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Disposition of amphotericin B in the isolated perfused rat liver

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Abstract

The hepatic disposition and biliary excretion of amphotericin B were investigated in the isolated perfused rat liver (IPRL). Bolus dose of 50 μg, 99 μg and 198 μg amphotericin B in lipoprotein-free perfusate and 198 μg amphotericin B in perfusate with 1 μM high-density lipoprotein (HDL) or 1 μM low-density lipoprotein (LDL) were examined in the IPRL. Amphotericin B concentration in perfusate was measured using a validated HPLC assay. Amphotericin B was eliminated from the perfusate in a biexponential manner. The hepatic clearance (CL_H) increased in proportion to the dose administered (0.27 ± 0.05 mL min^{-1} at low dose, 0.54 ± 0.23 mL min^{-1} at medium dose and 1.06 ± 0.24 mL min^{-1} at high dose), indicating non-linear hepatic disposition of amphotericin B. The hepatic extraction ratio of amphotericin B was very low (0.066 ± 0.015). Tissue-to-perfusion partition coefficient, calculated at 120 min, increased 1.5 fold from 9.8 ± 1.7 at low dose to 15.9 ± 6.4 at high dose, suggesting the significant uptake and extensive retention of amphotericin B in the liver. Biliary excretion made only minor contribution to amphotericin B elimination in the IPRL, representing around 1–3% of the dose administered. No metabolites were detected in perfusate, bile and liver samples. The hepatic disposition of amphotericin B was not affected by the presence of HDL and LDL in the perfusate. In conclusion, the hepatic disposition of amphotericin B demonstrates restrictive elimination and is concentration-dependent, consistent with carrier-mediated uptake, and lipoproteins do not influence amphotericin B hepatobiliary disposition.

Introduction

Amphotericin B is a macrocyclic polyene antifungal drug derived from *Streptomyces nodosus*. Its conventional formulation, amphotericin B deoxycholate (Fungizone), remains the therapeutic cornerstone for many fungal infections. Amphotericin B pharmacokinetics is characterised by extensive tissue distribution, high protein binding (91–95%) and a long terminal elimination half-life in man of more than 15 days (Janknegt et al 1992; Groll et al 1998). In man the elimination of amphotericin B has not been fully elucidated, but biliary excretion would be expected to contribute to its elimination since the drug has a high molecular weight of > 924 g mol^{-1} and is polar on one side of the lactone ring with seven hydroxyl groups. The metabolic fate of amphotericin B is unknown and no metabolites have been identified (Coukell & Brogden 1998). Furthermore, a recent study of 2 mg kg^{-1} liposomal amphotericin B (AmBisome) in healthy subjects attempted to identify metabolites using LC/MS/MS assay, but detected no peaks attributable to possible amphotericin B metabolites (Bekersky et al 2002).

Amphotericin B is known to associate with plasma proteins, including lipoproteins that commonly transport lipids (Wasan et al 1994a). These lipoproteins also bind and subsequently transport a number of water-insoluble compounds, including amphotericin B (Kwong & Wasan 2002). Recent reports suggest that increased nephrotoxicity associated with amphotericin B is related to increased uptake of amphotericin B by renal LLC PK1 cells via high-affinity LDL receptors ($K_d = 0.054$ ng mL^{-1}; 96 000 sites per cell) (Wasan & Lopez-Berestein 1994; Wasan et al 1994b; Wasan 1996). In contrast, HDL and amphotericin B complex is less toxic to LLC PK1 renal cells due to low-affinity HDL receptors ($K_d = 71.43$ ng mL^{-1}; 2 sites per cell). Thus, the possibility of amphotericin B and lipoprotein complex, especially HDL and LDL, may enhance drug

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transport and uptake into hepatic cells via HDL or LDL receptors, leading to higher intracellular amphotericin B availability for metabolism or biliary excretion.

Therefore, the primary aim of this study was to investigate the hepatic disposition and biliary excretion of amphotericin B using the isolated perfused rat liver (IPRL). A secondary aim was to examine the influence of lipoproteins (HDL and LDL) on the hepatobiliary disposition of amphotericin B.

Materials and Methods

Materials

Amphotericin B (purity 82%), LDL and HDL were purchased from Sigma Chemical Co. (Sydney, Australia). Amphotericin B deoxycholate (Fungizone) was obtained from Bristol-Myers Squibb (Victoria, Australia). All solvents were HPLC grade and other chemicals were analytical grade.

