J. Pharm. Pharmacol. 2000, 52: 1011–1016
Received November 17, 1999
Accepted April 20, 2000

Uptake and Dispersion of Metformin in the Isolated Perfused Rat Liver

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Abstract

Although metformin is a widely used oral antihyperglycaemic, the exact mechanisms of its cellular uptake and action remain obscure. In this study the hepatic extraction and disposition kinetics of metformin were investigated by use of an isolated in-situ rat liver preparation. The liver was perfused in single-pass mode with protein-free Krebs bicarbonate medium at a flow rate of 20 mL min^{-1}.

During constant infusion with 1 mg L^{-1} metformin hydrochloride the hepatic uptake of metformin approached equilibrium within 10 min. The steady-state availability, F, determined from the ratio of outflow concentration to input concentration, was $0{\cdot}99 \pm 0{\cdot}02$ (mean $\pm$ s.d., $n = 4$). The outflow profile of metformin resulting from a bolus injection of 25 μg into the portal vein, had a sharp peak then a slower declining terminal phase. The mean transit time (MTT; $49{\cdot}5 \pm 14{\cdot}5$, $n = 6$) and normalized variance (CV^2; $4{\cdot}13 \pm 0{\cdot}05$) of the hepatic transit times of metformin were estimated by numerical integration from the statistical moments of the outflow data. The volume of distribution of metformin in the liver ($1{\cdot}58 \pm 0{\cdot}28$ mL (g liver)$^{-1}$) was estimated from its MTT. The volume of distribution is greater than the water space of liver, indicating that metformin enters the hepatic aqueous space and becomes distributed among cellular components. The magnitude of CV^2 for metformin is greater than for the vascular marker sucrose, suggesting that distribution of metformin into hepatic tissue is not instantaneous.

In conclusion, hepatic uptake of metformin is rate-limited by a permeability barrier. Although metformin is accumulated in the liver, the organ does not extract it.

Metformin (1,1-dimethylbiguanide), an oral antihyperglycaemic agent used to treat non-insulin-dependent diabetes mellitus (type II) since 1957, inhibits hepatic gluconeogenesis, reduces intestinal glucose absorption, and increases peripheral glucose uptake and utilization. It also has lipid-reducing effects that are independent of its antihyperglycaemic effect (Bailey & Turner 1996; Scheen 1996). Metformin can have adverse effects, especially lactic acidosis, which might be related to high circulating concentrations of the drug. Although accumulation and lactic acidosis increase with impairment of renal function (Sambol et al 1995), the incidence of lactic acidosis with metformin is rare compared with that associated with other biguanides, e.g. phenformin. In 1995, metformin received FDA approval and in recent years there has been renewed interest in the drug (Scheen 1996).

Despite the longstanding availability of metformin, very little information is available on its pharmacokinetics in man and its disposition in different tissues or organs (Scheen 1996). By use of the radiolabelled drug Pentikäinen et al (1979) demonstrated that metformin is not metabolized and is excreted completely unchanged in urine after intravenous injection of 500 mg metformin hydrochloride in healthy volunteers. Tucker et al (1981) reported that cumulative urinary excretion of metformin after intravenous injection of 250 mg to healthy subjects was not complete (approx. 80%) and suggested that metformin might undergo metabolism. Study in rats demonstrated that metformin is rapidly distributed, and accumulated by the liver after oral administration. Further examination by use of subcellular preparations revealed that the hepatic distribution of metformin was mainly in
the cytosol; this closely parallels the distribution of lactate dehydrogenase (Wilcock et al 1991). Despite these results the mechanism of metformin-induced lactic acidosis is not yet clearly estab-

lished. Babich et al (1998) recently reported metformin-induced acute hepatitis and it is, therefore, of great interest to explore the hepatic disposition of metformin.

The purpose of this investigation was to examine the hepatic uptake, distribution and metabolism of metformin. An isolated perfused rat liver preparation was employed to study hepatic extraction of metformin under steady-state infusion and the disposition kinetics of metformin were characterized by use of the impulse–response technique.

