J. Pharm. Pharmacol. 1999, 51: 585–589
Received August 14, 1998
Accepted December 19, 1998

Effect of Pentobarbital Anaesthesia on Intestinal Absorption and Hepatic First-pass Metabolism of Oxacillin in Rats, Evaluated by Portal–Systemic Concentration Difference

SHINYA UEDA, KIYOSHI YAMAOKA AND TERUMICHI NAKAGAWA

Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan

Abstract

The effects of anaesthesia on intestinal drug absorption and hepatic first-pass metabolism in rats were investigated by observing the difference in the drug concentration between portal and systemic bloods. Oxacillin and pentobarbital were selected as a model drug and as an anaesthetic, respectively. Rats were divided into a conscious control group and an anaesthetized group. All rats were cannulated simultaneously in the portal vein and in the femoral artery, and oxacillin was orally administered after its intra-arterial injection (double dosing). For the anaesthetized group, pentobarbital was intrasubcutaneously administered twice, first before intra-arterial injection and again before oral administration of oxacillin. The arterial blood alone was sampled from the cannula in the femoral artery before oral administration, whereas the arterial and portal bloods were simultaneously sampled from both cannulated sites after oral administration. Oxacillin concentrations in plasma were assayed by HPLC.

The anaesthesia increased the absolute bioavailability (F), the mean absorption time (MAT) and the hepatic recovery ratio (F_H), but caused little change in the local absorption ratio into the portal system (F_a) and the total clearance (CL). The hepatic clearance (CL_H) was significantly decreased, resulting in an apparent small change in $CL-CL_H$ which is considered to be renal clearance.

By this method, it was shown directly that an increase in F due to pentobarbital anaesthesia was attributable to the significant increase in F_H. It is expected that the method is useful not only to evaluate the effect of anaesthesia on the first-pass effect, but also to assess the effect of co-administration of drugs on first-pass metabolism.

Drugs are administered to patients usually in a conscious condition, but are given to laboratory animals in an anaesthetized condition. Urethane, pentobarbital and ether are often used as anaesthetics. However, it has been suggested that anaesthesia can affect the disposition of a co-adminstered drug, for example the effects on intestinal absorption, hepatic first-pass metabolism and renal elimination. It has been shown by intestinal perfusion experiments that urethane, ether, ketamine and pentobarbital decreased intestinal absorption of both D- and L-glucose, although L-glucose is barely absorbed (Yuasa et al 1993). Yuasa et al (1995) reported on the basis of a theory by Amidon et al (1988) that 5-fluorouracil was absorbed through both passive and carrier-mediated pathways, and that the former was inhibited and the latter was promoted by pentobarbital. However, the effect of pentobarbital on intestinal absorption can differ between the perfusion experiment and living animals. In addition, since the bioavailability of a drug is affected not only by intestinal absorption but also by hepatic first-pass metabolism, the effect of pentobarbital anaesthesia should be evaluated on both first-pass processes. In-vivo, pentobarbital blocks the enterohepatic circulation of diclofenac (Fukuyama et al 1994; Tabata et al 1996). This was attributed to the inhibitory effect of pentobarbital on the biliary excretion of diclofenac (Fukuyama et al 1996).

Recently, an in-vivo experimental technique was developed to estimate separately the intestinal absorption kinetics into the portal system and the hepatic first-pass metabolism in a conscious rat.

Correspondence: K. Yamaoka, Graduate School of Phamaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan.

The method is based on the difference in drug concentration between the portal and systemic blood of rat (Hoffman et al 1995; Tabata et al 1995; Fujieda et al 1996; Sawai et al 1997a). By administering an intra-arterial dose of a drug followed by an oral dose to the same conscious rat in a short time-interval, we developed a new method for the simultaneous evaluation of intestinal absorption and hepatic first-pass effect of drugs from a single conscious rat (Ito et al 1997; Sawai et al 1997b).

