Effect of Fasting on Temporal Variation in the Nephrotoxicity of Amphotericin B in Rats

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Evidence for temporal variation in the nephrotoxicity of amphotericin B was recently reported in experimental animals. The role of food in these variations was determined by studying the effect of a short fasting period on the temporal variation in the renal toxicity of amphotericin B. Twenty-eight normally fed and 28 fasted female Sprague-Dawley rats were used. Food was available ad libitum to the fed rats, while the fasted animals were fasted 12 h before and 24 h after amphotericin B injection to minimize stress for the animals. Water was available ad libitum to both groups of rats, which were maintained on a 14-h light, 10-h dark regimen (light on at 0600 h). Renal toxicity was determined by comparing the levels of excretion of renal enzyme and the serum creatinine and blood urea nitrogen (BUN) levels at the time of the maximal (0700 h) or the minimal (1900 h) nephrotoxicity after the intraperitoneal administration of a single dose of dextrose (5%; control group) or amphotericin B (50 mg/kg of body weight; treated group) to the rats. The nephrotoxicities obtained after amphotericin B administration at both times of day were compared to the nephrotoxicities observed for time-matched controls. In fed animals, the 24-h urinary excretion of N-acetyl-β-D-glucosaminidase and β-galactosidase was significantly higher when amphotericin B was injected at 0700 and 1900 h. The excretion of these two enzymes was reduced significantly (P < 0.05) in fasting rats, and this effect was larger at 0700 h (P < 0.05) than at 1900 h. The serum creatinine level was also significantly higher (P < 0.05) in fed animals treated at 0700 h than in fed animals treated at 1900 h. Fasting reduced significantly (P < 0.05) the increase in the serum creatinine level, and this effect was larger in the animals treated at 0700 h. Similar data were obtained for BUN levels. Amphotericin B accumulation was significantly higher (P < 0.05) in the renal cortices of fed rats than in those of fasted animals, but there was no difference according to the time of injection. These results demonstrated that fasting reduces the nephrotoxicity of amphotericin B and that food availability is of crucial importance in the temporal variation in the renal toxicity of amphotericin B in rats.

Amphotericin B is a polyene macrolide antifungal agent with a broad spectrum of activity. It remains the most effective agent for the treatment of serious systemic mycoses (11, 13, 23). The amphipathic property of the amphotericin B chemical structure facilitates its binding to sterols of the cell membrane, which induces disruption of the membrane’s integrity and cell death (4, 6). Although this antifungal agent binds preferentially to ergosterol (the sterol of the fungal cell wall), it also binds to cholesterol (the sterol of mammalian cell membranes) (6, 12, 28). Despite its high nephrotoxic potential and the recent introduction of newer antifungal agents, amphotericin B remains the drug of choice for the treatment of severe systemic fungal infections (10, 11, 13, 29).

The clinical use of amphotericin B is limited by a dose-dependent renal toxicity that is reported to be found in up to 80% of treated patients (13, 25). The clinical presentation of nephrotoxicity includes azotemia, renal tubular acidosis, impairment of the ability to concentrate urine, electrolyte abnormalities such as hypokalemia, and sodium and magnesium wasting. Several factors that modulate amphotericin B toxicity were described over the last few years, and different approaches to reducing the renal toxicity of this drug have recently been identified. Salt supplementation (18, 25) and en-capsulation of amphotericin B into liposomes (19–21) were shown to reduce significantly the adverse effects of amphotericin B in patients.

Another approach that might contribute significantly to a reduction in the incidence of amphotericin B toxicity is the time of the day that the drug is given. In fact, a marked circadian stage dependence of murine survival time was observed following acute and chronic administration of amphotericin B (26). Furthermore, we showed that the renal toxicity of amphotericin B (10 mg/kg of body weight for 10 days) was higher when the drug was injected into rats at 0700 h than at any other time of day (17). Thus, the temporal variation in amphotericin B nephrotoxicity could be of clinical importance because this antifungal agent is usually given to humans as a once-daily injection (13).

The objective of the present study was to determine the effect of fasting on the temporal variation in the nephrotoxicity of amphotericin B. The renal toxicity of amphotericin B was thus investigated in fed and in fasted rats receiving a single injection of the antifungal agent at the beginning of their resting period (0700 h) or activity period (1900 h).

MATERIALS AND METHODS

Animals and treatment. Fifty-six female Sprague-Dawley rats (Charles River Breeding Laboratories Inc., Montréal, Québec, Canada) that weighed between 184 and 224 g and that were housed on a 14-h light, 10-h dark cycle (light on at 0600 h) were used in this study. A week after their arrival, the animals were divided into two groups of 28 rats each. The first group had free access to food (Rat Laboratory Chow pellets; Charles River Breeding Laboratories Inc.) and
performed in triplicate on antibiotic medium 12 (Difco Laboratories, Detroit, Canada). The intracortical accumulation of amphotericin B was determined in each time-matched control group. Cholesterol, HDL, and TG levels in the plasma of the fed and fasted rats were measured with an RA 500 Tecknicon apparatus, and these results were determined at the time of the single injection of amphotericin B since the renal toxicity of amphotericin B is influenced by serum lipid levels. All animals were killed by decapitation 72 h after the injection of amphotericin B or dextrose. At the time of killing, blood was collected and centrifuged, and the serum was quickly frozen at −20°C to determine serum creatinine and BUN levels. These substances are late and less sensitive markers of renal toxicity. A midline abdominal incision was made, and both kidneys were removed and rinsed out into two parts. The cortices of both kidneys were dissected out, and a piece of tissue was immediately frozen in dry ice for determination of amphotericin B concentrations.

