofenac in the portal and jugular veins were measured at time intervals. The absorption rate from the intestinal tract into the portal system was determined using the portal–venous difference in plasma concentrations of diclofenac, considering 40% partitioning of diclofenac into erythrocytes. ***Results.*** After one hour, the plasma concentration in the portal vein was always higher than that in the jugular vein in awakening rats with intact EHC (portal–venous blood concentration difference). No portal–venous difference was observed in awakening rats with bile-duct cannulation. Therefore, it was concluded that this portal–venous concentration difference was not due to the hepatic clearance but to diclofenac reabsorption from the intestinal tract. ***Conclusions.*** Appropriately 40% of the dose of diclofenac was reabsorbed over 8 hours from the intestinal tract into the portal system. By comparing the reabsorbed amounts in the portal system and in the systemic circulation, the hepatic extraction ratio in vivo (F_H) of diclofenac was estimated to be 63%.

KEY WORDS: portal–venous blood concentration difference; enterohepatic circulation; diclofenac; portal system; pharmacokinetics.

INTRODUCTION

Many drugs are subject to the first-pass effect, when absorbed from the GI tract into systemic circulation. The organs responsible for this effect, that is, the intestine, the liver and the lung, are serially arranged and can potentially reduce the extent of bioavailability. To understand the process of absorption from the GI tract into the systemic circulation, it is necessary to estimate the contribution of these organs to the first-pass effect. The contribution of each organ has been assessed indirectly by comparison of AUC values obtained by the administration through different routes (1,2), for example, the intravenous and oral routes to evaluate systemic availability (3,4), and intravenous and intraportal infusions to estimate the hepatic extraction ratio (5,6). Direct evaluation of the contribution of various organs to the first-pass effect has been limited. The contribution of the lung was predicted by measuring the arterial-venous blood concentration difference (7). There are reports estimating the contribution of the intestine and the liver to the first-pass effect by the portacaval transposition method, that is, by exchanging the portal vein with the inferior vena cava (8,9). There are few papers to estimate absorbed drug amount from the GI tract into the portal system. Single-pass perfusion methods in situ through the intestine have been attempted to evaluate elimination from the GI tract (10,11). Recently, a method using a perfused rat intestine-liver preparation in situ was developed to directly ascertain the contributions of the intestine and the liver in the first-pass effect (12,13). However, the obtained data may not reflect the absorption characteristics from the GI-tract into the portal system in the living rat. Diclofenac has been used as a marker for enterohepatic circulation (14,15,16). Enterohepatic circulation (EHC), has been studied experimentally using the linked-rat method (17), and evaluated theoretically (18–21). The present report introduced a new technique for evaluating the amount of drug absorbed from the intestinal tract into the portal system by simultaneously measuring the portal and venous plasma concentrations. Diclofenac, which is known to be subject to EHC, was used as the marker drug.

MATERIALS AND METHODS

Chemicals

Diclofenac sodium was obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Heparin was obtained from Novo Industries (Denmark). Other reagents used for the animal experiment and for the assay of diclofenac were of reagent grade.

Animal Experiment

Male Wistar rats weighing from 180 to 232 g were used in this study. Rats were fasted for 15 hr were anesthetized with a light ether. After the abdomen was opened through a middle incision, the first cannula filled with heparinized (100 IU/mL) normal saline was implanted in the upper part on the portal system through the pyloric vein (6). The free end of the cannula was drawn out through the incision on the abdomen. At the same time, the right jugular vessel of each rat was cannulated and the free end of the second cannula filled with heparinized (100 IU/mL) normal saline, was subcutaneously conducted and drawn out at the top of the neck. Then, the rats were divided to two groups, one with bile-duct cannulation (group A) and the other with intact enterohepatic circulation (group B). In group A, a third cannula was implanted into the bile duct and the free end of the cannula was drawn out through the incision on the abdomen. Each rat was held in the Bollman gauge and was allowed to recover for 30 min. Water was freely taken by every rat. After all surgeries, diclofenac sodium (5 mg/kg) dissolved in an aqueous solution of 30% polyethylene glycol was instanta-

[1] Faculty of Pharmaceutical Science, Kyoto University, Sakyo-ku, Kyoto 606, Japan.
[2] To whom correspondence should be addressed.

0724-8741/95/0600-0880$07.50/0

neously administrated into the venous system through the cannula in the jugular vein. Blood samples (0.11 mL) were drawn simultaneously from both cannulas through the portal vein and the jugular vein at 5, 15 and 30 min, and at 1, 2, 3, 4, 6 and 8 hr after administration. Concerning the group with bile duct cannulation, plasma samples were measured for the first 1 hr. At each sampling, the volume of blood reduction was supplemented with an equal volume of saline. After the blood samples were centrifuged for 5 min at 2000 g, the separated plasma samples were stored at −20°C until analysis.