Materials and Methods

Chemicals

Metformin hydrochloride (Lot 84H0451) was purchased from Sigma (St Louis, MO). HPLC-grade acetonitrile was obtained from BDH (Poole, UK). All other reagents were commercial products of analytical grade.

Animals

Male Sprague–Dawley rats (238–278 g; Animal Breeding Centre, National Cheng Kung University) were maintained with standard laboratory pellets and water freely available. The study protocol complied with the Institutional Guidelines on Animal Experimentation of National Cheng Kung University.

Liver perfusion

The in-situ perfused rat liver preparation was similar to that described elsewhere (Chou et al 1993, 1995; Chou & Rowland 1997). Under intraperitoneal anaesthesia with urethane ($1{\cdot}5\,g\,kg^{-1}$), the bile duct was cannulated with PE10 polyethylene tubing (i.d. 0·28 mm, o.d. 0·61 mm). The portal vein was then rapidly cannulated by use of a Medicut intravenous catheter placement unit (16-gauge; 1·7 mm o.d. × 45 mm). The liver was perfused ($20\,mL\,min^{-1}$) in single-pass mode with Krebs–Henseleit bicarbonate buffer (pH 7·4), containing $3\,g\,L^{-1}$ glucose and saturated with humidified carbogen gas (O_2/CO_2 95% : 5%). The superior vena cava was cannulated through the right atrium without interruption of portal perfusion. The inferior vena cava was ligated above the renal portal vein. All experiments were conducted at 37°C in a temperature-controlled cabinet. Viability of the liver was assessed by monitoring gross appearance, flow recovery, bile production and inflow and outflow perfusate pH throughout the experiment. The wet liver weight was determined immediately after an experiment.

Disposition kinetics

Steady-state infusion. After a 15-min initial stabilization period, metformin hydrochloride ($1\,mg\,L^{-1}$) was infused for 25 min (n = 4) to assess its hepatic extraction. Outflow perfusate samples were collected between 5 and 25 min at 2- to 5-min intervals.

Bolus injection. The methodology essentially followed that used for the indicator dilution method used in previous studies (Chou et al 1993, 1995; Chou & Rowland 1997). During constant perfusion of drug-free perfusate metformin hydrochloride ($1\,mg\,mL^{-1}$ in water; 25 μg) was injected as a bolus dose into the portal vein, via the inflow cannula (n = 6). The total effluent was automatically collected at one-second intervals, by use of a motor-driven carousel (Pan Chun Scientific, Taiwan) for 2 min, and thereafter collected into 1·5-mL Eppendorf tubes at increasing time intervals for a further 2 min.

Metformin assay

The concentrations of metformin (expressed as the hydrochloride) in the outflow perfusate samples were measured by use of a validated HPLC method based on that of Cheng & Chou (1999). The HPLC system consisted of an L-7100 pump, L-7200 autosampler, and L-7400 UV detector (all from Hitachi), a Bio-Rad column oven, and an SISC data station (Scientific Information Service, Taipei, Taiwan). The analytical column, 5 μm Hypersil HS silica, 25 cm × 4·6 mm i.d., was protected by a precolumn (Hichrom silica H5, 12·5 mm × 3 mm i.d.) The column temperature was maintained at 40°C. The mobile phase was 25 : 75 (v/v) acetonitrile–water containing 0·03 M $(NH_4)_2HPO_4$ (pH adjusted to 7·0 with orthophosphoric acid). The flow-rate was $1\,mL\,min^{-1}$. The detector wavelength was set at 240 nm. Outflow sample (20–50 μL) was injected directly into the HPLC. Quantitation of metformin was by measurement of the area of the drug peak and least squares analysis of the calibration curve constructed by use of blank perfusate. The calibration curve of the logarithmic-transformed peak area against logarithmic-transformed amount injected was linear ($R^2 > 0{\cdot}998$) over the range 1 to 5000 ng with within-day and between-day coefficients of variation below 15%.