The purpose of the present investigation was to evaluate quantitatively the effect of anaesthesia on the first-pass processes of a drug by applying this method. Oxacillin, a β-lactam antibiotic, is known to be considerably eliminated via a single-path through the liver and has no pharmacological effect on the portal system. The local disposition of oxacillin through the liver is reported using a perfusion experiment with a pulse-input (Yano et al 1990; Ohata et al 1996). Therefore, oxacillin and pentobarbital were selected as a target drug and an anaesthetic, respectively. The statistical test by analysis of variance was attempted to judge the effect of anaesthesia on the first-pass processes of oxacillin through the intestine and the liver.

Materials and Methods

Chemicals

Oxacillin was purchased from Sigma Co. (St Louis, MO). Sodium pentobarbital solution (Nembutal for animal injection) was purchased from Abbott Laboratories (North Chicago, IL). Heparin was obtained from Novo Industries (Denmark). Other chemicals and reagents were of analytical or HPLC grade.

Animal experiment

Healthy male Wistar rats, 235–276 g, were purchased from Shimizu Experimental Materials Co. Ltd (Kyoto, Japan) and allowed free access to standard chow and water. The rats were starved overnight, with free access to water, before the experiments. Under light anaesthesia with ether, a midline incision was made to open the abdomen of rats. A PE10 catheter filled with heparinized saline (100 U mL^{-1}) was inserted into the portal vein from the junction of portal vein and inferior pancreaticoduodenal vein, and the tip of a catheter was placed close to the liver. The catheter was secured to the mesentery with a drop of cyanoacrylate adhesive (Aron Alpha; Sankyo Co. Ltd, Japan). The free end of the catheter was exteriorized through a small puncture using an 18-gauge needle in the side abdominal wall. The right femoral artery of each rat was also cannulated, and the free side of the catheter filled with heparinized saline (100 units mL^{-1}) was subcutaneously conducted and exteriorized at the back of the leg. Each rat was held in a Bollman cage and was allowed to recover from the ether anaesthesia for at least 3 h. A small volume of arterial blood was collected from each rat for measuring haematocrit (H_t) and centrifuged for 5 min at 2000 *g*. The operated rats were divided into a conscious group and an anaesthetized group. All rats received intra-arterially oxacillin (25 mg mL^{-1}, 50 mg kg^{-1}), followed 2 h later by oral administration of oxacillin (25 mg mL^{-1} in saline) at the same dosage. For rats in group B, pentobarbital (30 mg kg^{-1}) was administered subcutaneously 30 min before the intra-arterial dose and 30 min before the oral dose of oxacillin (at twice the dosage of pentobarbital), to maintain anaesthesia. Blood (80 μL) from the femoral artery was sampled at 5, 15, 30, 60 and 120 min after the intra-arterial dose. Blood (80 μL) from both the femoral artery and the portal vein was sampled at 15, 30, 60, 90, 120 and 240 min after oral administration. After centrifugation for 5 min at 2000 *g*, separated plasma specimens were analysed on the same day. In a preliminary experiment, oxacillin concentrations were compared between portal and systemic bloods from 0 to 2 h after intra-arterial administration. Since it was found that the concentration difference between portal and arterial bloods was negligibly small, the arterial concentrations only were measured from 0 to 2 h.

Investigation of drug distribution to erythrocytes

When a drug is sufficiently distributed to 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 ($\overline{Q}_p$) in the following equation:

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

where H_t is the haematocrit value and k_b is the partition ratio of the drug between erythrocytes and plasma.

The partition ratio (k_b) of oxacillin between plasma and erythrocytes was evaluated using heparinized whole blood. After pre-incubation of 0·3 mL blood at 37°C, a saline solution of oxacillin (10 μL) was added to produce the standard blood sample of oxacillin. The blood samples were incubated for 10 min at 37°C, and centrifuged for 5 min at 2000 *g*. The plasma concentrations were measured by HPLC to estimate C_b/C_p. The partition ratio of oxacillin, according to equation 1, was calculated to be $0{\cdot}097 \pm 0{\cdot}014$.