The research adhered to the "Principles of Laboratory Animals Care" (NIH publication #85-23, revised 1985).

Experiment for Distribution into Erythrocyte

When a drug is widely distributed in the erythrocytes and the plasma concentration (C_p) is measured instead of the blood concentration (C_b), the blood flow rate (Q_b) can be replaced by the effective plasma flow rate ($\overline{Q}_p$) by the following equation (7).

$$\overline{Q}_p/Q_b = C_b/C_p = (1 - H_t)(1 + k_b) \quad (1)$$

where H_t is the hematocrit (0.46) and k_b is the partition ratio of the drug between erythrocyte and plasma.

The partition ratio (k_b) of diclofenac between plasma and erythrocytes was evaluated using heparinized whole blood. After pre-incubation of 0.5 ml blood at 37°C, a small volume of drug saline solution was added to produce the standard blood solutions (10, 35, 50 and 75 μL/mL). The blood samples were incubated for 10 min at 37°C, then centrifuged for 5 min at 2000 g, and the plasma concentrations were measured by HPLC to estimate C_b/C_p. The k_b of diclofenac was calculated according to Eq. (1). Thus, k_b of diclofenac was estimated to be 0.41 ± 0.04.

Assay Procedure

The diclofenac concentration in plasma was determined by HPLC reported by El-Sayed et al (22). HPLC (LC-10A, Shimadzu Co., Kyoto, Japan) was used with a stationary phase of Chemcosorb 5-ODS-H (150 × 4.6 mm id). The detector wavelength, the flow rate and the column temperature were 280 nm, 1.0 ml/min and 40°C, respectively. The peak area was measured with Chromatopac C-R6A (Shimazdu, Co.). The mobile phase was composed of H_2O: CH_3CN (1:1, v/v) which was adjusted at pH = 3.3 with small amount of acetic acid. Calibration lines were freshly prepared by spiking plasma with diclofenac, making four points in a range from 0.01 to 20 μL/mL. All correlation coefficients were above 0.999.

Data Analysis

The absorption rate dA(t)/dt from the intestinal tract into the portal system was calculated by Eq. (2).

$$\begin{aligned} dA(t)/dt &= Q_b\,(C_b^P(t) - C_b^v(t)) \\ &= Q_p(C_p^P(t) - C_p^v(t)) \end{aligned} \quad (2)$$

where Q_b and $\overline{Q}_p$ are blood and effective plasma flow rates in the portal vein, respectively, and $C_b(t)$ and $C_p(t)$ are the time courses of blood and plasma concentrations, respectively. The superscripts p and v specify portal and venous concentrations, respectively. Since blood flow rate Q_b was given as 9.8 mL/min for the rat (250 g) (23), the effective plasma flow rate $\overline{Q}_p$ was calculated according to the following equation by taking the weight of the rat into the consideration.

$$\overline{Q}_p = 9.8 \cdot (1 - H_t) \cdot (1 + k_b) \cdot W_t/250 \quad (3)$$

where W_t is the weight of the rat.

The absorption ratio to the dosing amount was calculated by a trapezoidal integration according to Eq (4).

$$F_a(t) = \int_0^t \frac{dA(t)}{dt}\,dt \Big/ D \quad (4)$$

where $F_a(t)$ is the absorption ratio and D is the dose of diclofenac sodium.

The area under the curve (AUC) and the mean residence time (MRT) were calculated by the trapezoidal integration without extrapolation (24), because the time profile around the secondary peak was too unstable to extrapolate. Since the plasma concentrations at 8 hr were approximately 1% of the maximal concentration, it was assumed that the truncation errors were negligible.

