Local Absorption Kinetics of Levofloxacin from Intestinal Tract into Portal Vein in Conscious Rat Using Portal-Venous Concentration Difference

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Purpose. The local absorption kinetics of levofloxacin from the intestinal tract was quantitatively evaluated by simultaneously measuring the portal and venous plasma concentrations in a conscious rat.
Methods. The venous and upper portal blood vessels were cannulated through the jugular and pyloric veins, respectively. After oral or intravenous administration of levofloxacin, portal and venous concentrations of levofloxacin were simultaneously monitored. The absorption rate from the intestine into the portal system was calculated from the portal-venous difference in the plasma concentration of levofloxacin, considering the distribution of levofloxacin into erythrocytes. Portal blood flow rate was newly measured by an electromagnetic flow meter.
Results. There was little portal-venous difference after an intravenous dose of levofloxacin. In contrast, after oral administration, the plasma concentration in the portal vein was constantly higher than that in the jugular vein, demonstrating that this difference was caused by the intestinal absorption of levofloxacin.
Conclusions. Approximately 90% levofloxacin was absorbed as the intact form from the intestinal tract into the portal system. By considering the bioavailability of levofloxacin in rat, the hepatic extraction ratio in vivo of levofloxacin was estimated to be 30%. The mean local absorption time ($\bar{t}_a$) was 1.44hr which coincided almost with the mean absorption time (MAT).

KEY WORDS: levofloxacin; portal-venous blood concentration difference; first-pass effects; electromagnetic blood flow meter.

INTRODUCTION

Levofloxacin, S(-)enantiomer of ofloxacin, is a fluoroquinolon antibiotics for oral use. Levofloxacin is twice as potent as ofloxacin in vitro (1). Levofloxacin is active against most aerobic Gram-positive and Gram-negative organisms and demonstrates a moderate activity against anaerobes (2). This drug is rapidly absorbed after oral administration. The study of the enantioselective ofloxacin disposition demonstrated that the bioavailability of levofloxacin is the same as that of ofloxacin (close to 100%) and the urinary excretion is the main pathway in healthy humans (3,4). In contrast, the study in vitro using the homogenate of rat liver demonstrated that the considerable amount of levofloxacin was converted to its glucuronide (5), suggesting the biliary excretion of levofloxacin. However, there is no report that demonstrates the absorption of levofloxacin from the intestinal tract into the portal system and the hepatic first-pass effect in vivo.

Recently, a new analytical technique was developed to determine directly the intestinal absorption of drug into the portal system by sampling both portal and systemic bloods (6,7). The portal blood concentration is always higher than the systemic blood concentration, when a drug is absorbed from the intestinal tract into the portal system. In this analysis, the accurate estimation of the portal blood flow rate is extensively important to calculate the absorption rate of drug into the portal system.

In the present study, we newly measure the portal blood flow rate of rats with an electromagnetic blood flow meter. The intestinal absorption of levofloxacin into the portal system and the first-pass effect on absorbed levofloxacin through the liver into the systemic circulation are separately evaluated by measuring simultaneously the portal and venous concentrations in a conscious rat.

MATERIALS AND METHODS

Chemicals and Reagents

Levofloxacin and DR-3354[R-(+)-ofloxacin] were kindly supplied by Daiichi Seiyaku Co. LTD. (Japan). Diphenylphosphinyl chloride and L-leucinamide were purchased from Sigma Chemical Company (St. Louis. MO), triethylamine from Wako Pure Chemical Industries Ltd. (Japan). All other chemicals and reagents were of analytical grade.

Measurement of Portal Blood Flow Rate

Male Wistar rats (n = 17), weighing 202–303g, were used for the measurement of portal blood flow rate. Rats were anesthetized by an intraperitonial injection of pentobarbital sodium (50mg/kg). After the abdomen was opened through a midline incision, the upper portal vein was dissected free from surrounding tissues, and the interval of 5min was taken for the rat to recover from surgical shock. A probe, i.e. a perivascular flow sensor (internal diameter 0.6mm; Skalar Medical, Delft, the Netherlands), was placed around the portal vein. The signals for the portal blood flow rate were measured for 3min with a compact electromagnetic flowmeter (MDL1401; Skalar Medical). The measurement was attempted twice at an interval of 15min, and the mean value was considered to be an individual portal blood flow rate. During the interval of 15min, the probe was removed from the portal vein which was placed back into the abdomen. After the second measurement was completed, the liver weight was recorded.