Animals

Male Sprague-Dawley rats (Gore Hill, NSW, Australia), 286 ± 23 g, were housed in a 12-h light–dark cycle and controlled temperature environment with free access to standard laboratory chow and water. The study was approved by the Animal Ethics Committee of the University of Sydney.

IPRL study

The perfusion apparatus was housed in a thermostatically controlled cabinet. Perfusate solution (100 mL) was recirculated at 16 mL min^{-1} using a peristaltic pump (MasterFlex; Cole-Parmer Instrument Company, Chicago, IL). The perfusion medium was passed over an oxygenator exchanged with Carbogen (95% O_2–5% CO_2) before delivery to the liver. Inlet and outlet oxygen concentrations in the perfusate were measured with an oxygen meter (ORION, Model 830; Extech Equipment Pty, Ltd, Australia). Rats were anaesthetized intraperitoneally with Nembutal 60 mg kg^{-1} (Merial Australia Pty, Ltd). After a midline incision, the bile duct was first cannulated with PE 10 tubing. The portal vein was then exposed and cannulated with 18 G catheter and perfusion was initiated immediately with Hartmann's solution (Baxter Healthcare Pty, Ltd, Sydney, Australia). The thorax was then cut open to expose the right atrium. PE 220 tubing was then inserted through the right atrium into the upper portion of the inferior vena cava (IVC) and tied. Liver was isolated and perfusion medium was changed to Krebs-Henseleit bicarbonate solution, which contained 1% bovine serum albumin (BSA, fraction V, Sigma, Sydney, Australia) and 0.1% glucose. Perfusion flow was maintained at a hydrostatic pressure of 13–15 cm of H_2O to ensure adequate oxygen supply. Liver viability was judged on the basis of gross macroscopy, oxygen consumption (> 1.8 μmol min^{-1} (g liver)$^{-1}$), pH of perfusate (7.2–7.4) and bile flow (5 μL min^{-1}).

IPRL was allowed to equilibrate for 15–20 min after connection to the perfusion apparatus. Taurocholic acid (TCA) 60 mM in Krebs solution was infused into the reservoir at 0.5 mL h^{-1} using a syringe infusion pump (Harvard Apparatus, Holliston, MA) to maintain bile flow. In the first experiment, fixed doses of 50, 99 and 198 μg of Fungizone were administered as a bolus into the perfusate reservoir, yielding initial concentrations of 0.50, 0.99 and 1.98 μg mL^{-1}, which correspond to human therapeutic concentrations (Groll et al 1998). In the second experiment, the Krebs-Henseleit perfusate containing 1 μM HDL or LDL was re-circulated for 10 min before 198 μg amphotericin B was injected to achieve a concentration of 1.98 μg mL^{-1}. Serial 0.5-mL samples were withdrawn from the perfusate reservoir at 0, 2, 5, 8, 12, 15, 20, 30, 50, 80 and 120 min; another 0.5 mL of corresponding perfusate solution was sampled from the IVC exit at the same time. Samples were immediately replaced by the same volume of drug-free perfusion medium. Bile was collected before amphotericin B administration and at 30-min intervals in tared polypropylene tubes; bile volume was estimated gravimetrically, assuming a density of 1.0 g mL^{-1}. At the end of the experiment, the liver was excised, blotted dry and weighed.

Amphotericin B protein binding

Amphotericin B binding to BSA was determined by ultrafiltration at concentrations of 0.50, 0.99 and 1.98 μg mL^{-1} in Krebs-Henseleit solution containing 1% BSA. Separation of the unbound drug from BSA was performed by centrifugation with a fixed angle rotor in a micropartition device with a 30-KDa cutoff (Centrifree, Millipore, Sydney, Australia) at 1500 rev min^{-1} for 20 min. Samples (200 μL) were taken before and after centrifugation and assayed for amphotericin B concentration by HPLC. The unbound drug percentage (f_u) was determined using the following formula: $f_u = 100 \times C_U/C_{TOT}$, where C_U is the drug concentration in ultrafiltrate and C_{TOT} is the drug concentration in perfusate before centrifugation.