RESULTS AND DISCUSSION

Figures 1(A) and 1(B) present the time courses of portal and venous plasma concentrations of diclofenac in rats with bile-duct cannulation to prevent EHC (group A), and in rats with intact EHC (group B). Each point in the figures represents the mean of 4 rats. While a portal–venous concentration difference was not observable in Fig. 1 (A), the portal concentration was larger than the venous concentration after 1 hr following the intravenous administration in Fig. 1(B). Diclofenac is known to be subject to EHC and is reabsorbed with approximately 1 hr lag time which is required for movement through the intestinal tract as a glucuronide and for its

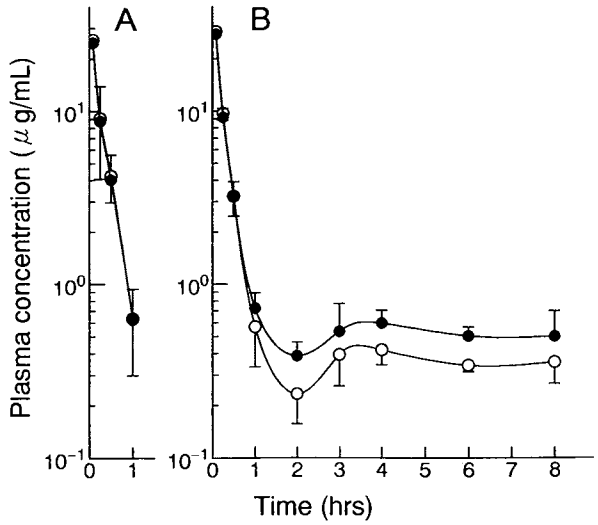

Fig. 1. Mean plasma concentration time courses of diclofenac after intravenous administration (5 mg/kg) with bile-duct cannulation (A), with intact enterohepatic circulation (B). ● and ○ represent portal and venous blood, respectively. Each point represents mean and SD (n = 4).

deglucuronization (16). Therefore, the concentration difference after 1 hr is due to the reabsorption of diclofenac from the intestinal tract into the portal system. Tables 1(A) and 1(B) present AUC and MRT of the time course of portal and venous plasma concentrations in group A and group B, respectively. There was a difference in AUC but no difference in MRT between the portal and the venous concentrations in group A, while both AUC and MRT significantly differed from those in group B at a 5% level of significance on two-way ANOVA. AUC of the time course of portal concentration was 2.5% smaller than that of venous concentrations in group A, which was presumably due to the portal blood being slightly diluted with substances such as water absorbed from the GI tract. It is noted here that every rat in the Bollman gauge was freely taking water during this study. Thus, the definite portal–venous concentration difference in Fig. 1 (B) and Table 1(B) is explained not by the hepatic clearance but by the reabsorption of diclofenac from the intestinal tract into the portal system. In the group with intact EHC, the finite-time AUC of the portal plasma calculated using data for the first 1 hour was 9.02 ± 1.42 μg · hr/mL, while that of the venous plasma was 10.0 ± 2.74 μg · hr/mL. Differences in finite-time AUC values of portal and venous plasma were not significant between the group with bile-duct cannula and that with intact EHC at a 5% level of significance on one-way ANOVA. The coincidence in the time courses of plasma concentration within the first 1 hour demonstrates that the effect of enterohepatic circulation of diclofenac is negligible within the first 1 hour.

Figures 2(A) and 2(B) present the time courses of the absorption rate and the absorption ratio of diclofenac, respectively, according to Eqs (2) and (3) using the portal–venous concentration difference in Fig. 1(B). The present result agrees with that of the previous study (16). It is shown in Fig. 2(B) that approximately 40% of the diclofenac dose

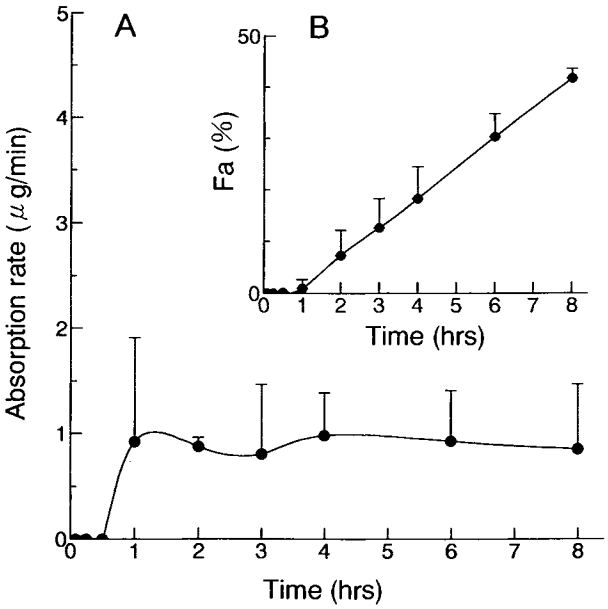

Fig. 2. Predicted time courses of absorption rate (A) and absorbed ratio [F_a(%)] (B). Each point represents mean and SD (n = 4).