Data analysis

Steady-state infusion. The availability (the fraction escaping liver extraction) of metformin was calculated from the ratio of outflow concentration to input concentration.

Bolus injection. The outflow concentration of the solutes at the midpoint time of the collection interval, C(t), was converted to frequency outflow, f(t), by use of equation 1. (Chou et al 1995; Chou & Rowland 1997).

$$f(t) = \frac{C(t) \cdot Q}{Dose} \quad (1)$$

where Q is the perfusate flow rate. The maximum value of f, f_{max}, and the time, t_{max}, at which it occurred were observed. The mean transit time (MTT) and the relative spread of the transit times (CV^2) were estimated from the statistical moments of the outflow profiles (equations 2, 3).

$$MTT = \frac{\int_0^\infty t \cdot C(t)dt}{\int_0^\infty C(t)dt} \quad (2)$$

$$CV^2 = \frac{\int_0^\infty t^2 \cdot C(t)dt}{MTT^2 \cdot \int_0^\infty C(t)dt} - 1 \quad (3)$$

The moments were calculated by numerical integration, by linear interpolation and extrapolation to infinite time, after subtracting the mean transit delay caused by the tubing (1·5 s). The variance of the tubing transit time was negligible compared with that of the liver outflow data. The apparent volume of distribution of drug within the liver was estimated by use of equation 4:

$$V = Q \times MTT \quad (4)$$

Results

Figure 1 shows the profile of mean availability against time for metformin during constant infusion of 1 mg L^{-1}. The hepatic distribution of metformin approached equilibrium within 10 min. The steady-state availability, F, was 0·99 ± 0·02 (mean ± s.d., n = 4).

The profile of frequency outflow against time for metformin after bolus injection into the portal vein is shown in linear and semi-logarithmic plots in Figure 2. It is apparent from Figure 2 that the profile of metformin was biphasic with a characteristic sharp peak, which eluted over the first 20 s, followed by a slow-eluting flat tail.

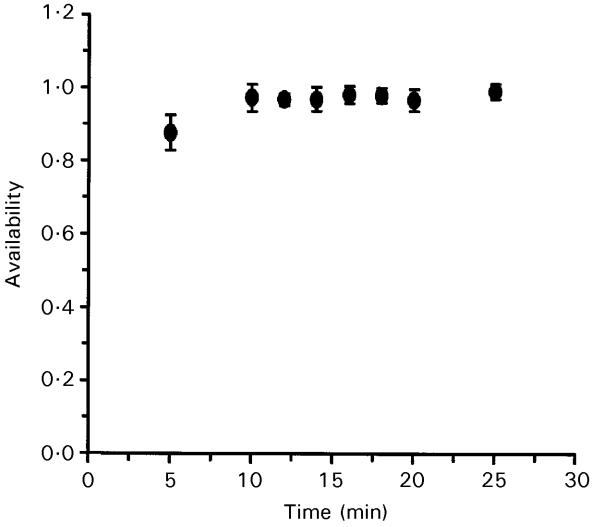

Figure 1. Availability (mean ± s.d.) of metformin during constant infusion of 1 mg L^{-1} in the isolated perfused rat liver.

The peak occurred rapidly at 5·3 s (t_{max}) with average maximum value (f_{max}) of 0·156 s^{-1} (Table 1). The biphasic shape of the profile of metformin is clearly apparent from the semi-logarithmic plot. Approximately 70% of the injected dose eluted during the first 20 s, the remaining 30% declining exponentially in the terminal phase. Recovery of metformin was complete. After 210 s 92·8% of the metformin had been recovered; this suggests not only that this time was sufficient to ensure essentially full recovery of administered material but also, because the time was four times the MTT, was sufficient to provide a good estimate of moment parameters.

