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

EVAN H. KWONG, 1 MANISHA RAMASWAMY, 1 EMILY A. BAUER, 2 SCOTT C. HARTSEL, 2 AND KISHOR M. WASAN 1 *

Division of Pharmaceutics and Biopharmaceutics, Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3, 1 and Department of Chemistry, University of Wisconsin-Eau Claire, Eau Claire, Wisconsin 2

Received 9 October 2000/Returned for modification 24 February 2001/Accepted 29 March 2001

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.

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum creatinine</th>
<th>% Change from baseline</th>
<th>AUC$_{0-48}$ (μg·h/ml kg)</th>
<th>$V_{ss}$ (ml/kg)</th>
<th>CL (ml/h/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FZ</td>
<td>Prior to drug administration: 60 ± 12</td>
<td>51.7</td>
<td>11.3 ± 2.5</td>
<td>1,251 ± 187</td>
<td>88.9 ± 19.4</td>
</tr>
<tr>
<td>HFZ</td>
<td>57 ± 7</td>
<td>13.2</td>
<td>3.1 ± 0.3</td>
<td>1,277 ± 112</td>
<td>313 ± 28</td>
</tr>
<tr>
<td></td>
<td>10 h following drug administration: 91 ± 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Data are means ± standard deviations (n = 6 for FZ; n = 4 for HFZ). Doses (1 mg/kg) were given to female New Zealand White rabbits (2.5 to 3 kg). Serum creatinine is a measure of kidney toxicity. Increases in serum creatinine suggest elevation in kidney toxicity.

$^p < 0.05$ versus value prior to drug administration.

$^P < 0.05$ 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; Eco-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 methodology used in this study. Reported because it was below the limit of detection of the AmpB high-pressure liquid chromatography methodology used in this study.

**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.](http://aac.asm.org/)

\[\text{AmpB concentration (µg/ml) vs. Time (hours)}\]
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.

ACKNOWLEDGMENTS

This work was supported with a grant from the Canadian Institutes of Health Research (grant MT-14484 to K.M.W.) and a grant from the National Science Foundation (MCB-9603582 to S.C.H.). Evan H. Kwong was supported by the Rx&D Health Research Foundation/Canadian Institutes of Health Research. Emily A. Bauer was supported by the Ronald E. McNair Scholars Program. We thank Michael Boyd for his surgical assistance.
REFERENCES