Animal Experiment

Male Wistar rats weighing 225–248g were used in this study. After 16hr fasting, the abdomen of each rat under a light ether anesthesia was opened through a midline 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 midline incision. Simultaneously, the right jugular vessel of each rat was cannulated and the free end of second

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cannula with heparinized (100 IU/ml) normal saline was subcutaneously conducted and drawn out at the top of the neck to prevent its removal by the rat. Each rat was held in the Bollman gauge and was allowed to recover 2hr from the ether anesthesia. Then, levofloxacin dissolved in sodium phosphate buffer (pH = 7.0) was administered orally or intravenously at a dose of 20mg/kg. Blood samples (0.11ml) were collected simultaneously from the portal and jugular vessels at 15, 30, 45min, 1, 2, 4, 6, 8hr after an oral administration, or at 5, 15, 30min, 1, 2, 4hr after an intravenous administration. After each sampling, the reduced blood volume was supplemented with an equal volume of saline. After the centrifugation for 5min at 2000g, the plasma samples separated were stored at −20°C until the analysis.

Levofloxacin Uptake by Rat Erythrocytes

When a drug considerably distributes into 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 ($\bar{Q}_p$) by the following equation (6,8).

$$\frac{\bar{Q}_p}{Q_b} = \frac{C_b}{C_p} = R_B \tag{1}$$

The partition coefficient (R_B) of levofloxacin between whole blood and plasma was evaluated using heparinized whole blood (8). After pre-incubation of 1ml blood at 37°C, a small volume of drug solution (20μl) was added to produce the standard blood solutions (0.5, 2.5 and 10μg/ml). The blood samples were incubated for 5min at 37°C, then were centrifuged for 5min at 2000g, and the plasma concentration was measured by HPLC to estimate R_B. Each measurement was repeated three times. Since R_B values were almost the same among the three different concentrations, R_B of levofloxacin was estimated to be 1.17 ± 0.08 averaged among all concentrations.

Assay Procedure

Levofloxacin concentration was determined by HPLC, using R(+)ofloxacin as an internal standard according to the previous report (9). The plasma sample (50μl) was mixed with 0.5ml of phosphate buffer (pH 7.0) containing the internal standard in a test tube and the mixture was shaken with dichloromethane (1.5ml) for 20min. After dichloromethane extract (1ml) was transferred to another test tube, diphenylphosphinyl chloride, triethylamine and L-leucinamide were successively added to the extract to synthesize the amide of ofloxacin. The generated amide was then extracted into 1N HCl (200μl). The extracted solution (50–100μl) was injected on a reverse-phase column (Nucleosil 5C18 particle size 5μm, 15cm × 4.6mm id., Macherey-Nagel, Düren, Germany) using LC-3A HPLC system (Shimadzu, Kyoto, Japan). The mobile phase was the mixture of 0.2M phosphate buffer contained tetraethyl ammonium (pH 1.85) and acetonitrile, 8:2 (v/v). The effluent was monitored with a fluorescence detector (RF-550A, Shimadzu) using 298nm for excitation and 458nm for emission.

Data Analysis

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

$$\begin{aligned} dA_a(t)/dt &= Q_b(C_b^p(t) - C_b^v(t)) \\ &= \bar{Q}_p(C_p^p(t) - C_p^v(t)) \end{aligned} \tag{2}$$

where Q_b and $\bar{Q}_p$ are blood and effective plasma flow rates in portal vein, respectively. $C_b(t)$ 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.

The local moments for the absorption rate-time curve are defined by the following Eqs. (3) and (4).

$$F_a = \int_0^\infty \frac{dA_a(t)}{dt} \cdot dt \Big/ \text{Dose} = \bar{Q}_p(AUC^p - AUC^v)/\text{Dose} \tag{3}$$

$$\begin{aligned} \bar{t}_a &= \int_0^\infty t \cdot \frac{dA_a(t)}{dt} \cdot dt \Big/ \int_0^\infty \frac{dA_a(t)}{dt} \cdot dt \\ &= \frac{MRT^p \cdot AUC^p - MRT^v \cdot AUC^v}{AUC^p - AUC^v} \end{aligned} \tag{4}$$

where F_a is the local absorption ratio from the intestinal tract into the portal system, $\bar{t}_a$ is the mean local absorption time from gastrointestinal tract into the portal system. The superscripts p and v specify portal and venous, respectively.

The extent of bioavailability (F) and the mean absorption time (MAT) of levofloxacin are calculated by comparing moments of the time courses after an oral administration and those after an intravenous administration. Relationships between local moments and global moments are represented as follows (10).

$$F = \frac{AUC^v_{p.o.}}{AUC^v_{i.v.}} = F_a \cdot F_H \tag{5}$$

$$MAT = MRT^v_{p.o.} - MRT^v_{i.v.} = \bar{t}_a + \bar{t}_H \tag{6}$$

where F_H is the hepatic recovery ratio, $\bar{t}_H$ is the mean hepatic transit time. Subscripts p.o. and i.v. mean the oral and intravenous administration, respectively.

