Heat-Treated Fungizone Retains Amphotericin B Antifungal Activity Without Renal Toxicity in Rats Infected with *Aspergillus fumigatus*

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Purpose. The purpose of this study was to assess the antifungal activity and renal and hepatic toxicity of amphotericin B (AmpB) following administration of Fungizone (FZ) and a heat-treated form of FZ (HFZ) to rats infected with *Aspergillus fumigatus.*

Methods. Infected rats were administered FZ and HFZ at a dosing regimen of 1 mg/kg IV once daily for 4 consecutive days. Following administration the number of colony forming units (CFUs) of *Aspergillus fumigatus* in different organs and serum creatinine concentrations were determined.

Results. FZ and HFZ had similar overall effectiveness in decreasing the total number of *Aspergillus fumigatus* CFUs found in all organs analyzed compared to controls. Except for the serum creatinine concentrations reported in the nontreated infected control rats, none of the treatment groups tested displayed a greater than 50% increase in serum creatinine.

Conclusions. Taken together, these findings suggest that HFZ at 1 mg/kg once daily × 4 days appears to be as effective as FZ as an antifungal agent without renal toxicity.

KEY WORDS: antifungal activity; *Aspergillus fumigatus*; Fungizone; heat-treated amphotericin B; rats.

INTRODUCTION

Invasive aspergillosis has recently developed into a widespread life-threatening fungal infection commonly found in immunocompromised patients (i.e., AIDS) (1). Amphotericin B-deoxycholate micellar formulation, Fungizone (FZ; Bristol Myers Squibb, Nutley, NJ), has been used for more than 45 years in the treatment of a variety of systemic fungal infections (i.e., candidasis, leishmaniasis, and aspergillosis) (1–8) and despite its dose-dependent kidney toxicity remains one of the most widely used drug for these infections (2,5–7). Less toxic liposomal and lipid-associated amphotericin B (AmpB) formulations have been developed [e.g., AmBisome, Abelcet (also known as amphotericin B lipid complex, ABLC), Amphocil], and although they have proven to reduce AmpB-induced kidney toxicity (8–14), their use has been limited by their high expense.

An inexpensive alternative FZ is the heat treatment (70°C for 20 min) of FZ that produces a "superaggregated" form of AmpB commonly referred to as heat-treated Fungizone (HFZ) (15–19). As 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 (15,17). They further reported that this heat-induced "superaggregated" form of AmpB was more stable in the presence of high- and low-density lipoproteins, whereas FZ is less stable and more dynamic with the aggregate dissociating to a greater extent in the presence of either lipoprotein (17). Further studies using human monocytes reported that HFZ provokes less release of tumor necrosis factor-alpha (TNFα) while retaining AmpB-induced antifungal activity compared to FZ (17,20). It has been speculated that the non-aggregated form of AmpB and the release of TNFα may be involved with side effects associated with AmpB administration (15–17,20).

Gaboriau *et al.* have reported that HFZ exhibits significantly lower *in vitro* cytotoxicity against human colon cancer cells without diminishing its cytotoxic effect against *Leishmania donovani* (16). Petit *et al.* have reported that HFZ has a superior therapeutic index than FZ in murine models of systemic candidiasis and leishmaniasis (18,19). We have reported that HFZ administration to rabbits significantly lowered AmpB-induced renal toxicity and modified AmpB plasma pharmacokinetics and tissue distribution compared to FZ administration (21). In addition, our group has recently shown that HFZ decreases AmpB-induced renal cytotoxicity without modifying AmpB's antifungal activity in cell culture (22). However, to date few studies investigating the antifungal activity of HFZ *vs.* FZ following administration to rats infected with *Aspergillus fumigatus* have been reported. Therefore, the purpose of this study was to assess the antifungal activity of HFZ vs. FZ following administration in experimental systemic aspergillosis. We hypothesize that HFZ will have similar antifungal activity without observable toxicity as FZ.

MATERIALS AND METHODS

Aspergillus fumigatus isolated from a patient with disseminated aspergillosis (provided by the BC Center for Disease Control, F1048) was used to infect the rats. The inoculum was grown for 48 h at 37°C on Sabouraud dextrose agar. Spores were harvested from the agar using glass beads and suspended in pyrogen-free saline. Spore suspensions were standardized to 1% transmission at 540 nm (LKB Ultraspec II) (10,15). The *Aspergillus fumigatus* inoculum [1.3 to 2.3 × 10^7 colony-forming units (CFU)] was injected through the jugular vein of male albino Sprague Dawley rats (350–400 g). The jugular vein of the rat was cannulated by a similar method used for rabbits (21). After 48-h post-*Aspergillus* injection a single intravenous (IV) dose of either FZ, HFZ (1 mg AmpB/kg) or an equivalent volume of normal saline (vehicle control) was administered once daily for 4 days.