Drug assay

Amphotericin B concentrations in perfusate, ultrafiltrate, liver homogenate and bile were determined by high-performance liquid chromatography (Alak et al 1996). Perfusate, ultrafiltrate and bile samples, with nitroaniline as the internal standard, were extracted with acetonitrile. Following mixing and centrifugation, the supernatant was directly injected into the HPLC system. Extraction of amphotericin B from the liver homogenate was accomplished using solid-phase extraction (C_{18} Maxi-clean, 300 mg; Alltech, Sydney, Australia). Sample extracts were prepared according to Wang et al (1992) and Lee et al (2001). The liver was first homogenized using 0.2 M ice-cold sodium acetate buffer (pH 4.5) followed by vigorous vortex-mixing with acetonitrile. After centrifugation at 2100 rev min^{-1}, 4 °C for 10 min, the supernatant (0.6 mL) was loaded onto pre-conditioned cartridges, which were then washed with 2 mL of methanol–10 mM phosphate buffer pH 7.4 (20:80, v/v).

Amphotericin B retained on the cartridge was then eluted using 2 mL of 10 mM sodium acetate–acetic acid buffer (pH 3.6, including 10 mM EDTA)–acetonitrile (60:40, v/v) including 0.585 $\mu g\,mL^{-1}$ *p*-nitroaniline as internal standard. Finally, the elutes were dried under nitrogen, reconstituted in 200 μL CH_3CN–H_2O (30:70, v/v) and a sample (20 μL) was injected into the HPLC system for quantification. Chromatographic separation of all samples was performed on LiChrospher 100RP-18 (5 μm; E. Merck, Germany) with a mobile phase consisting of 10 mM sodium acetate–acetic acid buffer (pH 3.6, including 10 mM EDTA)–acetonitrile (58:42, v/v) delivered at 0.8 $mL\,min^{-1}$ using an LC-10AS pump (Shimadzu Oceania, Sydney). Peaks were detected at 405 nm using SPD-10A*vp* UV detector (Shimadzu Oceania, Sydney). Amphotericin B concentrations in unknown samples were determined from the slope of the calibration plots of the peak area ratio of amphotericin B to nitroaniline versus amphotericin B concentrations. The linearity of assay was shown across the concentration range of 0.05–48.72 $\mu g\,mL^{-1}$ for perfusate, 0.12–97.44 $\mu g\,g^{-1}$ for liver homogenate and 0.06–5.0 $\mu g\,mL^{-1}$ for bile. The correlation coefficients of the standard curves for all matrices were 0.99. Limit of quantitation (LOQ) for perfusate, liver homogenate and bile were 50 $ng\,mL^{-1}$, 120 ng $(g\ liver)^{-1}$ and 60 $ng\,mL^{-1}$, respectively. The recovery of amphotericin B from perfusate was 86% and 74% from liver homogenate. The intra-day assay variability was 1.7% for perfusate and 7.5% for liver homogenate, while the corresponding inter-day variability was 2.8% and 4.0%.

Pharmacokinetic analysis

Amphotericin B perfusate concentration–time data were fitted to the biexponential equation ($C = Ae^{-\alpha t} + Be^{-\beta t}$) using SCIENTIST software (Version 2.0; MicroMath Scientific Software, Salt Lake City, UT). The area under the perfusate concentration–time curve from zero to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-\infty} = A/\alpha + B/\beta$ (González-Martin et al 1997; Hong et al 1998; Kato et al 1999). Hepatic clearance (CL_H) was determined as $CL_H = dose/AUC_{0-\infty}$. The half-lives of faster and slower distributional phases ($t_{1/2\alpha}$ and $t_{1/2\beta}$) were estimated as $0.693/\alpha$ and $0.693/\beta$, respectively. The hepatic extraction ratio (E_H) was calculated as $E_H = CL_H/Q_H$. The estimated tissue-to-perfusion partition coefficient (K_p) was determined as $C_{120,\,liver}/C_{120,\,out}$, where $C_{120,\,liver}$ and $C_{120,\,out}$ were the amphotericin B concentrations in liver and in perfusate exiting the liver at 120 min of perfusion, respectively. The mass balance of amphotericin B in the IPRL system was calculated by adding the amount of amphotericin B in perfusate, liver and bile during perfusion and relating them to the dose of amphotericin B administered.

Statistical analysis

The data are presented as mean ± standard deviation; statistical analysis was conducted using Microsoft Excel employing analysis of variance followed by Tukey test at the $P < 0.05$ level of significance.