Analytical procedure

To determine oxacillin concentration in the plasma, 30 μL of the plasma was vortexed vigorously for

20 s with 100 μL methanol, followed by centrifugation at 2000 g for 5 min. Supernatant (50 μL) was submitted to HPLC analysis. An HPLC (LC-10A, Shimadzu Co., Kyoto, Japan) was used with a stationary phase of Capcellpak C_{18} (250 mm $\times$ 4·6 mm i.d.; Shiseido, Tokyo, Japan), and a mobile phase of 10 mM acetate buffer (10 mM acetic acid–10 mM sodium acetate, 1 : 1) with methanol (1 : 1 (v/v)). The detection wavelength, flow rate and column temperature were 220 nm, 1·0 mL min^{-1} and 40°C, respectively. The detection response was recorded on a Chromatopac C-R6A instrument (Shimadzu Co.). The detection limit was 0·1 μg mL^{-1}. Calibration lines were prepared by mixing plasma with oxacillin, making five different concentrations in a range from 0·2 to 100 μg mL. The correlation coefficients of the calibration lines were greater than 0·999.

Data analysis

The absorption rate from the intestinal tract into the portal system, dA(t)/dt, was calculated by the following equation:

$$\begin{aligned} dA(t)/dt &= Q_b(C_b^{por}(t) - C_b^{art}(t)) \\ &= \bar{Q}_p(C_p^{por}(t) - C_p^{art}(t)) \end{aligned} \quad (2)$$

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. Superscripts 'por' and 'art' specify portal and arterial vessels, respectively. The effective plasma flow rate $\overline{Q}_p$ was calculated according to equation 3, below, by taking the weight of the rat (W_t) into consideration:

$$\overline{Q}_p = 15{\cdot}3(1 - H_t) \times (1 + k_b) \times W_t/250 \quad (3)$$

where the blood flow rate at portal vein (Q_b) in equation 1 was assumed to be 15·3 mL min^{-1} per 250 g body weight, which was measured with a compact electromagnetic flowmeter (Ito et al 1997). Equations 4 and 5 define the local moments for the absorption rate–time curve:

$$F_a = \int_0^\infty \frac{dA(t)}{dt} dt/D = \overline{Q}_p\left(AUC^{por} - AUC^{art}\right)/D \quad (4)$$

$$\begin{aligned} \bar{t}_a &= \int_0^\infty t \cdot \frac{dA(t)}{dt} dt \Big/ \int_0^\infty \frac{dA(t)}{dt} dt \\ &= \frac{MRT^{por} \cdot AUC^{por} - MRT^{art} \cdot AUC^{art}}{AUC^{por} - AUC^{art}} \end{aligned} \quad (5)$$

where F_a is the absorption ratio of dose from the intestinal lumen to the portal system, $\bar{t}_a$ is the mean local absorption time from the gastrointestinal tract to the portal system and D is the dose of oxacillin. The extent of bioavailability (F) and the mean absorption time (MAT) of oxacillin are calculated according to equations 6 and 7, assuming the pulmonary local disposition is negligible:

$$F = AUC_{po}/AUC_{ia}^{art} = F_a \times F_H \quad (6)$$

$$MAT = MRT_{po}^{art} - MRT_{ia}^{art} = \bar{t}_a + \bar{t}_H \quad (7)$$

where AUC is the area under the curve, MRT is mean residence time, F_H is the hepatic recovery ratio and t_H is the mean hepatic transit time which is considered to be negligible. Superscript 'art' specifies arterial blood and subscript 'po' and 'ia' specify oral and intra-arterial administration, respectively. The clearances were calculated by the following equations:

$$CL = D/AUC_{ia}^{art} \quad (8)$$

$$CL_H = Q_p(1 - F_H) \quad (9)$$

where CL is total plasma clearance, and CL_H is hepatic plasma clearance.

Plasma flow rate through the portal vein (Q_p) was calculated according to equation 10:

$$Q_p = 15{\cdot}3(1 - H_t) \times W_t/250 \quad (10)$$

where 15·3 is portal blood flow rate (mL min^{-1}) which is the same as in equation 3. AUC and MRT contributions due to the first intra-arterial dose were calculated using a linear trapezoidal method with extrapolation to infinite time. Because the contributions arising after the second oral dose includes the time-course due to the first intra-arterial dose, the predicted plasma concentration due to the intra-arterial dose was subtracted from the concentration in the second phase after the second oral dose, making the second dosing time the new origin.

