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Heat Treatment of Amphotericin B Modifies Its Serum Pharmacokinetics, Tissue Distribution, and Renal Toxicity following Administration of a Single Intravenous Dose to Rabbits

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The purpose of this investigation was to determine the serum pharmacokinetics, tissue distribution, and renal toxicity of amphotericin B (AmpB) following administration of a single intravenous dose (1 mg/kg of body weight) of Fungizone (FZ) and a heat-treated form of FZ (HFZ) to New Zealand White female rabbits. FZ solutions were heated at 70°C for 20 min to produce HFZ. Blood samples were obtained before drug administration and serially thereafter. After collection of the 48-h blood sample, each rabbit was humanely sacrificed and the right kidney, spleen, lungs, liver, and heart were harvested for AmpB analysis. Serum creatinine levels were measured before and 10 h after drug administration. AmpB concentrations in the serum and tissues were analyzed using high-performance liquid chromatography. FZ administration to rabbits resulted in a greater-than-50% increase in serum creatinine concentrations compared to baseline. However, HFZ administration resulted in no difference in serum creatinine concentrations compared to baseline. The AmpB area under the concentration-time curve (AUC) after HFZ administration was significantly lower than the AmpB AUC in rabbits administered FZ. However, AmpB systemic total body clearance was significantly greater in rabbits administered HFZ than in rabbits administered FZ without any differences in volume of distribution at steady state. Kidney tissue AmpB concentrations, although not significantly different, were greater in rabbits administered FZ than in rabbits administered HFZ. Likewise, lung and spleen AmpB concentrations, although not significantly different, were greater in rabbits administered FZ than in rabbits administered HFZ. However, liver AmpB concentrations were significantly lower in rabbits administered FZ than in rabbits administered HFZ. No significant differences in heart AmpB concentration between rabbits administered FZ and those given HFZ were found. These findings suggest that the pharmacokinetics, tissue distribution, and renal toxicity of AmpB are modified following administration of HFZ. HFZ could be an improved low-cost AmpB drug delivery system that has a potentially higher therapeutic index than FZ.

Amphotericin B (AmpB) is a polyene macrolide antibiotic used for the treatment of systemic fungal infections commonly found in immunocompromised patients (i.e., those with AIDS), cancer patients, and diabetics (2–4, 8, 13, 16). The conventional AmpB-deoxycholate micellar formulation, Fungizone (FZ) (Bristol-Myers Squibb, Princeton, N.J.), has been used for over 45 years, and, despite its dose-dependent kidney toxicity, it remains the most widely used drug for the treatment of most systemic fungal infections (2, 8, 13). In addition, less-toxic liposomal and lipid-associated AmpB formulations have been developed (e.g., AmBisome, Abelcet, and Amphocil), and, although they have been proven to reduce AmpB-induced kidney toxicity (6, 16–20), their use has been limited by their high expense.

A potentially simple and inexpensive alternative is the heat treatment (70°C for 20 min) of FZ to produce a "superaggregated" form of AmpB commonly referred to as heat-treated Fungizone (HFZ) (1, 5, 7, 11, 12). As recently reported by Hartsel et al., this new self-associated form of AmpB is spectroscopically different from FZ, with a blue-shifted absorption maximum and a uniquely characteristic circular dichroism spectrum (1, 7).

Gaboriau et al. have reported that HFZ exhibits significantly lower in vitro cytotoxicity against mammalian cells without diminishing its cytotoxic effect against fungal cells (5). In addition, Petit et al. have recently reported that HFZ has a therapeutic index superior to that of FZ in murine models of systemic fungal infections (11, 12). However, to date little is known about the pharmacokinetics, tissue distribution, and renal toxicity of AmpB following administration of a single intravenous dose of HFZ to rabbits. Thus, the objective of this study was to evaluate the serum pharmacokinetics, tissue distribution and renal toxicity of AmpB following administration of a single intravenous (i.v.) bolus dose of HFZ and FZ to rabbits.