The area under the curve (AUC) and the mean residence time (MRT) of the plasma time courses in venous and portal blood vessels were calculated by the trapezoidal integration without extrapolation (10), because the time profile at the terminal phase was too unstable to extrapolate.

All experimental results were expressed as the arithmetic mean and standard deviation of four rats. The statistical analysis was performed by two-way analysis of variance (ANOVA) at a 5% significant level.

RESULT

Portal Blood Flow in Rats

The portal blood flow rates were estimated to be 13.3 ± 2.0ml/min per body weight (250g) and 1.60 ± 0.28ml/min per liver weight, which were very close to those in the literature (11). In the preceding paper, we adopted 9.8ml/min as the portal blood flow rate, which seems to be an under-estimate (6). The effective portal plasma flow rate ($\bar{Q}_p$) was calculated according to the following equation by taking the rat weight(W_t).

$$\bar{Q}_p = R_B \cdot Q_b = 13.3 \cdot R_B \cdot Wt/250 \tag{7}$$

Absorption of Levofloxacin in Conscious Rat

Fig. 1A presents the time courses of portal and venous plasma concentrations of levofloxacin after intravenous administration into rats. Each point in the figure represents the mean of four rats. Although the concentration in the venous plasma seems to be slightly higher than that in the portal plasma, the difference is small. This small difference means that the intestinal absorption of levofloxacin is negligible after intravenous administration (6).

Fig. 1B presents the time courses of portal and venous plasma concentrations of levofloxacin after oral administration into rats. As found in the figure, the portal concentration is constantly higher than the venous concentration. This portal-venous difference demonstrates the definite absorption of levofloxacin from the intestinal tract into the portal system.

Table 1 presents AUC and MRT of time courses of the portal and venous plasma concentrations after intravenous administration (IV group) and oral administration (PO group). There was a slight difference in AUC and little difference in MRT between the portal and venous concentrations in IV group at a significant level (5%) of two-way ANOVA. AUC of the time course of portal concentration is ~10% smaller than that of venous concentration in IV group (Table 1A), which is presumably explained by the dilution of portal blood with substances such as water from the intestinal tract. Every rat in the Bollman gauge was freely taking water during the experiment. On the other hand, both AUC and MRT were significantly different between the portal and venous concentrations in PO group (Table 1B). AUC (12.8 ± 1.1μg · hr/ml) of the time course of portal concentration is greater than that (8.15 ± 0.75μg · hr/ml) of venous concentration, and MRT (2.25 ± 0.33hr) of the former is smaller than that (2.72 ± 0.53hr) of the latter. This portal-venous concentration difference (P-V difference) is due to the absorption of levofloxacin from the intestinal tract into the portal system. F_a values in Eq.(3) and $\bar{t}_a$ in Eq.(4) were estimated to be 87.6 ± 11.1% and 1.44 ± 0.21hr, respectively. Fig.2 shows time courses of the absorption rate and the cumulative absorption ratio (F_a) of levofloxacin, according to Eq.(2).

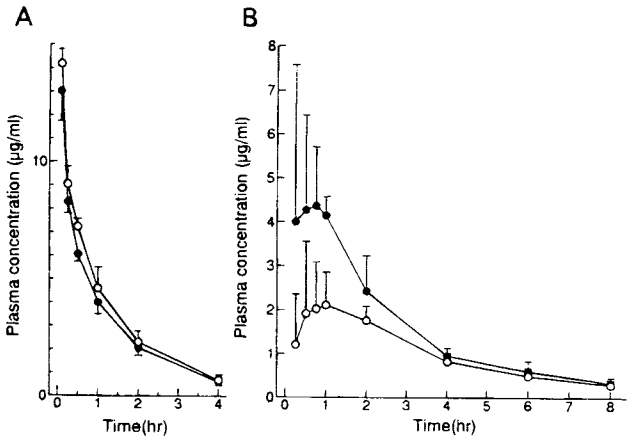

Fig. 1. A:Mean plasma concentration time courses of levofloxacin after intravenous administration (20mg/kg). B:Plasma concentration time courses of levofloxacin after oral administration (20mg/kg). ● and ○ represent portal and venous blood, respectively. Each point represents mean and SD (n = 4).