All experimental results are expressed as the arithmetic mean and standard deviation from four rats. The statistical analysis was performed by one-way analysis of variance, regarding 5% as the acceptable level of significance.

Results

Figure 1 shows the time-courses of portal and arterial plasma concentrations of oxacillin in conscious rats and anaesthetized rats. Figure 2 shows the absorption rate–time curve and the cumulative absorption ratio–time curve in conscious and anaesthetized rats, with the time of oral dosing regarded as the origin. Table 1 shows AUC and MRT of the time-course of oxacillin concentrations

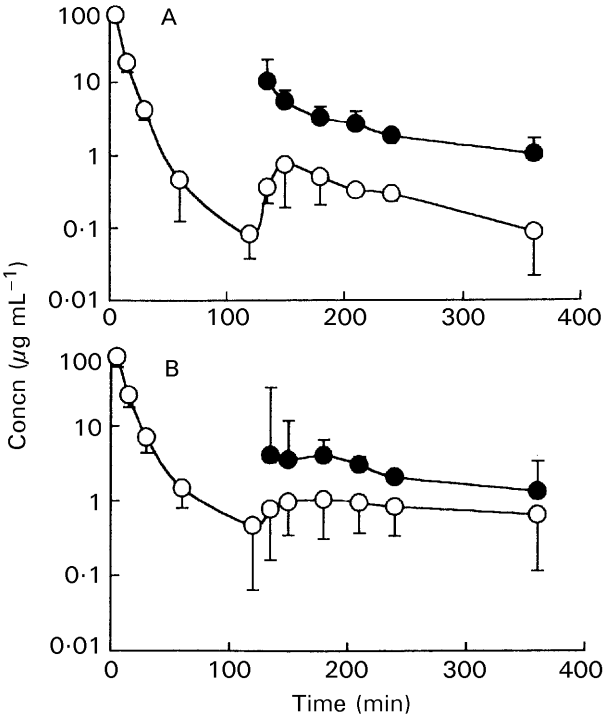

Figure 1. Plasma concentration–time courses of oxacillin in conscious rats (A) and anaesthetized rats (B). The symbols specify portal plasma (●) and arterial plasma (○). Each point represents the mean ± s.d. (n = 4).

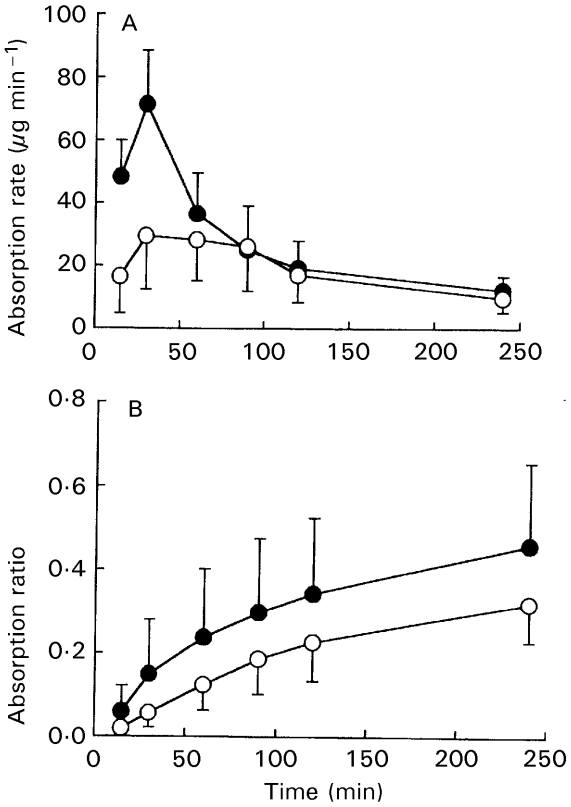

Figure 2. Predicted time-courses of oxacillin absorption rate (A) and cumulative absorption amount (B) into the portal system of rats. The symbols specify conscious (●) and anaesthetized (○) groups. Each point represents the mean ± s.d. (n = 4).