Results

Liver perfusion

The hepatic disposition profiles of amphotericin B at the three doses (50, 99 and 198 μg) are shown in Figure 1. In the IPRL circuit without the liver, amphotericin B perfusate concentrations decreased less than 15% during the 2-h perfusion period, indicating no significant loss or degradation of the drug within the perfusion system. In the presence of the liver, the disappearance of amphotericin B from the perfusate was biexponential (Figure 1). Distinct differences in the perfusate concentration–time profiles can be attributed to a more rapid initial distribution phase at the lowest amphotericin B dose. The initial distribution half-lives ($t_{1/2\alpha}$) were 3.9 ± 2.0 min, 6.1 ± 0.8 min and 5.9 ± 3.1 min for the low, medium and high amphotericin B doses, while the half-life of later phase ($t_{1/2\beta}$) was inversely related to dose, significantly increasing to 613 ± 186 min at 50 μg from 132 ± 57 min at 198 μg ($P < 0.05$). Chromatograms from the perfusate samples did not show any additional new peaks that might be attributable to metabolites of amphotericin B.

Table 1 summarizes the pharmacokinetic parameters of amphotericin B. The $AUC_{0-\infty}$ did not increase in direct proportion to the dose. CL_H and E_H increased proportionally to the dose, being significantly different at 198 μg amphotericin B from those at the lower doses ($P < 0.05$). No significant changes in amphotericin B kinetic parameters were noted for the livers perfused with lipoproteins compared with control livers.

The percentage of amphotericin B unbound in perfusate containing 1% BSA was 18.9 ± 2.1% at low dose,

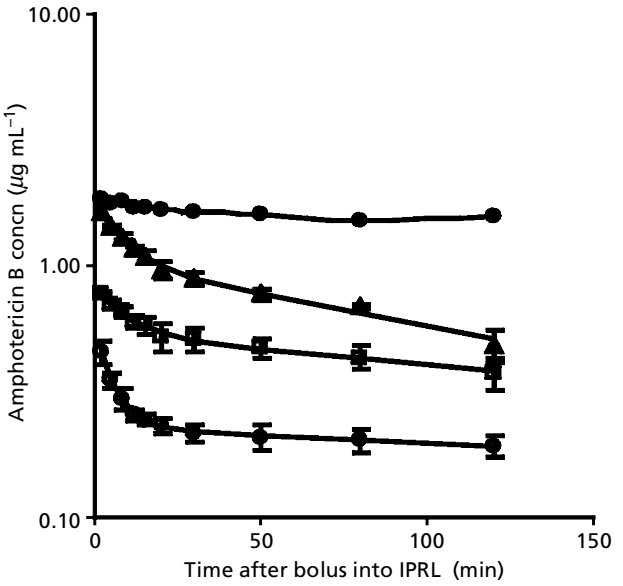

Figure 1 Amphotericin B perfusate concentrations after introduction of amphotericin B bolus into the IPRL reservoir. ●, Control (198 μg amphotericin B without liver); △, 198 μg amphotericin B; □, 99 μg amphotericin B; ○, 50 μg amphotericin B. Data are mean ± s.d., n = 4 for each dose.

Table 1 Hepatic extraction of amphotericin B under various experimental conditions.

Parameter	Amphotericin B dose (μg)			198 μg Amphotericin B	
	50	99	198	LDL (1 μM)	HDL (1 μM)
No. of livers	4	4	4	5	4
Liver weight (g)	12.08 ± 1.57	12.98 ± 1.19	13.96 ± 0.48	13.10 ± 1.68	12.98 ± 0.90
$AUC_{0-\infty}$ ($\mu g\,mL^{-1}\cdot min$)	193.1 ± 33.3	214.4 ± 92.9	196.9 ± 55.4	169.8 ± 35.1	206.8 ± 44.5
CL_H ($mL\,min^{-1}$)	0.27 ± 0.05	0.54 ± 0.23	1.06 ± 0.24[a]	1.21 ± 0.25	0.99 ± 0.20
E_H	0.017 ± 0.003	0.034 ± 0.015	0.066 ± 0.015[a]	0.075 ± 0.016	0.062 ± 0.012
$t_{1/2\alpha}$ (min)	3.9 ± 2.0	6.1 ± 0.8	5.9 ± 1.3	4.4 ± 1.5	4.6 ± 2.6
$t_{1/2\beta}$ (min)	613 ± 186[b]	276 ± 134	132 ± 57	96 ± 27	121 ± 46
K_p	9.76 ± 1.71	10.26 ± 3.94	15.88 ± 6.43	19.65 ± 7.00	14.05 ± 1.22

Results are given as mean ± s.d. [a]$P < 0.05$ vs 50 and 99 μg amphotericin B values; [b]$P < 0.05$ vs 99 and 198 μg amphotericin B values.

