from a patient treated with ofloxacin. No significant interfering peaks derived from biological substances were seen on the chromatograms.

Calibration graphs for ofloxacin in human skin tissue and serum were linear over the ranges 250–3000 ng g^{-1} and 50–2000 ng mL^{-1}, respectively. The limits of detection for ofloxacin were 100 ng g^{-1} in skin tissue and 20 ng mL^{-1} in serum. The results of recovery studies are given in Table 1. The coefficient of variation for the concentrations of ofloxacin in the patients samples ranged from 0·4 to 1·6%. The concentration of ofloxacin in skin tissue samples and serum samples was determined in patients receiving 200–300 mg ofloxacin by mouth before surgery. Fig. 3 shows that the correlation between results with skin tissue ofloxacin (y) and serum ofloxacin (x) was very good, giving the regression: $y = 0{\cdot}785x + 126{\cdot}9$ ng mL^{-1} ($r = 0{\cdot}84$, $n = 30$). The average concentration ratio of ofloxacin in skin tissue vs serum was 0·84. Several workers have reported serum and skin tissue levels of ofloxacin in man (Tomizawa et al 1984; Takahashi et al 1984). However, no coefficient of correlation was obtained in this paper, because the sample number was not sufficient for statistical treatment. Our results suggest that the therapeutic basis of treatment of secondary wound-infection by oral administration of ofloxacin is firmly established in man. In conclusion, a simple, practical and accurate HPLC method has been developed for the determination of ofloxacin in human skin tissue and serum without prior extraction. The good distribution of ofloxacin from blood to skin tissue after oral administration using our method is useful for pharmacokinetic studies in skin tissue and serum in patients.

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Moment analysis of hepatic local disposition of allopurinol and oxipurinol: metabolism kinetics from allopurinol to oxipurinol in the rat isolated perfused liver

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Abstract—Drug metabolism in the liver was examined by the rat isolated perfused liver using the single-pass bolus-input technique. The test compounds, allopurinol and its metabolite oxipurinol, were independently introduced into the liver from the portal vein, and the concentration profiles in the venous outflow were monitored and kinetically analysed by moment theory. The recovery ratios of allopurinol and oxipurinol after the individual administration of each drug were estimated to be 0·17 (±0·08 s.d.) and 1·03 (±0·02 s.d.), respectively. The outflow recovery ratio of oxipurinol as the metabolite after allopurinol administration was estimated to be 0·80 (±0·07 s.d.). These results indicate that the combined outflow recovery of the precursor and the metabolite after allopurinol administration is almost 100% in the rat liver.

Allopurinol and oxipurinol have been used extensively as potent inhibitors of xanthine oxidase, an enzyme that converts hypoxanthine to xanthine, and xanthine to uric acid, for the medical treatment of hyperuricaemia and gout (Elion et al 1963; Rundles et al 1963; Elion 1966; Hille & Massey 1981). Allopurinol is metabolized by xanthine oxidase in the liver to oxipurinol (Elion et al 1966; Krenitsky et al 1967). There are very few reports on the hepatic local disposition of allopurinol and oxipurinol, the target organ of which is the liver. The purpose of this study is to evaluate in detail the local disposition of allopurinol and oxipurinol in the liver, and to elucidate the metabolic kinetics from allopurinol to oxipurinol by means of single-pass rat-liver perfusion experiments following impulse administration into the portal vein. Several models for the analysis of both local disposition and metabolism kinetics during an organ pass have been proposed of which the well-stirred model and the parallel-tube model (Pang & Rowland 1977) are the simplest. A dispersion model has also been used in the analysis of the outflow dilution curve following an impulse input (Roberts &

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Rowland 1986). However, since the application of such models to elimination kinetics and metabolite formation is limited to the steady-state conditions, the moment theory has been adopted as the model-independent method (Yamaoka et al 1978; Kakutani et al 1985), by which the outflow concentration curves after a bolus injection of drug into the liver was kinetically analysed.