in portal and arterial plasma, following double dosing of oxacillin. In both groups, AUC in the portal plasma was significantly greater than that in the arterial plasma after oral administration; F was increased from 0·0536 to 0·114 and MAT from 74·5 min to 103 min by pentobarbital anaesthesia. The change in F_a was statistically insignificant, whereas F_H was significantly increased from 0·128 to 0·410. The change in CL was statistically insignificant, whereas CL_H was significantly decreased from 28·2 to 20·0 mL min^{-1} kg^{-1}. $CL-CL_H$, which is considered to be renal clearance, was unaffected (12·8 vs 14·6 mL min^{-1} kg^{-1}) by pentobarbital anaesthesia.

Discussion

The disposition of a drug can differ between conscious and anaesthetized animals. In the present

Table 1. Moment characteristics of oxacillin after intra-arterial and oral administration (50 mg kg^{-1}) to conscious rats and anaesthetized rats.

Route	Parameter	Conscious rats	Anaesthetized rats
Intra-arterial	AUC (μg min mL^{-1})	1260 ± 240	1510 ± 370
	MRT (min)	8·49 ± 0·47	11·9 ± 1·4
Oral	Portal AUC (μg min mL^{-1})	631 ± 233	558 ± 148
	Portal MRT (min)	79·3 ± 19·4	99·2 ± 22·9
	Artery AUC (μg min mL^{-1})	66·4 ± 21·0	185 ± 120
	Artery MRT (min)	83·0 ± 18·9	115 ± 14
	F_a	0·439 ± 0·179	0·301 ± 0·090
	F_H	0·128 ± 0·031	0·410 ± 0·176*
	F	0·0536 ± 0·0150	0·114 ± 0·046*
	MAT (min)	74·5 ± 18·8	103 ± 13*
	$\bar{t}_a$ (min)	78·8 ± 19·4	91·5 ± 25·9
	CL (mL min^{-1} kg^{-1})	41·0 ± 7·8	34·5 ± 7·8
	CL_H (mL min^{-1} kg^{-1})	28·2 ± 1·5	20·0 ± 5·7*
	$CL-CL_H$ (mL min^{-1} kg^{-1})	12·8 ± 8·2	14·6 ± 4·1

Each value represents mean ± s.d., n = 4. *$P < 0·05$ compared with control.

experiment, pentobarbital and oxacillin were selected as the model pair of an anaesthetic and a target drug. It was predicted from an intestinal perfusion experiment that pentobarbital can reduce the bioavailability of a drug which is absorbed through passive transport (Yuasa et al 1993). Because oxacillin is absorbed from the intestine into the portal system through passive transport (Rollo 1972; Barza & Weinstein 1976), it is expected that the bioavailability of oxacillin would be reduced by pentobarbital anaesthesia. In contrast to this expectation, we found that pentobarbital significantly increased the extent (F) of bioavailability, although F_a was statistically unchanged in Table 1. Because the hepatic recovery ratio (F_H) was considerably increased by pentobarbital, it is concluded that an increase in F is attributable to an increase in F_H. Pentobarbital can decrease the portal flow rate, which may decrease the hepatic first-pass effect. However, Hadengue et al (1988) demonstrated that the portal flow rate was almost unchanged by pentobarbital. It has also been reported that the hepatic elimination of diclofenac is inhibited by pentobarbital (Fukuyama et al 1996; Tabata et al 1996). Yano et al (1990) showed, in a perfusion experiment, that the hepatic recovery ratio (F_H) of oxacillin was 43·7% after pentobarbital anaesthesia. Using the present method, F_H was 12·8% in conscious rats and 41·0% in anaesthetized rats. We conclude that the hepatic elimination of oxacillin is inhibited by pentobarbital. The rate of bioavailability, given as mean residence time (MRT), is significantly retarded by pentobarbital, although $\bar{t}_a$ was statistically unchanged.

In conclusion, by means of the described method, we could demonstrate that the increase in F due to pentobarbital anaesthesia was attributable to the significant increase in F_H. The method is expected to be applicable not only to the evaluation of the effect of anaesthesia on the first-pass effect, but also to the assessment of the effect of co-administration of drugs on first-pass metabolism.