18.6 ± 1.6% at medium dose and 17.6 ± 3.1% at high dose (n = 4), indicating that amphotericin B protein binding was concentration-independent over this dose range.

Biliary excretion

Biliary excretion represented a relatively minor fraction of the dose administered and appeared to be dependent upon dose. The cumulative biliary excretion of amphotericin B tended to decrease dose-dependently, from 3.24 ± 1.01% and 2.20 ± 2.06% at low and medium dose to 1.14 ± 0.90% at high dose (Table 2); however, these data were not found to be significantly different, probably due to large inter-rat variation and limited sampling times. Decreased amphotericin B excretion in bile was observed in lipoprotein-containi ng perfusate (0.35 ± 0.13% in LDL, 0.54 ± 0.32% in HDL) compared with that (1.14 ± 0.90%) in control (Table 2). Bile flow was progressively diminished even with continuous TCA perfusion (data not shown). No additional peaks were detected in bile samples.

Liver distribution

At the end of all IPRL experiments, a relatively higher amount of amphotericin B was observed in liver tissue, indicating significant uptake and retention of amphotericin B by the liver. Dose-dependent increase of amphotericin B in liver was observed from 20.60 ± 1.51 μg at low dose, 45.87 ± 13.52 μg at medium dose to 103.23 ± 16.32 μg at high dose ($P < 0.05$). In addition, around 50% of amphotericin B dose was found in the livers, independent of whether or not the livers were perfused with lipoproteins (Table 2). K_p was high and averaged 12.0 ± 5.0 over the dose range studied (Table 1).

Discussion

Current therapeutic regimens for amphotericin B are somewhat empirical, partly due to limited information about its disposition kinetics. Furthermore, there is evidence to suggest that the association of amphotericin B with lipoproteins can alter the drug's tissue distribution. To fully understand the hepatic handling of amphotericin B the re-circulation isolated perfused rat liver (IPRL) was employed in this study. This experimental approach alleviates the confounding effects of other organ systems, plasma constituents and routes of elimination present in the intact animal and has been of value in studies of the hepatic disposition of a number of compounds (Liu et al

Table 2 Recovery of amphotericin B after administration of Fungizone to the IPRL.

	% Recovery of dose				
Amphotericin B dose (μg)	50	99	198	198 + LDL (1 μM)	198 + HDL (1 μM)
Perfusate	48.2 ± 4.5[b]	46.9 ± 6.1	32.2 ± 4.5	34.4 ± 8.7	39.2 ± 2.3
Liver	41.6 ± 3.0	46.3 ± 13.7	52.1 ± 8.2[a]	60.3 ± 18.8	48.0 ± 3.5
Bile	3.24 ± 1.01	2.20 ± 2.06	1.14 ± 0.90	0.35 ± 0.13	0.54 ± 0.32
Total	93.1 ± 3.5	95.5 ± 6.6	85.5 ± 3.2	95.1 ± 21.6	87.7 ± 5.4

Results are given as mean ± s.d. [a]$P < 0.05$ vs 50 and 99 μg amphotericin B values; [b]$P < 0.05$ vs 99 and 198 μg amphotericin B values.

2000; Farabos et al 2001), especially those with low hepatic extraction ratio (Mehvar 1997). A limitation of the IPRL system is that the in-situ preparation is only viable for 2 h. This has implications for the reliable assessment of pharmacokinetic parameters for drugs such as amphotericin B that show low clearance and slow distribution. In this regard the parameters generated in this study must be viewed with a degree of caution but this does not exclude meaningful comparisons between doses and treatments.