Fungizone (purchased from Vancouver General Hospital Department of Pharmacy; contains 50 mg of AmpB for every 41 mg of sodium deoxycholate) was reconstituted with sterile water. Heat-treated FZ (HFZ) was prepared by heating FZ solutions for 20 min in water-bath at 70°C as previously described (15–17).

All of the animals used in the present study were cared for in accordance with the principles promulgated by the Canadian Council on Animal Care and the University of British

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Table I. Treatment Groups in the Study

Treatment groups	N	Dosage
Vehicle control (normal saline; NS)	12	1 ml
Fungizone (FZ)	8	1 mg/kg
Heat-treated fungizone (HFZ)	6	1 mg/kg

Prior to, 24, 48, and 144 h after FZ, HFZ, or NS administration, serum samples were obtained for serum creatinine measurements as an indirect evaluation of renal function and for serum AST measurements as an indirect evaluation of hepatic function. Following the 144 h blood collection, each rat were sacrificed by injecting a single intraperitoneal dose of sodium pentobarbital (300 mg/kg); the kidneys, spleen, lung, liver, heart, and brain were removed, blot dried, and weighed. The degree of antifungal activity was determined by measuring the number of colony forming units (CFU) of *Aspergillus fumigatus* in whole blood and all tissues.

Columbia. A total of 48 male albino Sprague-Dawley rats (weight range, 350 to 400 g; Charles River Canada, Montreal, Quebec, Canada) were housed in an animal facility with a 12-h dark-light cycle and controlled temperature and humidity. The availability of water and food (Purina rat chow) was unrestricted throughout the duration of the study. Renal function was measured by determining serum creatinine (SCr) concentrations prior to and 48 h and 144 h after administration of the drugs or normal saline (NS). For the purposes of this study, the criterion for measurable kidney toxicity was set as a 50% increase in serum creatinine concentration from baseline. To assess antifungal activity (Table I), brain, lung, heart, liver, spleen and kidney sections (1 g of each tissue) were homogenized with normal saline (2 ml; concentration of 0.5 g tissue/1 ml) (Heidolph diax 900) for 5 min on ice. Ten-fold serial dilutions of 0.1 ml homogenate were spread plated onto Saboraud dextrose agar plates and incubated for 48 h at 37°C. Surviving colonies of *A. fumigatus* were counted (CFU/ml homogenate, corrected for tissue weight).

The number of CFUs in tissues and serum creatinine concentration prior to and following administration were compared between each treatment group by analysis of variance (INSTAT2; GraphPad Inc.). Critical differences were assessed by Tukey *post hoc* tests (4). A difference was considered significant if the probability of chance explaining the results was reduced to less than 5% ($p < 0.05$). All data were expressed as a mean ± standard deviation.

RESULTS AND DISCUSSION

Fungizone and heat-treated Fungizone at a dosing regimen of 1 mg/kg IV once daily for 4 consecutive days had similar overall effectiveness in decreasing the total number of *Aspergillus fumigatus* CFUs found in all organs analyzed compared to controls (Table II). Except for the serum creatinine concentrations reported in the nontreated infected control rats, none of the treatment groups tested displayed a greater than 50% increase in serum creatinine (Table III).

The use FZ as therapy for invasive aspergillosis has resulted in a response rate of around 40% among a variety of patient populations' (1). However, dose-dependent renal toxicity has limited the aggressive use of this AmpB formulation. Since HFZ appears to be less toxic and as equally as effective as FZ in cell culture studies (22), it has been hypothesized that administering HFZ at higher doses than FZ could be achieved to eradicate all of the fungal infection without the associated AmpB-induced nephrotoxicity. The purpose of this study was to assess the antifungal activity of HFZ vs. FZ following administration within rats infected with *Aspergillus fumigatus*.