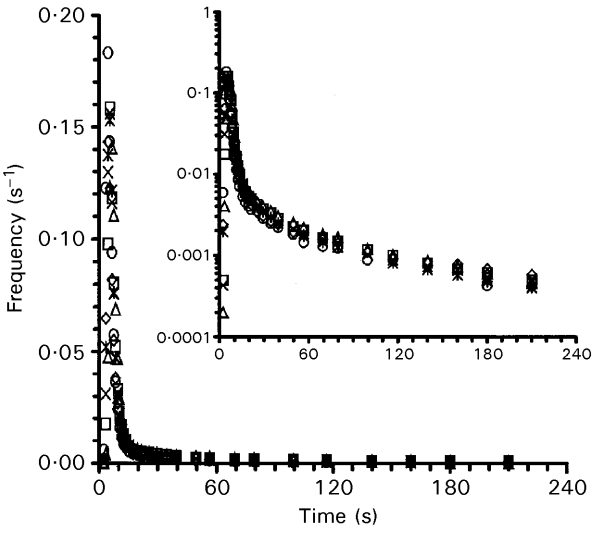

Figure 2. Linear and semi-logarithmic (inset) profiles of observed frequency outflow against time for metformin, after bolus injection into the portal vein of single-pass perfused rat livers.

Table 1. Observational and statistical moment parameters for metformin outflow profiles after bolus administration.

	1	2	3	4	5	6	Mean ± s.d.
Mass of liver (g)	9·1	9·8	10·7	10·2	10·3	13·3	10·6 ± 1·4
Perfusate flow rate (mL min^{-1})	20·3	20·6	20·3	20·6	20·7	20·2	20·5 ± 0·2
Maximum frequency outflow (s^{-1})	0·14	0·16	0·15	0·18	0·16	0·14	0·16 ± 0·02
Time of maximum frequency outflow (s)	6·5	5·5	5·5	4·5	5·5	4·5	5·3 ± 0·8
Mean transit time (s)	43·8	48·3	40·4	36·4	51·3	76·9	49·5 ± 14·5
Volume of distribution (mL (g $liver^{-1}$))	1·63	1·69	1·28	1·22	1·72	1·95	1·58 ± 0·28
Normalized variance of hepatic transit time	3·39	4·27	4·51	4·79	3·89	3·91	4·13 ± 0·05

Results from statistical moment analysis are presented in Table 1. The volume of distribution ($1{\cdot}58 \pm 0{\cdot}28$ mL $(g\,liver)^{-1}$, $n = 6$) is greater than the water space of the liver, indicating that metformin enters the total aqueous space and becomes distributed among the cellular components. The magnitude of the normalized variance (CV^2; $4{\cdot}13 \pm 0{\cdot}05$) for metformin was greater than that for the vascular marker sucrose, suggesting that distribution of metformin into hepatic tissue is not instantaneous.

Discussion

Choice of compound and method

In this study both steady-state infusion and bolus injection were used to assess the disposition of metformin in the isolated perfused rat liver. The steady-state infusion approach, which primarily focuses on examining clearance rather than distribution, is the better method for exploring elimination of metformin. The single-pass indicator dilution method provides rich information on the transient kinetics of uptake and distribution (Goresky 1983; Rowland & Evans 1991). Metformin is a hydrophilic weak base with a partition coefficient, logP (*n*-octanol/aqueous buffer, pH 7·4), of −1·43 (Craig 1990). The pK_a of metformin (11·5) is extremely high (Craig 1990; Scheen 1996) and the compound is therefore predominantly ionized and occurs as the cation at physiological pH. The lipophilicity and degree of ionization of metformin suggest that its crossing of hepatocyte membranes is limited and that there might be a hepatocyte permeability barrier for metformin. Metformin does not bind to plasma protein but does become substantially distributed in red blood cells (Sambol 1995; Scheen 1996). Because extraction of metformin during a single pass through the liver is negligible, and no protein and red blood cells were added to the perfusate, the hepatic uptake and distribution can be explored without the complications of plasma protein binding, red blood cell partition and hepatic elimination.