This study found that, in the IPRL, amphotericin B exhibits unusual non-linear kinetic behaviour with low extraction ratio (E_H), low hepatic clearance (CL_H) and extensive uptake and retention in the liver. The amphotericin B hepatic disposition involves the initial uptake across the sinusoidal membrane of the hepatocyte followed by retention in hepatic tissue or biliary excretion through canalicular membrane. Furthermore, no peaks were detected in the HPLC chromatograms that might be attributed to amphotericin B metabolite(s) in IPRL perfusate, bile and liver samples. Approximately 90% of the amphotericin B dose is accounted for in mass balance calculations from this study, which suggests that metabolism plays a minor role in amphotericin B elimination. The hepatic clearance (CL_H) calculated in this study must be considered a hybrid parameter predominantly describing the sinusoidal hepatic uptake clearance and to a lesser extent the metabolic clearance and biliary clearance. This observation is important for interpreting the dose dependency of CL_H observed in this study, which is consistent with the presence of a carrier-mediated uptake mechanism for amphotericin B in the liver. Recently, two potential candidates for the carrier-mediated hepatic uptake of bulky organic substances in the rat were identified: OCT1 (Gründemann et al 1994) and OATP (Oude et al 1995). The latter carrier protein, surprisingly, also seems to accommodate amphipathic organic compounds. It is, therefore, reasonable to speculate that the observed non-linearity of amphotericin B uptake into the liver from the perfusate reflects the carrier-mediated uptake. The relatively long half-life of the terminal portion of amphotericin B perfusate concentration is a likely reflection of slow redistribution of amphotericin B from the liver. CL_H was found to increase proportionally to the increasing dose with statistically significant difference being achieved at higher dose. Such an increase has been observed in rats following an intravenous infusion of amphotericin B in-vivo. Chow et al (1995) observed that the systemic clearances (CL) of $3.52 \pm 0.72\,mL\,min^{-1}\,kg^{-1}$ at a steady-state concentration (C_{ss}) of $0.066 \pm 0.020\,\mu g\,mL^{-1}$ and of $4.03 \pm 0.67\,mL\,min^{-1}\,kg^{-1}$ at C_{ss} of $0.543 \pm 0.150\,\mu g\,mL^{-1}$. The half-life ($t_{1/2\beta}$) increased significantly at the lowest dose, which is in agreement with the observation by Chow et al (1995) indicating a relatively long $t_{1/2\beta}$ of 866 ± 102 min at C_{ss} of $0.543 \pm 0.150\,\mu g\,mL^{-1}$. Furthermore, the low E_H is also consistent with previous studies in man, which reported that amphotericin B plasma clearance is at least 50-fold lower than plasma flow to either the kidney or the hepatic-portal system (Davies & Morris 1993; Bekersky et al 2002).

In-vivo studies in rats indicate that most of the amphotericin B dose recovered from tissues was found in the liver (Wang et al 1995; Echevarria et al 2000). In man, only one study was able to quantify the amount of amphotericin B distributed into the liver because of methodological and ethical problems (Christiansen et al 1985). Concentrations of amphotericin B in tissues obtained at autopsy from eight patients were measured by HPLC. Highest concentrations of the drug were found in the liver and the amount of amphotericin B in the patient's liver was 41% of the total dose, which is consistent with the amphotericin B extensive hepatic retention observed in this study (K_p).

The failure of this study to demonstrate metabolism of amphotericin B is consistent with other reports, which found that the urinary and faecal clearances of unchanged amphotericin B amounted to 75% of the total clearance observed in man after 1 week of a single infusion of $2\,mg\,kg^{-1}$ Fungizone (Bekersky et al 2002). Although the sensitivity of the metabolites screen employed is limited, it also suggests the absence of major metabolites after amphotericin B administration.

Biliary excretion of amphotericin B was low in this study compared with the previous observation where the total cumulative faecal recovery of amphotericin B from day 1 to day 28 after intravenous administration of $1\,mg\,kg^{-1}$ Fungizone daily was 20% of the total dose administered while only 3.95% was accounted for in urine (Wang et al 1995). This discrepancy may be because the IPRL system cannot be maintained viable beyond 2 h of perfusion and it is possible that additional amounts of amphotericin B can be excreted into the bile after 2 h. Even using taurocholic acid (TCA) infusion, bile flow gradually decreased over time. This is expected because amphotericin B itself has been shown to reduce bile flow and bile acid excretion in the IPRL (Gaeta et al 1989). Carrier-mediated processes, which translocate substances from hepatocytes into bile through the canalicular membrane, are accountable for the saturation of biliary excretion (Han et al 2001). The characteristics of these canalicular transporters differ considerably from those responsible for uptake into the hepatocytes (Nathanson & Boyer 1991). Those transporters responsible for amphotericin B hepatobiliary translocation need to be further identified.