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MATERIALS AND METHODS

Chemicals and plasma. The commercially available lyophilized powder form of AmpB-deoxycholate (FZ) was purchased from Bristol-Myers Squibb Canada

TABLE 1. Serum creatinine and pharmacokinetic parameters of AmpB after administration of a single i.v. dose of FZ and HFZ[a]

Treatment	Serum creatinine		AUC_{0-48} ($\mu g \cdot h/ml$)	V_{ss} (ml/kg)	CL (ml/h/kg)
	Concn (μmol/liter)		% Change from baseline		
	Prior to drug administration	10 h following drug administration			
FZ	60 ± 12	91 ± 4[b]	51.7	11.3 ± 2.5	1,251 ± 187	88.9 ± 19.4
HFZ	57 ± 7	65 ± 5	13.2	3.1 ± 0.3[c]	1,277 ± 112	313 ± 28[c]

[a] Data are means ± standard deviations ($n = 6$ for FZ; $n = 4$ for HFZ). Doses (1 mg/kg) were give to female New Zealand White rabbits (2.5 to 3 kg). Serum creatinine is a measure of kidney toxicity. Increases in serum creatinine concentration suggest elevation in kidney toxicity.
[b] $P < 0.05$ versus value prior to drug administration.
[c] $P < 0.05$ versus value for FZ treatment.

Inc. and reconstituted with 10 ml of distilled water (final concentration of 5 mg/ml). For all stability and activity studies, a 100 μM solution of FZ in phosphate-buffered saline at pH 7.4 was made. HFZ was prepared by heating FZ solutions for 20 min in a water bath at 70°C as previously described (1, 5, 7).

Rabbit model. New Zealand White female rabbits (2.5 to 3.0 kg; Jeo-Bet Rabbits Ltd., Aldon, British Columbia, Canada; $n = 10$) used for this study were cared for in accordance with the principles promulgated by the Canadian Council on Animal Care and the University of British Columbia. They were housed within individual metabolism cages in an animal facility with a 12-h dark-light cycle and controlled temperature and humidity. Water and food (Purina rabbit chow 5001) were unrestricted throughout the study. This was an "ideal animal model" because no kidney or liver function and hematological-profile abnormalities were observed in age-matched New Zealand White rabbits and blood samples were obtained without significant changes in blood flow (9). Furthermore, rabbits were the appropriate experimental animals to use in these studies because the behavior and structure of their systemic proteins and lipoproteins are similar to those of humans (10).

The operative technique for chronic catheter insertion was modified from that of Walsh and coworkers (15) to include a heparin lock device (Harvard Apparatus Canada, Saint-Laurent, Quebec, Canada) (20). Briefly, a 2-cm incision was made in the right anterolateral cervical region about 3 cm posterior to the angle of the jaw to expose the external jugular vein. A segment of the vein was freed from subcutaneous fat just below the bifurcation of the internal and external maxillary veins. A catheter was then flushed with sterile saline and inserted carefully through an incision in the external jugular venous wall until the catheter cuff was continuous with the vein wall. Two silk suture ties were used to ligate the Silastic catheter to the external jugular vein. After two-way flow was confirmed, the catheter was flushed with 1 ml of heparin (1,000 U/ml). Rabbits were then brought to the recovery room for postoperative observation.

Measurement of AmpB. Serum and tissue samples were obtained and processed for AmpB analysis as previously described (16, 18–20). AmpB levels in serum and tissues were determined by high-pressure liquid chromatography using an external calibration curve as previously described (16, 18–20).

Assessment of renal function. To assess renal function, serum creatinine concentrations prior to and 10 h following the administration of FZ or HFZ were measured by standard enzymatic reactions (Sigma Chemical, St. Louis, Mo.). For the purposes of this study and based on our preliminary studies with rats (18), rabbits (20), and humans (16) the criterion for measurable kidney toxicity was set at a 50% increase in serum creatinine concentration from baseline. Ten hours was chosen because initial studies demonstrated that following administration of a single i.v. bolus of FZ (1 mg/kg of body weight) to rabbits serum creatinine reached its maximum elevation from baseline 10 h following administration of the dose (data not shown).