Materials and methods

Allopurinol and oxipurinol were obtained from Sigma Chemical Co. (St Louis, MO). All other reagents of analytical grade used for the Krebs-Ringer bicarbonate buffer and for the mobile phase in HPLC were obtained from commercial sources.

A high-performance liquid chromatograph (LC-6A, Shimadzu Co., Kyoto, Japan) equipped with a UV-detector, SPD-2A (Shimadzu), and an integrated data analyser, Chromatopac C-R6A (Shimadzu), was used with a Capcell Pak C18 column (25 cm × 4·6 mm i.d., Shiseido Co. Ltd, Tokyo, Japan) for the simultaneous determination of allopurinol and oxipurinol concentrations in the outflow samples. The mobile phase consisted of 0·02 M potassium dihydrogen phosphate adjusted to pH 3·65 with orthophosphoric acid (Boulieu et al 1984). The flow rate was at 1·0 mL min^{-1}. The detection wavelength was 254 nm, and the column temperature was 40°C. A 100-μL portion of each outflow sample was injected. The correlation coefficients of the calibration line for both drugs were above 0·999 over the concentrations measured in this experiment.

Male Wistar rats, 182–221 g, with free access to standard rat food and water were supplied by Shimizu Laboratory Supplies Co. Ltd, (Kyoto, Japan). The single-pass perfusion experiments using a rat isolated liver were performed in-situ according to the Mortimore perfusion method (Mortimore & Tieze 1959). Male Wistar rats were anaesthetized with pentobarbitone (Nembutal, Abbott Laboratories, USA) and the common bile duct was cannulated with a polyethylene tube (120 × 0·3 mm i.d., 0·6 mm o.d.). The perfusate of Krebs–Ringer bicarbonate buffer (pH 7·4) containing 10 mM glucose and saturated with 95% O_2–5% CO_2 was maintained at 37°C during the in-situ perfusion experiments. The portal vein was rapidly catheterized with a polyethylene tube (1·67 mm o.d.) and the perfusate was delivered into the liver through a portal vein cannula by a peristaltic roller pump (RP-N3, Furue Science Co. Ltd, Tokyo, Japan) at a constant flow rate. Albumin or red blood cells were not included in the perfusate so as to avoid their influence on the drug disposition in the liver. The flow rate of the perfusate was maintained at 15·0 ± 0·21 mL min^{-1}. The flow recovery was 99·3% (± 0·7). After a stabilization period of 20 min, 250 μL allopurinol solution (0·100 mg mL^{-1}, 0·735 mM) or oxipurinol solution (0·100 mg mL^{-1}, 0·657 mM) in the perfusate buffer was introduced instantaneously into the liver through a portal vein cannula using a six-port rotary valve injector. The outflow samples were collected at intervals of approximately 1 s for 2 min from a cannula inserted into the thoracic vena cava inferior. The exact sampling time was calculated from the eluent volume of each outflow sample at a constant flow rate. The viability of the liver was monitored by bile flow throughout the experiments. The wet liver weight was 8·3 ± 0·6 g. The void time through the inlet and outlet catheter was subtracted from the obtained sampling time data. The variance of catheter transit time was less than 0·09 s^2 which was about 0·26% or less compared with the variance of the liver perfusion data. Thus, the broadening of the injected sample in the injector loop and catheter was neglected in analysing the outflow data.

All the drug disposition processes in the liver were assumed to be linear. The outflow time profiles of allopurinol and oxipurinol from livers after the impulse input of the respective drugs were evaluated in a model-independent fashion based on moment theory. The moments of the outflow profile of precursor, i.e. the recovery ratio F_H, the mean transit time $\bar{t}_H$, and the relative variance $\sigma^2/\bar{t}_H^2$ are defined as follows (Yamaoka et al 1978; Kakutani et al 1985):

$$F_H = \int_0^\infty C_{pre}(t)dt/(M/Q) \quad (1)$$

$$\bar{t}_H = \int_0^\infty t \cdot C_{pre}(t)dt \Big/ \int_0^\infty C_{pre}(t)dt \quad (2)$$