References

Amidon, G. L., Sinko, P. J., Fleisher, D. (1988) Estimating human oral fraction dose absorbed: a correlation using rat intestinal membrane permeability for passive and carrier-mediated compounds. Pharm. Res. 10: 651–654

Barza, M., Weinstein, L. (1976) Pharmacokinetics of the penicillins in man. Clin. Pharmcokinet. 1: 297–308

Fujieda, Y., Yamaoka, K., Ito, T., Nakagawa, T. (1996) Local absorption kinetics of levofloxacin from intestinal tract into portal vein in conscious rat using portal-venous concentration difference. Pharm. Res. 13: 1201–1204

Fukuyama, T., Yamaoka, K., Nakagawa, T. (1994) A new analysis method for disposition kinetics of enterohepatic circulation of diclofenac in rats. Drug Metab. Dispos. 22: 479–485

Fukuyama, T., Yamaoka, K., Tabata, K., Nakagawa, T. (1996) Inhibitory effect of pentobarbital on biliary excretion of diclofenac in a rat liver perfusion system. J. Pharm. Pharmacol. 48: 734–737

Hadengue, A., Lee, S. S., Koshy, A., Girod, C., Lebrec, D. (1988) Regional blood flows by the microsphere method: reproducibility in portal hypertensive rats and influence of a portal vein catheter. Proc. Soc. Exp. Biol. Med. 187: 461–468

Hoffman, D. J., Seifert, T., Borre, A., Nellans, H. N. (1995) Method to estimate the rate and extent of intestinal absorption in conscious rats using an absorption probe and portal blood sampling. Pharm. Res. 12: 889–894

Ito, T., Yamaoka, K., Nakagawa, T. (1997) Short-period double-dosing for simultaneous evaluation of intestinal absorption and hepatic disposition in a single conscious rat using cephalexin as test drug. J. Pharm. Pharmacol. 49: 1189–1194

Ohata, Y., Yamaoka, K., Yasui, H., Nakagawa, T. (1996) Consideration on moments of outflow profile in liver perfusion system with change in perfusate flow rate using oxacillin as model drug. Biol. Pharm. Bull. 19: 83–87

Rollo, I. M. (1972) Physiological disposition of some semi-synthetic penicillins. Can. J. Physiol. Pharmacol. 50: 986–998

Sawai, Y., Yamaoka, K., Takemura, A., Nakagawa, T. (1997a) Moment analysis of intestinal first-pass metabolism by portal-systemic concentration difference in single conscious rat using 5′-deoxy-5-fluorouridine and 5-fluorouracil as model drug system. J. Pharm. Sci. 86: 1269–1272

Sawai, Y., Yamaoka, K., Ito, T., Nakagawa, T. (1997b) Simultaneous evaluation of intestinal absorption and hepatic extraction of 5-fluorouracil using portal-systemic concentration difference by short-period double dosing in a single conscious rat. Biol. Pharm. Bull. 20: 1313–1316

Tabata, K., Yamaoka, K., Fukuyama, T., Nakagawa, T. (1995) Evaluation of intestinal absorption into the portal system in enterohepatic circulation by measuring the difference in portal-venous blood concentrations of diclofenac. Pharm. Res. 12: 880–883

Tabata, K., Yamaoka, K., Yasui, H., Fukuyama, T., Nakagawa, T. (1996) Influence of pentobarbitone on in vivo local disposition of diclofenac in rat liver. J. Pharm. Pharmacol. 48: 866–869

Yano, Y., Yamaoka, K., Minamide, T., Nakagawa, T., Tanaka, H. (1990) Evaluation of protein binding effect on local disposition of oxacillin in rat liver by a two-compartment dispersion model. J. Pharm. Pharmacol. 42: 632–636

Yuasa, H., Matsuda, K., Watanabe, J. (1993) Influence of anesthetic regimens on intestinal absorption in rats. Pharm. Res. 10: 884–888

Yuasa, H., Matsuda, K., Watanabe, J. (1995) Influence of anesthetic regimens on the intestinal absorption of 5-fluorouracil in rats. Biol. Pharm. Bull. 18: 747–752