Amphotericin B is an example of a drug that is insoluble in water and binds to plasma lipoproteins in-vivo and in-vitro, which is believed to have a major impact on the efficacy and safety of amphotericin B. Previous reports demonstrate that amphotericin B or LDL-associated amphotericin B is more toxic to LLC PK1 renal cells than HDL-associated amphotericin B. This increased nephrotoxicity is the consequence of amphotericin B association with LDL, which enhances the ability of amphotericin B to damage kidney cells mediated through the LDL receptors (Wasan et al 1994a; Wasan & Lopez-Berestein 1994; Wasan et al 1994b; Wasan 1996). Therefore, it is expected that alteration of amphotericin B distribution in the lipoprotein-treated livers would have been seen if there was such an involvement of lipoprotein receptors. Instead, the effects of LDL and HDL on the pattern of amphotericin B perfusate concentrations, biliary excretion and its metabolism are negligible, indicating lipoproteins have no influence on amphotericin B disposition in the IPRL. Previous studies

of the effects of LDL on ciclosporin metabolism using IPRL in rats treated with 17α-ethinylestradiol, which markedly increases LDL receptors, further confirmed that LDL-receptors are not involved in drug disposition in liver (Prueksaritanont et al 1992).

Conclusions

In summary, this investigation of amphotericin B disposition in the IPRL has indicated that amphotericin B exhibits unusual characteristics of hepatic disposition. It exhibits non-linear pharmacokinetics, with low E_H and extensive distribution into the liver. Contrary to the usual pattern, CL_H increases with dose. In any case, the liver appears to serve as the body's primary reservoir of amphotericin B, from which free amphotericin B slowly and gradually releases into the systemic circulation. The addition of lipoproteins (LDL and HDL) to the IPRL perfusate had no statistical influence on the hepatobiliary disposition of amphotericin B. Further studies will be needed to fully elucidate the mechanism of amphotericin B hepatic disposition.

References

Alak, A., Moy, S., Bekersky, I. (1996) A high-performance liquid chromatographic assay for the determination of amphotericin B serum concentrations after the administration of AmBisome, a liposomal amphotericin B formulation. *Ther. Drug Monit*. **18**: 604–609

Bekersky, I., Fielding, R., Dressler, D. E., Lee, J. W., Buell, D. N., Walsh, T. J. (2002) Pharmacokinetics, excretion, and mass balance of liposomal amphotericin B (AmBisome) and amphotericin B deoxycholate in humans. *Antimicrob. Agents Chemother*. **46**: 828–833

Chow, H. H., Wu, Y., Mayersohn, W. (1995) Pharmacokinetics of amphotericin B in rats as a function of dose following constant-rate intravenous infusion. *Biopharm. Drug Dispos*. **16**: 461–473

Christiansen, K. J., Bernard, E. M., Gold, J. W., Armstrong, D. (1985) Distribution and activity of amphotericin B in humans. *J. Infect. Dis*. **152**: 1037–1043

Coukell, A. J., Brogden, R. N. (1998) Liposomal amphotericin B: therapeutic use in the management of fungal infections and visceral leishmaniasis. *Drugs* **55**: 585–612

Davies, B., Morris, T. (1993) Physiological parameters in laboratory animal and humans. *Pharm. Res*. **10**: 1093–1095

Echevarria, I., Barturen, C., Renedo, M. J., Troconiz, I. F., Dios-Vieitez, M. C. (2000) Comparative pharmacokinetics, tissue distributions, and effects on renal function of novel polymeric formulations of amphotericin B and amphotericin B-deoxycholate in rats. *Antimicrob. Agents Chemother*. **44**: 898–904

Farabos, C., Haaz, M. C., Gires, P., Robert, J. (2001) Hepatic extraction, metabolism, and biliary excretion of irinotecan in the isolated perfused rat liver. *J. Pharm. Sci*. **90**: 722–731

Gaeta, G. B., Utili, R., Adinolfi, L. E., Tripodi, M. F., Esposito, V. (1989) Effects of amphotericin B on the excretory function and the colloid clearance capacity of the perfused rat liver. *J. Hepatol*. **8**: 344–350

González-Martin, G., Domínguez, A. R., Guevara, A. (1997) Pharmacokinetics and hepatotoxicity of diclofenac using an isolated perfused rat liver. *Biomed. Pharmacother*. **51**: 170–175

Groll, A. H., Piscitelli, S. C., Walsh, T. J. (1998) Clinical pharmacology of systemic antifungal agents: a comprehensive review of agents in clinical use, current investigational compounds, and putative targets for antifungal drug development. *Adv. Pharmacol*. **44**: 343–500

Gründemann, D., Gorboulev, V., Gambaryan, S. (1994) Drug excretion mediated by a new prototype of polyspecific transporter. *Nature* **372**: 549–552