Steady-state availability

The availability of metformin increases sharply to 90% (approx.) 5 min after the start of constant infusion. The distribution equilibrium was achieved after 10 min. The steady-state availability of metformin determined in this study (0·99) was close to unity, indicating that hepatic clearance of metformin is extremely low. These results confirm a finding from an in-vivo study that metformin is essentially not metabolized in the liver (Pentikäinen et al 1979). This rules out the possibility that hepatic metabolism accounts for the incomplete urinary recovery of metformin after intravenous administration (Tucker et al 1981). Because the delay caused by the void volume of the tubing and apparatus of the perfusion system for constant infusion is 1·5 min (approx.), the practical time to reach steady state would be 3·5 min (210 s). This result is in agreement with that from the bolus studies, in which 93% of the injected dose was recovered within 210 s.

Uptake kinetics

Metformin has two-compartmental distribution characteristics in the liver, in which uptake is limited by permeation rate, and it becomes significantly distributed among the hepatic tissue at a low bolus dose (25 μg). Visual inspection of the outflow curve for metformin indicated that most of the metformin passes through the liver without entering hepatic cells. This is mainly because of limited hepatocyte membrane permeability and rapid flow washout from the vascular compartment. The slowly eluting tail, the so-called returning component (Chou et al 1995; Goresky 1983), represents the fraction of the total dose that has entered the cells and returned to the vascular space. To assess the relative influence of the throughput and returning components on the shape of the outflow profile, the area under the frequency out-

flow curve from time zero to 20 s was calculated. On the basis of the result it was estimated that throughput and returning component comprise roughly 70% and 30%, respectively, of the injected material.

Dispersion and permeability

The profiles of metformin were indicative of typical permeability-rate-limited distribution characteristics; this was also reflected by the statistical moment parameter, CV^2. The high value of CV^2 for metformin is consistent with the possibility of efflux limitation. Roberts & Rowland (1986) presented expressions for CV^2 showing that CV^2 consists of two components, that associated with vascular dispersion and that associated with limited hepatocyte permeability. Vascular dispersion is a characteristic of hepatic morphology alone (Rowland & Evans 1991; Roberts et al 1998). The value of CV^2 for metformin, which is about 6–7 times greater than that for sucrose (Chou et al 1995), is therefore consistent with the presence of a permeability barrier.

Hepatic distribution

The distribution volume of metformin is 1·58 mL $(g\,liver)^{-1}$, approximately twice the total aqueous space, 0·7 mL $(g\,liver)^{-1}$ (Pang et al 1988). This implies that metformin enters the total aqueous space of the liver and becomes distributed among the cellular components, even though uptake is rate-limited by a membrane barrier. This finding is in agreement with the subcellular distribution in rat liver described by Wilcock et al (1991), who found that after oral administration metformin is rapidly distributed into rat liver with an hepatic concentration 3–4 times that in plasma. The relative subcellular distribution of metformin was similar 30 min (first sampling time) and 4 h after dosing; it was highest (78%) in the cytosol and lowest (2–3%) in the nuclear fraction (Wilcock et al 1991). Similar results were obtained for salicylic acid by use of the indicator–dilution method (Hussein et al 1994). Salicylic acid occurs predominantly in the anionic form at physiological pH and its apparent partition coefficient at pH 7·4 ($\log D = -2{\cdot}17$) is similar to that of metformin. In common with metformin, the permeability of the hepatocyte membrane to salicylic acid is low, it becomes distributed between vascular and cellular space, and has a volume of distribution (1·4 mL $(g\,liver)^{-1}$) very similar to that of metformin. Because significant partition of metformin into hepatocytes seems to contradict the presence of a membrane barrier, it has been suggested that metformin uses an active trans-membrane transport mechanism in the liver (Wilcock et al 1991). Further work is needed to explore the possibility of a specific mechanism for hepatic transport of metformin. In summary, this study demonstrates that the hepatic disposition of metformin has two-compartmental distribution characteristics and that hepatic uptake of metformin is rate-limited by a permeability barrier. Although metformin is accumulated in the liver, it is not metabolized by the organ.

Acknowledgements

This work is supported by a grant from the National Science Council of Taiwan. We thank Hue-Yu Wang for technical assistance and Ching-Ling Cheng for her helpful comments and support.

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