Experimental design. New Zealand White rabbits (2.5 to 3 kg) were administered either a single i.v. dose of FZ ($n = 6$) or HFZ ($n = 4$) (1 mg of AmpB/kg) through the jugular vein. Preliminary studies have shown that an FZ dose of 1 mg/kg is sufficient to treat experimental candidiasis and yet exhibits measurable kidney toxicity (16–20). Following FZ and HFZ administration serial blood samples were obtained and stored in centrifuge tubes prior to and 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, 24, and 48 h after the injection. Serum was harvested and stored at 4°C prior to analysis to prevent any redistribution of drug. After collection of the 48-h sample, each rabbit was humanely sacrificed and the heart, spleen, liver, right kidney, and lungs were removed, dried, and weighed. Each organ was stored at −20°C until analysis.

Pharmacokinetic analysis. The pharmacokinetic parameters total body clearance (CL) and volume of distribution at steady state (V_{ss}) were estimated by compartmental analysis using the WINNONLIN nonlinear estimation program (14). It was concluded that the AmpB serum concentration data fit a two-compartment model based on "goodness-of-fit" and residual-sum-of-squares estimations using the WINNONLIN program. In addition, an independent criterion (the Akaike information criterion) for determination of the goodness of fit was used. Concentrations of AmpB in serum were plotted against time on log-linear graph paper (14). The area under the AmpB concentration-time curve from 0 to 48 h (AUC_{0-48}) was estimated by the trapezoidal rule (14).

Statistical analysis. AmpB serum pharmacokinetics, tissue concentration, and serum creatinine concentration for treatment groups were compared by an unpaired t test (INSTAT; GraphPad). Critical differences were assessed by Tukey post hoc tests. A difference was considered significant if the probability of chance explaining the results was reduced to less than 5% ($P < 0.05$). All data are expressed as means ± standard deviations.

RESULTS

A single i.v. dose of FZ to rabbits resulted in a greater-than-50% increase in serum creatinine concentrations compared to baseline (Table 1). However, following administration of a single i.v. dose of HFZ to rabbits no difference in serum creatinine concentrations compared to baseline was observed (Table 1).

The AmpB AUC after administration of a single i.v. dose of HFZ in rabbits was significantly lower than the AUC in rabbits administered FZ (Fig. 1 and Table 1). However, AmpB sys-

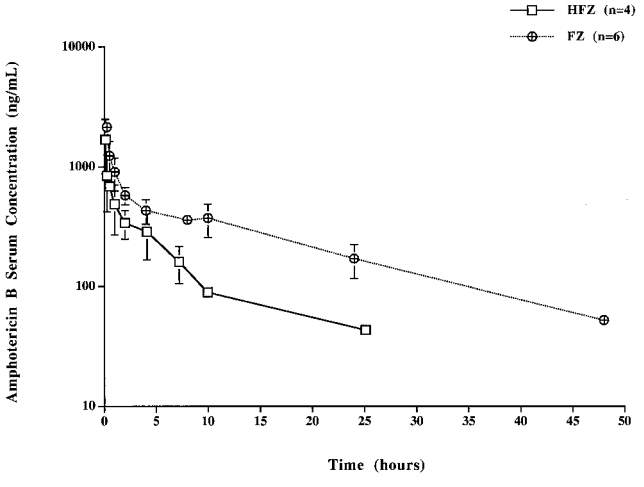

FIG. 1. AmpB concentration in serum versus time following administration of a single i.v. bolus of either FZ or HFZ (1 mg of AmpB/kg) to rabbits. Values are means ± standard deviations. Note that the 48-h time point following HFZ administration was not reported because it was below the limit of detection of the AmpB high-pressure liquid chromatography methodology used in this study.

TABLE 2. Tissue AmpB distribution following administration of a single i.v. dose (1 mg/kg) of FZ and HFZ to New Zealand White female rabbits (2.5 to 3.0 kg)[a]

Tissue	Level of AmpB (μg/g of tissue) after treatment with:	
	FZ	HFZ
Kidney	0.94 ± 0.20	0.61 ± 0.44
Liver	3.07 ± 0.70	5.29 ± 1.17[b]
Lung	0.82 ± 0.20	0.43 ± 0.29
Spleen	5.60 ± 3.70	2.53 ± 1.67
Heart	0.06 ± 0.05	0.095 ± 0.011

[a] Data are means ± standard deviations ($n = 6$ for FZ, and $n = 4$ for HFZ).
[b] $P < 0.05$ versus value for FZ.

temic CL was significantly greater in rabbits administered HFZ than in rabbits administered FZ without any differences in V_{ss} (Table 1).