Table 1. Moment Characteristics of Levofloxacin After Intravenous or Oral Administration. A and B Correspond to Data After Intravenous Administration (IV group) and Oral Administration (PO group)

(A) Intravenous administration group

	RAT-A	RAT-B	RAT-C	RAT-D	Mean ± SD
Weight (g)	225	248	240	238	238 ± 9.5
AUC (μ g · hr/ml)					
Portal	11.5	13.7	11.7	12.2	12.3 ± 1.0
Venous	12.4	15.7	12.4	14.0	13.6 ± 1.6
MRT (hr)					
Portal	1.00	1.08	0.97	0.95	1.00 ± 0.06
Venous	0.98	1.09	0.96	0.97	1.00 ± 0.06

(B) Oral administration group

	RAT-1	RAT-2	RAT-3	RAT-4	Mean ± SD
Weight (g)	225	225	225	240	229 ± 7.5
Qp (ml/min)	14.0	14.0	14.0	15.0	14.3 ± 0.4
AUC (μ g · hr/ml)					
Portal	11.4	12.6	14.0	13.3	12.8 ± 1.1
Venous	7.04	8.57	8.66	8.32	8.15 ± 0.75
MRT (hr)					
Portal	2.17	1.84	2.34	2.64	2.25 ± 0.33
Venous	2.72	2.08	2.71	3.37	2.72 ± 0.53
F_a (%)	81.6	75.4	100.0	93.3	87.6 ± 11.1
$\bar{t}_a$ (hr)	1.28	1.33	1.74	1.42	1.44 ± 0.21

DISCUSSION

In the local absorption study in vivo, the estimation of the portal blood flow rate is the most important to predict accurately the absorption time course from the intestinal tract into the portal system. The portal blood flow rate (Q_b) reported in the

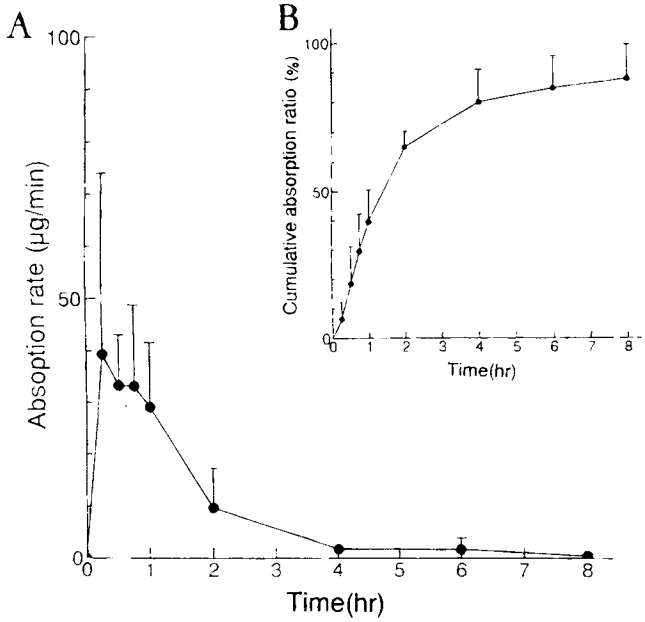

Fig. 2. Predicted time course of absorption rate (dA_a/dt) and cumulative absorption ratio (F_a). Each point represents mean and SD (n = 4).

literature extremely fluctuates. Q_b estimated in the present investigation using anesthetized rats well coincides with those using the electromagnetic and microsphere methods (11). There is a possibility that Q_b is different between the anesthetized and conscious rats. Hedengue et al. demonstrated that Q_b in rats anesthetized with pentobarbital was the same as that in conscious rats (12). Therefore, we adopted 13.3ml/min per 250g as the portal blood flow rate in conscious rats.

It is well known that new quinolones are effectively absorbed from the intestinal tract into the systemic circulation in human (13). The absorption of quinolones, which are moderately water-soluble, has been suggested to be connected with the special mechanism such as a transport system in the blush-border membrane and the saturability in the intestinal absorption was also demonstrated (14). The local absorption ratio (F_a) and the mean local absorption time ($\bar{t}_a$) of levofloxacin were 88% and 1.44hr, respectively. The absolute bioavailability (F) defined by Eq.(5) was calculated to be 59% from the AUC values in IV and PO groups. Since the hepatic recovery ratio (F_H) through the liver is given by F/F_a, F_H was estimated to be 68%. The study using ^{14}C-labeled levofloxacin demonstrated that 57% of an oral dose was found in faeces of rat, and 57% of an oral dose was excreted into the bile of bile-cannulated rat (15). The present result that levofloxacin was elimated by rat liver well agreed with the bile-excretion in literature. The mean absorption time (MAT) defined by Eq.(6) is calculated to be 1.7hr. It is demonstrated that the absorption of levofloxacin is flip-flop, i.e. MAT is larger than MRT (=1.0hr) in IV group (Table 1A). Although MAT is slightly greater than $\bar{t}_a$, the difference may be negligible, i.e. the hepatic mean transit ($\bar{t}_H$) is close to zero in Eq.(6). The prediction for the local absorption of levofloxacin is consistent with the preceding findings that levofloxacin is rather slowly but completely absorbed from the intestinal tract (3,4,15).

In conclusion, the present analysis using levofloxacin as a model drug gave a reasonable result and it is expected that the method of P-V difference offers a means to estimate the absorption kinetics from the intestinal tract through the portal vein into the systemic circulation in the conscious rat.

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