was reabsorbed from the intestinal tract. Since the plasma concentration was negligible at 8 hr, it is speculated that a considerable amount of diclofenac (or its metabolites) is eliminated through the feces and urine (25). We previously demonstrated by comparison of the AUC values in the groups with and without EHC that about 25% of diclofenac was reabsorbed from the intestinal tract into the systemic circulation over 8 hr (16). Thus, the hepatic recovery ratio in vivo (F_H) of diclofenac is estimated to be 0.63 (= 0.25/0.40). It is shown that about half of diclofenac is extracted by the

Table 1. Moment Characteristics of Diclofenac in Two Groups over 8 hrs Intravenous Administration. A and B Present Moments in Rats with Bile–Duct Cannula and Those with Intact Enterohepatic Circulation, Respectively

	RAT-A	RAT-B	RAT-C	RAT-D	Mean ± SD
A: Group with bile-duct cannula					
Weight (g)	196	200	232	221	212 ± 16
Q_p (mL/min)	5.86	5.98	6.94	6.61	6.35 ± 0.49
AUC (μg · hr/mL)					
Portal blood	9.71	7.60	7.81	9.09	8.55 ± 1.02
Venous blood	9.85	7.81	8.11	9.31	8.77 ± 0.97
MRT (hr)					
Portal blood	0.24	0.18	0.20	0.22	0.21 ± 0.02
Venous blood	0.24	0.18	0.20	0.22	0.21 ± 0.03
	RAT-1	RAT-2	RAT-3	RAT-4	Mean ± SD
B: Group with intact enterohepatic circulation					
Weight (g)	188	195	179	195	189 ± 8
Q_p (mL/min)	5.62	5.83	5.36	5.83	5.66 ± 0.23
AUC (μg · hr/mL)					
Portal blood	11.6	12.1	13.2	11.3	12.1 ± 1.0
Venous blood	10.8	11.0	12.3	10.4	11.1 ± 1.0
MRT (hr)					
Portal blood	1.48	1.77	1.54	1.46	1.60 ± 0.16
Venous blood	1.14	1.26	1.11	1.09	1.20 ± 0.09

first-pass effect through the liver. The hepatic plasma flow rate Q_p was calculated to be 4.23 mL/min by the following equation.

$$Q_p = 9.80\ (1 - Ht)\ Wt/250 \quad (5)$$

Thus, the hepatic plasma clearance CL_H is estimated to be 1.57 mL/min (=4.23 × 0.37). Total plasma clearance CL is estimated to be 2.01 mL/min (=1060/8.77/60) in the group with bile-duct cannula using the data in Table 1. It is concluded that diclofenac is mainly metabolized by the liver. Free diclofenac is negligible in the urine and the major portion is excreted as the metabolites in the urine (14,26). The present result is in agreement with previously published reports.

In conclusion, we succeeded in directly observing the absorption of a drug from intestinal tract into the portal system in the awakening rat, and it is expected that this new technique offers an effective tool for analyzing the kinetic process through the intestinal wall.