$$\sigma^2/\bar{t}_H^2 = \int_0^\infty (t - \bar{t}_H)^2 \cdot C_{pre}(t)dt \Big/ \int_0^\infty C_{pre}(t)dt \Big/ \bar{t}_H^2 \quad (3)$$

where M is the injected amount into the portal vein, Q is the flow rate of the perfusate, t is the sampling time, and $C_{pre}(t)$ (μM) is the outflow concentration of precursor, i.e. allopurinol or oxipurinol concentration after administration of each drug. On the other hand, the recovery of the outflow profile of metabolite transformed from precursor, i.e. oxipurinol appearing in the outflow after allopurinol administration into livers, $F_{H\,pre\to met}$, can be defined in model-independent fashion as follows:

$$F_{H\,pre\to met} = \int_0^\infty C_{pre\to met}(t)dt/(M/Q) = (1 - F_{H\,pre}) \cdot f_m \cdot F_{H\,met,pre} \quad (4)$$

where M is the injected amount of precursor (allopurinol) into the liver, $C_{pre\to met}(t)$ (μM) is the outflow concentration of metabolite (oxipurinol) appearing after the administration of precursor, $F_{H\,pre}$ is the recovery ratio of precursor, f_m is the fraction of precursor eliminated in the liver that is converted to metabolite, and $F_{H\,met,pre}$ is the apparent recovery ratio of metabolite transformed in the liver from precursor to metabolite that returns again to the venous outflow (Roberts & Rowland 1986). The moments are calculated by numerical integration using the trapezoidal rule and the extrapolation to infinite time based on a monoexponential equation (Yamaoka et al 1978; Kakutani et al 1985).

Results

Fig. 1 shows the typical venous outflow curves of allopurinol and oxipurinol from the liver after administration of allopurinol. Allopurinol and oxipurinol simultaneously appeared in the venous outflow, and the concentration of oxipurinol was always higher than that of allopurinol. The descending part in the outflow profile of allopurinol was described by a monoexponential equation, while that of oxipurinol was best fitted by a biexponential equation. Therefore, the moments of oxipurinol as metabolite were calculated using the extrapolation of the terminal phase to infinite time based on a corresponding monoexponential equation. On the other hand, Fig. 2 shows the typical venous outflow curve of oxipurinol from the liver after administration of oxipurinol itself. The descending part in the outflow profile of oxipurinol fitted a monoexponential equation.

Table 1 presents the local moments calculated from equations 1–3. Table 2 presents the local moments of oxipurinol as the metabolite from allopurinol calculated from equation 4.

Discussion

In the in-situ perfusion experiments, the kinetic analysis of hepatic drug metabolism has been studied by a constant infusion method (Pang & Gillette 1978; Xu & Pang 1989) or by a recirculation method (Pang & Gillette 1978; St-Pierre et al 1990)