Previous studies, primarily *in vitro* and animal models (16–22) have reported the benefits of HFZ in the treatment of systemic fungal infections. However, few studies have investigated the effectiveness and possible reduced toxicity of HFZ in a rat model of systemic *Aspergillus fumigatus*. Our investigation reports that HFZ at 1 mg AmpB/kg × 4 days has similar antifungal activity as FZ at 1 mg AmpB/kg × 4 days. Based on our selected criteria for kidney toxicity as a 50% increase in SCR from baseline, none of the treatment groups displayed measurable kidney toxicity (Table III). Results presented in this paper are consistent with results we observed in rabbits (21). In those studies, we investigated the influence of prior heat treatment of FZ on AmpB disposition, tissue distribution and renal toxicity in rabbits and observed significantly lower increases in serum creatinine concentrations from baseline following HFZ administration than FZ administration (21). This lack of change in serum creatinine concentrations indirectly suggested that HFZ does not damage the glomerular filtration of the kidney to the same extent FZ does. We further observed lower AmpB kidney concentrations following HFZ than FZ administration (21). Gaboriau *et al.* have reported that HFZ exhibits significantly lower cytotoxicity against other mammalian cells compared to FZ without diminishing its cytotoxic effect against fungal cells (16). Taken together, these findings suggest that the heat treatment of FZ into a "superaggregated" complex has reduced interaction with kidney cell membranes resulting in lower cytotoxicity (15).

In conclusion, this study assesses the antifungal activity and renal toxicity of FZ vs. HFZ in Experimental Systemic Aspergillosis. Our findings suggest that HFZ at 1 mg/kg once

Table II. Fungi Analysis of *Aspergillus fumigatus*–Infected Male Sprague-Dawley Rats Treated with Single Intravenous Doses of Normal Saline (Controls), Fungizone (FZ; 1 mg/kg × 4 days), and Heat-Treated Fungizone (HFZ; 1 mg/kg × 4 days)

	Infected tissues (CFU/0.5 g of homogenized tissue)						
Treatment groups	Brain	Lungs	Heart	Liver	Spleen	Kidney	All organs
Controls (n = 12)	2595 ± 1050	811 ± 458	164 ± 59	682 ± 418	936 ± 292	156 ± 40	5343 ± 1515
FZ (n = 8)	563 ± 451*	35 ± 9*	16 ± 4*	140 ± 60*	576 ± 45*	24 ± 7*	1354 ± 662*
HFZ (n = 6)	288 ± 228*	25 ± 7*	40 ± 15*†	265 ± 127	190 ± 70*†	58 ± 23*†	867 ± 352*

All rats were infected with 1.1–2.3 × 10^7 colony forming units (CFU)/0.3 ml/rat of *Aspergillus fumigatus* prior to initiation of treatment.
* $p < 0.05$ vs. nontreated controls.
† $p < 0.05$ vs. FZ using PCANOVA.
All data are presented as mean ± SEM.

Table III. Serum Creatinine (SCr) Analysis of *Aspergillus fumigatus*–Infected Male Sprague-Dawley Rats Treated with Single Intravenous Doses of Normal Saline (Controls), Fungizone (FZ; 1 mg/kg × 4 days), and Heat-Treated Fungizone (HFZ; 1 mg/kg × 4 days)

Treatment groups	SCr concentration (mg/dl)			
	Baseline†	48 h‡	144 h§	% Change from baseline¶
Controls (n = 6)	0.45 ± 0.12	0.40 ± 0.06	0.86 ± 0.40*	+91.1%
FZ (n = 6)	0.61 ± 0.20	0.94 ± 0.25*	0.62 ± 0.30	+1.6%
HFZ (n = 6)	0.48 ± 0.08	0.47 ± 0.08	0.40 ± 0.08	–16.7%

All rats were infected with 1.1–2.3 × 10^7 colony forming units (CFU)/0.3 ml/rat of *Aspergillus fumigatus* prior to initiation of treatment.

* $p < 0.05$ vs. baseline using PCANOVA

† Baseline SCr levels prior to the rats being infected with *Aspergillus fumigatus.*

‡ 48 h SCr levels 2 days after the rats were infected with *Aspergillus fumigatus* and prior to treatment.

§ 144 h SCr levels 2 days after the rats were infected with *Aspergillus fumigatus* after 4 days of treatment.

¶ % change in SCr levels between baseline and 144 h after the initiation of the infection.

All data are presented as mean ± SEM.

daily × 4 days exhibited similar antifungal activity as FZ in this animal model with no apparent renal toxicity.

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