Han, Y. H., Kato, Y., Watanabe, Y., Terao, K., Asoh, Y., Sugiyama, Y. (2001) Carrier-mediated hepatobiliary transport of a novel antifolate, N-[4-[(2,4-dianninopteridine-6-yl)methyl]-3,4-dihydro-2H-1,4-benzothiazin-7-yl]carbonyl-L-homoglutamic acid, in rats. *Drug Metab. Dispos*. **29**: 394–400

Hong, G., Bazin-Redureau, M., Gires, P., Scherrmann, J. M. (1998) Hepatic disposition and toxicity of cationized goat immunoglobulin G and FAB fragments in isolated perfused rat liver. *Drug. Metab. Dispos*. **26**: 661–669

Janknegt, R., de Marie, S., Bakker-Woudenberg, I., Crommelin, D. (1992) Liposomal and lipid formulations of amphotericin B: clinical pharmacokinetics. *Clin. Pharmacokinet*. **23**: 279–291

Kato, Y., Akhteruzzaman, S., Hisaka, A., Sugiyama, Y. (1999) Hepatobiliary transport governs overall elimination of peptidic endothelin antagonists in rats. *J. Pharmacol. Exp. Ther*. **288**: 568–574

Kwong, M., Wasan, K. M. (2002) Cholesteryl ester transfer protein facilitates the movement of water-insoluble drugs between lipoproteins: a novel biological function for a well-characterized lipid transfer protein. *Biochem. Pharmacol*. **64**: 1669–1675

Lee, J. W., Petersen, M. E., Lin, P., Dressler, D., Berkersky, I. (2001) Quantitation of free and total amphotericin B in human biologic matrices by a liquid chromatography tandem mass spectrometric method. *Ther. Drug Monit*. **23**: 268–276

Liu, Y., Bacon, E. R., Ballinger, K., Black, C. D., Illig, K., McIntire, G. L., Wang, P. P., O'Neil, N., Kinter, L., Desai, V. C. (2000) Pharmacokinetics and hepatic disposition of bis[1-(ethoxycarbonyl)propyl]5-acetylamino-2,4,6-triiodo-isophthalate in rats and isolated perfused rat livers. *Drug Metab. Dispos*. **28**: 731–736

Mehvar, R. (1997) Kinetics of hepatic accumulation of dextrans in isolated perfuse rat livers. *Drug Metab. Dispos*. **25**: 552–556

Nathanson, M. H., Boyer, J. L. (1991) Mechanism and regulation of bile secretion. *Hepatology* **14**: 551–566

Oude, E., Meijer, D. K., Kuipers, F. (1995) Hepatobiliary secretion of organic compounds: molecular mechanisms of membrane transprort. *Biochim. Biophys. Acta* **1241**: 215–268

Prueksaritanont, T., Hoener, B., Benet, L. Z. (1992) Effects of low-density lipoprotein and ethinyl estradiol on cyclosporine metabolism in isolated rat liver perfusion. *Drug Metab. Dispos*. **20**: 547–552

Wang, L. H., Smith, P. C., Anderson, K. L. (1992) High-performance liquid chromatographic analysis of amphotericin B in plasma, blood, urine and tissue for pharmacokinetic and tissue distribution studies. *J. Chromatogr*. **579**: 259–268

Wang, L. H., Fielding, R. M., Smith, P. C. (1995) Comparative tissue distribution and elimination of amphotericin B colloidal dispersion (Amphocil) and Fungizone after repeated dosing in rats. *Pharm. Res*. **12**: 275–283

Wasan, K. M. (1996) Modifications in plasma lipoprotein concentration and lipid composition regulate the biological activity of hydrophobic drugs. *J. Pharmacol. Toxicol. Methods* **36**: 1–11

Wasan, K. M., Lopez-Berestein, G. (1994) Modification of amphotericin B's therapeutic index by increasing its association with serum high-density lipoproteins. *Ann. N. Y. Acad. Sci*. **730**: 93–106

Wasan, K. M., Morton, R. E., Rosenblum, M. G., Lopez-Berestein, G. (1994a) Decreased toxicity of liposomal amphotericin B due to association of amphotericin B with high-density lipoproteins: role of lipid transfer protein. *J. Pharm. Sci.* **83**: 1006–1010

Wasan, K. M., Rosenblum, M. G., Cheung, L., Lopez-Berestein, G. (1994b) Influence of lipoproteins on renal cytotoxicity and antifungal activity of amphotericin B. *Antimicrob. Agents Chemother.* **38**: 223–227