Kidney AmpB tissue concentrations, although not significantly different, were greater in rabbits administered FZ than in rabbits administered HFZ (Table 2). Likewise, lung and spleen AmpB concentrations, although not significantly different, were greater in rabbits administered FZ than in those administered HFZ (Table 2). However, liver AmpB concentrations were significantly lower in rabbits administered FZ than in rabbits administered HFZ (Table 2). No significant differences in heart AmpB concentrations between rabbits administered FZ and HFZ were observed (Table 2).

DISCUSSION

AmpB remains one of the most effective and widely used antifungal agents for the treatment of systemic fungal infections such as candidiasis, histoplasmosis, and aspergillosis commonly found in patients who are immunocompromised, who have cancer, or who are diabetic. However, the administration of AmpB has been limited by its dose-dependent renal toxicity, which has not been predictable by monitoring of the serum drug concentration (16–20). Since heat-induced superaggregation of FZ reduces its in vitro toxicity (5), we studied the influence of prior heat treatment of FZ on AmpB disposition, tissue distribution, and renal toxicity in rabbits.

There were differences in AmpB disposition, tissue distribution, and AmpB-induced renal toxicity following the administration of HFZ to rabbits compared to values for rabbits administered FZ. AUC was decreased in rabbits administered HFZ compared to those administered FZ. This result could be explained by the fact that the systemic clearance of AmpB was significantly higher in rabbits administered HFZ than in rabbits administered FZ. Furthermore, the volume of distribution of AmpB following HFZ administration was not significantly different from that following FZ administration, suggesting that binding differences probably don't account for changes in disposition.

Since the heat treatment of FZ has been shown to decrease the toxicity of the drug to mammalian cells (11, 12), we hypothesized that HFZ administration would result in less AmpB-induced kidney toxicity than FZ administration. Consistent with this hypothesis we observed significantly lower increases in serum creatinine concentrations from baseline following HFZ administration than following FZ administration (Table 1). This lack of change in serum creatinine concentrations indirectly suggests that HFZ does not damage the glomerular filtration of the kidney to the same extent that FZ does.

In addition, we hypothesized that the lower AmpB-induced kidney toxicity observed following HFZ administration (Table 1) may be due to a lower concentration of AmpB recovered in the kidney and a greater concentration of drug recovered in other tissues such as the liver. We observed lower kidney AmpB concentrations with a statistically significantly greater concentration of AmpB in the liver following HFZ administration than following FZ administration. However, no statistically significant differences in lung, spleen, and heart AmpB concentrations were observed. Taken together, these findings suggest that the heat treatment of FZ to change it into a superaggregated complex would increase its clearance from the systemic circulation resulting in a greater distribution into the liver and possibly less AmpB found in the kidney. This decrease in kidney distribution may be one reason for the diminished AmpB-induced renal toxicity of HFZ. Hartsel and others have shown that heat treatment of FZ results in a condensation of monomeric and aggregated forms of AmpB (1, 7). This condensation results in a larger, physiologically stable superaggregated complex, which appears to be more susceptible to circulating macrophages, which in turn would deliver more drug to tissues rich in phagocytes such as the liver. However, they have shown that this superaggregated complex has reduced interaction with mammalian mimetic cell membranes, resulting in lower cytotoxicity (1). Furthermore, the observations that the liver AmpB concentration is increased and the AmpB AUC is decreased with no significant differences in V_{ss} following HFZ administration further support the hypothesis that the superaggregated form of AmpB has a higher disposition for the liver while sparing other tissues. Further work to test these hypotheses is warranted.

Although efficacy was not measured in this animal study, Gaboriau et al. have reported that HFZ exhibits significantly lower cytotoxicity against mammalian cells than FZ without its cytotoxic effect against fungal cells being diminished (5). Currently studies using an animal model with systemic fungal infection are being completed to address this concern.

In conclusion, we have demonstrated differences in the pharmacokinetics, liver distribution, and drug-induced renal toxicity of AmpB between single-i.v.-dose administrations of HFZ and FZ to rabbits. These findings suggest that heat-treated FZ could be an improved low-cost AmpB drug delivery system that has a potentially higher therapeutic index than FZ. However, a multiple-dose study with large doses to determine efficacy is warranted.

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