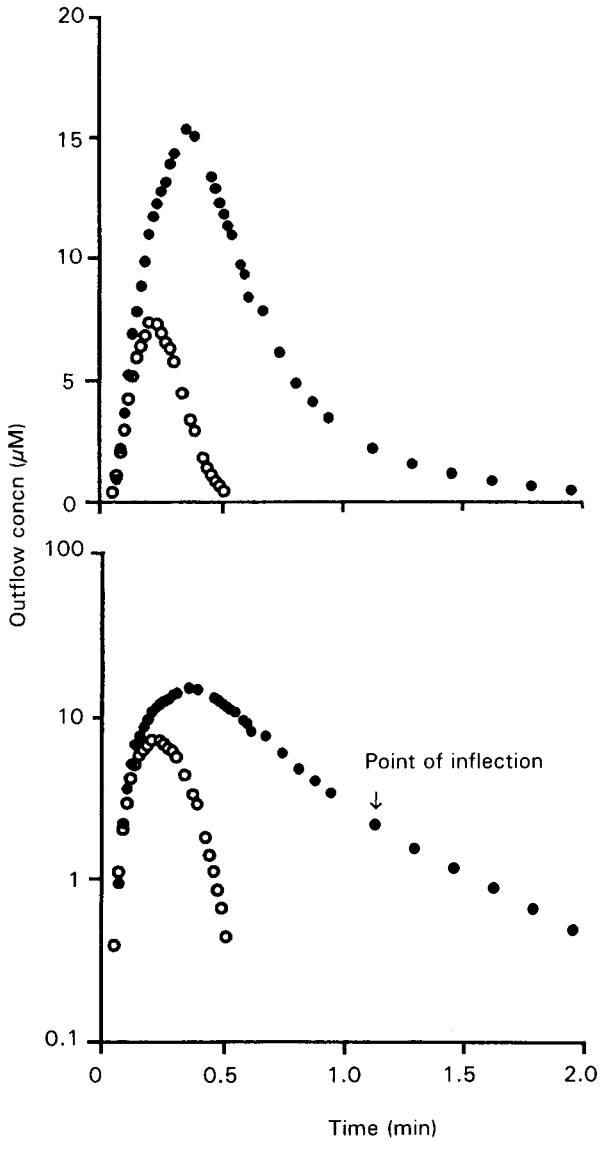

FIG. 1. Typical allopurinol (○) and oxipurinol (●) concentration vs time data after allopurinol administration. The bottom panel is the semi-logarithmic plot of the same data.

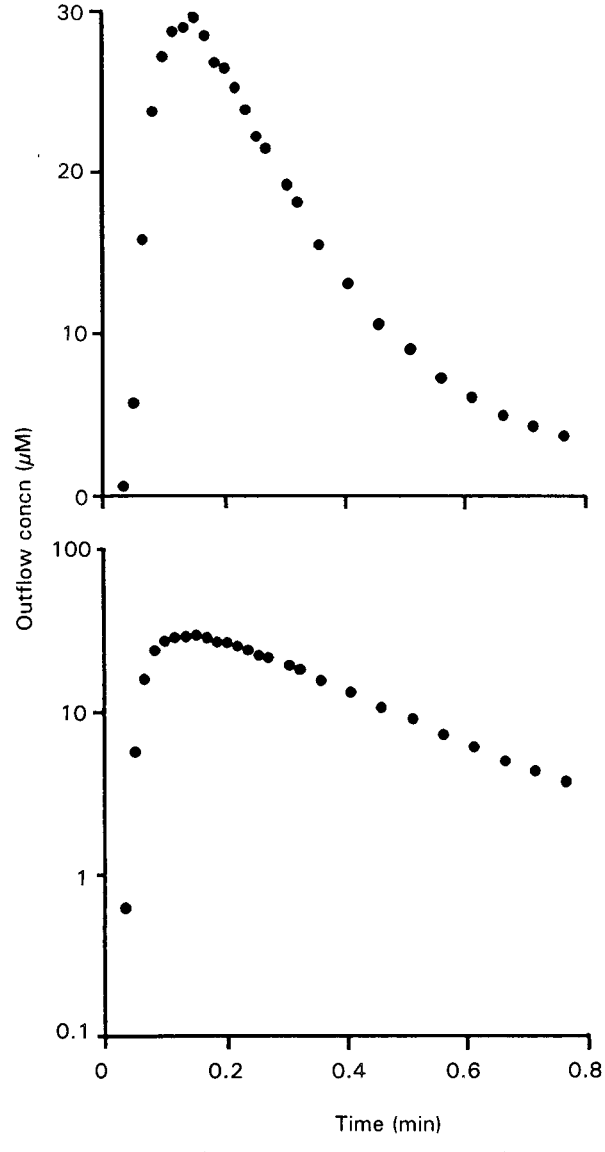

FIG. 2. Typical oxipurinol concentration vs time data after oxipurinol administration. The bottom panel is the semi-logarithmic plot of the same data.

in combination with several models. However, these analyses using a complicated model were not applied to evaluate the kinetics of elimination and metabolite formation in the single-pass bolus perfusion study. In the present study, a simple moment analysis was applied to estimate the outflow recovery ratio of precursor and metabolite, and the local disposition of metabolite generated from precursor in the liver after impulse administration. The metabolic characteristics of precursor into metabolite could be represented simply by the local zero moments, i.e. recovery or converted fraction.