REFERENCES

1. M. Rowland. Influence of route of administration on drug availability. *J. Pharm. Sci.* 61:70–74 (1972).
2. K. S. Pang and J. R. Gillette. Theoretical relationships between area under the curve and route of administration of drugs and their precursors for evaluating sites and pathways of metabolism. *J. Pharm. Sci.* 67:703–704 (1978).
3. P. A. Harris and S. Riegelman. Influence of the route of administration on the area under the plasma concentration-time curve. *J. Pharm. Sci.* 58:71–75 (1969).
4. M. Gibaldi, R. N. Boyes, and S. Feldman. Influence of first-pass effect on availability of drugs on oral administration. *J. Pharm. Sci.* 60:1338–1340 (1971).
5. S. L. Bramer, J. L.-S. Au, and M. G. Wientjes. Gastrointestinal and hepatic first-pass elimination of 2′, 3′-dideoxyinosine in rats. *J. Pharmacol. Exp. Ther.* 265:731–738 (1993).
6. T. Suzuki, Y. Saitoh, S. Isozaki, and R. Ishida. Drug absorption and metabolism study by use of portal vein infusion in the rat. I. Pyloric vein cannulation and its application to study of first-pass effect on bioavailability of Propranolol. *Chem. Pharm. Bull.* 20(12):2731–2735 (1972).
7. Y. Yano, K. Yamaoka, H. Yasui, and T. Nakagawa. Analysis of arterial-venous blood concentration difference of drugs based on recirculatory theory with FILT. *J. Pharmacokinetic. Biopharm.* 19:71–85 (1991).
8. D. J. Effeney, S. M. Pond, M.-W. Lo, B. M. Silber, and S. Riegelman. A technique to study hepatic and intestinal drug metabolism separately in the dog. *J. Pharmacol. Exp. Ther.* 221:507–511 (1982).
9. M.-W. Lo, D. J. Effeney, S. M. Pond, B. M. Silber, and S. Riegelman. Lack of gastrointestinal metabolism of propranolol in dogs after portacaval transposition. *J. Pharmacol. Exp. Ther.* 221:512–515 (1982).
10. J. T. Dolusio, N. F. Billups, L. W. Dittert, E. T. Sugita, and J. V. Swintosky. Drug Absorption I: An in situ rat gut technique yielding realistic absorption rates. *J. Pharm. Sci.* 58:1196–1200 (1969).
11. T. Hirasawa, T. Muraoka, A. Karino, M. Hayashi, and S. Awazu. Solvent drug in jejunal absorption of salicylic acid and antipyrine obtained by in situ single-pass perfusion method in rat. *J. Pharm. Dyn.* 7:246–253 (1984).
12. K. S. Pang, W. F. Cherry, and E. H. Ulm. Disposition of enalapril in the perfused rat intestine-liver preparation: absorption, metabolism and first-pass effect. *J. Pharmacol. Exp. Ther.* 233:788–795 (1985).
13. H. Hirayama, X. Xu, and K. S. Pang. Viability of the vascularly perfused, recirculating rat intestine and intestine-liver preparations. *Am. J. Physiol.* 255:G249–258 (1989).
14. J. E. Peris-Ribera, F. Torres-Molina, M. C. Garcia-Carbonell, J. C. Aristorena, and J. M. Pla-Delfina. Pharmacokinetics and bioavailability of diclofenac in the rat. *J. Pharmacokinetic. Biopharm.* 19:647–665 (1991).
15. T. Tsuchiya, M. Terakawa, K. Ishibashi, H. Noguti, and R. Kato. Disposition and enterohepatic circulation of diclofenac in dogs. *Arzneim.-Forsch./Drug Res.* 30:1650–1653 (1980).
16. T. Fukuyama, K. Yamaoka, Y. Ohata, and T. Nakagawa. A new analysis method for disposition kinetics of enterohepatic circulation of diclofenac in rats. *Drug. Metab. Dispos.* 22:479–485 (1994).
17. H. Kotaki, Y. Yamamura, Y. Tanimura, Y. Saitoh, F. Nakagawa, and Z. Tamura. Enterohepatic circulation of clioqinol in the rat. *J. Pharm. Dyn.* 7:420–425 (1984).
18. K. Singh, J. M. Orr, and F. S. Abbott. Pharmacokinetics and enterohepatic circulation of (E)-2-ene valproic acid in the rat. *J. Pharm. Dyn.* 13:622–627 (1990).
19. K. Singh, J. M. Orr, and F. S. Abbot. Pharmacokinetics and enterohepatic circulation of 2-n-propyl-4pentenoic acid in the rat. *Drug. Metab. Dispos.* 16:848–852 (1988).
20. T. A. Shepard and R. H. Reuning. An equation for the systemic availability of drugs undergoing simultaneous enterohepatic cycling, first-pass metabolism, and intestinal elimination. *Pharm. Res.* 4:195–199 (1987).
21. K. Yamaoka, M. Kanba, Y. Toyoda, Y. Yano, and T. Nakagawa. Analysis of enterohepatic circulation of cefixime in the rat by fast inverse Laplace transform (FILT). *J. Pharmacokinetic. Biopharm.* 18:545–559 (1990).
22. Y. M. El-Sayed, M. E. Abdel-Hameed, M. S. Suleiman, and N. M. Najib. A rapid and sensitive high-performance liquid chromatographic method for the determination of diclofenac sodium in serum and its use in pharmacokinetic studies. *J. Pharm. Pharmacol.* 40:727–729 (1988).
23. B. Davies, T. Morris. Physiological parameters in laboratory animals and humans. *Pharm. Res.* 10:1093–1095 (1993).
24. K. Yamaoka, T. Nakagawa, and T. Uno. Statistical moments in pharmacokinetics. *J. Pharmacokinetic. Biopharm.* 6:547–558 (1978).
25. W. Riess and H. Stierlin. Pharmacokinetics and metabolism of the anti-inflammatory agent Voltaren. *Scand. J. Rheumatology, Suppl.* 22:17–29, 1978.
26. H. Stierlin, J. W. Faigle, A. Sallmann, and W. Kung. Biotransformation of diclofenac sodium (Voltaren) in animals and in man. *Xenobiotica* 9, no. 10,601–610, 1979.