As shown in the results, the sum of the outflow recovery of allopurinol and oxipurinol as metabolite was approximately 100% of injected dose. Therefore, oxipurinol generated from allopurinol in the liver was considered to return completely to the venous outflow, while the outflow recovery of oxipurinol after administration of the metabolite was also estimated to be 100%. Since the distribution volume of a drug in the liver, V_H, is approximately given by $\bar{t}_H \cdot Q$ (Yano et al 1989), the V_H values of allopurinol and oxipurinol were evaluated to be about 42 and 65% of the liver, respectively. These V_H values are larger than the blood space, which corresponds to the sum of the sinusoid and space of the Disse occupying approximately 15% of the liver (Goresky 1963): hence, both drugs are predicted to partition into the liver tissue. These results suggest that substantially the whole fraction of allopurinol eliminated from the perfusate was

Table 1. Local moment characteristics for allopurinol and oxipurinol calculated from venous outflow curves after each drug administration in a single-pass rat liver perfusion system.

	Allopurinol (n = 4)	Oxipurinol (n = 4)
F_H (% dose)	16·7 ± 7·5	102·6 ± 1·7
$\bar{t}_H$ (s)	14·0 ± 1·0	21·5 ± 1·1
$\sigma^2/\bar{t}_H^2$	0·177 ± 0·026	0·598 ± 0·031

Values given are mean ± s.d. Numbers in parentheses represent the number of experiments for each perfusion system.

Table 2. Local moment characteristics for oxipurinol as metabolite calculated from venous outflow curve of oxipurinol after allopurinol administration in a single-pass perfusion system.

	(n = 4)
$F_{H\ pre \rightarrow met}$ (% dose)	80·1 ± 7·4
$1 - F_{H\ pre}$ (% dose)	83·3 ± 7·5
$f_m \cdot F_{H\ met,pre}$ (% dose)	96·2 ± 2·1

Values given are mean ± s.d. Numbers in parentheses represent the number of experiments for the perfusion system.

metabolized to oxipurinol in the liver by xanthine oxidase, and that the converted fraction of oxipurinol almost completely returned to the venous perfusate after appreciable distribution into the hepatocytes, i.e. cytoplasm or enzyme-binding, consistent with allopurinol and oxipurinol being mainly eliminated by the kidney in-vivo (Elion et al 1966, 1968; Krenitsky et al 1967).

Xanthine oxidase is localized in the cytoplasm near the sinusoidal membrane (Jarasch et al 1986). Accordingly, oxipurinol as a metabolite of allopurinol is expected to appear in the venous outflow without the delayed time compared with the outflow time course of allopurinol as precursor. In fact, the venous appearance of allopurinol and the converted oxipurinol was observed almost simultaneously, and the mean transit time of the converted oxipurinol was estimated to be 36·9 ± 3·5 s, which was approximately equal to the sum of the mean transit times of allopurinol and oxipurinol. Thus, it is presumed that the outflow pattern of metabolite after an impulse input of precursor may involve many complex sequential processes, i.e. uptake of precursor, association of precursor with enzyme, formation of metabolite, dissociation of metabolite from enzyme, and elimination and distribution of metabolite. In the present study, the local metabolic process of oxipurinol from allopurinol in the liver was generally evaluated with respect to extent and rate based on the moment theory. Although metabolite kinetics involves a series of complex processes as mentioned above, the calculated local moments are free from restrictions of the model analysis. The detailed analysis based on the pharmacokinetic model with several meaningful parameters should also be useful with the venous outflow patterns of precursor and metabolite in the single-pass liver perfusion study, using the bolus-input method.

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