Biochemical analysis. The activities of β-GAL and NAG were determined by the method of Maruhn (22). The results are presented as the percent difference in enzyme activities between the treated and the time-matched control animals. The baseline levels of the activities of these enzymes in urine were also determined at the time of the single injection of amphotericin B since the renal toxicity of amphotericin B is influenced by serum lipid levels.

Animals were housed in individual metabolic cages throughout the experiment, and urine was collected under mineral oil to avoid evaporation. The urine of each animal was collected over two periods of 24 h (0 to 24 and 48 to 72 h after the injections). The urine was collected, its volume was noted, and it was centrifuged (1,430 × g) for 15 min. The enzymatic activities of β-galactosidase (β-GAL) and N-acetyl-β-D-glucosaminidase (NAG) were measured in urine. The excretion of these enzymes in urine are early and sensitive markers of tubular damage.

Immediately before the injection of amphotericin B or dextrose, blood was collected from the retro-orbital plexus to measure lipid levels in plasma. The levels of cholesterol, high-density lipoproteins (HDLs), low-density lipoproteins (LDLs), and triglycerides (TGs) in the plasma of the fed and fasted rats were measured at the time of the single injection of amphotericin B or dextrose. The plasma cholesterol, HDL, LDL, and TG levels in the plasma of the fed and fasted rats were measured at the time of the single injection of amphotericin B (Fungizone; 30 mg/kg given intraperitoneally [i.p.]), whereas 5% dextrose (1 ml given i.p.) was given to the control animals. The injection time was 0700 or 1900 h because our previous study showed that this was the time of maximal (0700 h) and minimal (1900 h) amphotericin B nephrotoxicity (17). The single large dose of amphotericin B was used to be able to quantify renal toxicity by using early and sensitive markers of tubular damage (urinary enzymes) or late and less sensitive markers (creatinine and blood urea nitrogen [BUN] levels).

The baseline levels of the activities of these enzymes in urine were also determined at the time of the single injection of amphotericin B since the renal toxicity of amphotericin B is influenced by serum lipid levels. All animals were killed by decapitation 72 h after the injection of amphotericin B or dextrose. At the time of killing, blood was collected and centrifuged, and the serum was quickly frozen at −20°C to determine serum creatinine and BUN levels. These substances are late and less sensitive markers of renal toxicity. A midline abdominal incision was made, and both kidneys were removed and rinsed out into two parts. The cortices of both kidneys were dissected out, and a piece of tissue was immediately frozen in dry ice for determination of amphotericin B concentrations.

RESULTS

Urinary enzyme excretion. Figure 1 presents the percent change in the level of excretion of NAG and β-GAL in urine collected from 0 to 24 h after a single injection of amphotericin B. Fed and fasted control animals showed similar baseline levels of the activities of these enzymes in urine. For the fed rats, the excretion of NAG was significantly higher (P < 0.05) in the urine of rats treated with amphotericin B at 0700 and 1900 h than in their time-matched controls. Figure 1A also shows a significant temporal variation in the toxicity of the drug: amphotericin B treatment increased the level of NAG excretion by 390% ± 60% at 0700 h and by 240% ± 90% at 1900 h (P < 0.05). Figure 1A also shows that fasting reduced the level of excretion of NAG compared with that for the time-matched controls at both times of day and that this effect was more important at 0700 h than at 1900 h. Fasting abolished the time-dependent variations in NAG excretion. In urine collected from 48 to 72 h, the NAG activity was significantly higher in fed and fasted rats treated at 0700 h than in their time-matched controls and animals treated at 1900 h (P < 0.05) (data not shown). These data were predictable since fasted rats had free access to food 24 h after the single injection of amphotericin B or dextrose.

The level of excretion of β-GAL in urine measured from 0 to 24 h after amphotericin B injection is presented in Fig. 1B. Fed rats treated at 0700 and 1900 h had significantly higher levels of urinary excretion of β-GAL than their time-matched controls (P < 0.05), and this effect was greater when the antifungal agent was administered at 0700 h than when it was administered at 1900 h (P < 0.05). Figure 1B also illustrates the fact that fasting reduced the level of β-GAL excretion at both times of treatment but that the time-dependent variations...
in the nephrotoxicity persisted in these animals because the 
\( \beta \)-GAL activities measured at 0700 were still significantly 
higher \((P < 0.05)\) than the activities found in the urine of rats 
injected at 1900 h. No significant difference in the level of 
excretion of \( \beta \)-GAL in urine collected from 48 to 72 h after 
amphotericin B administration was found (data not shown).

Renal function. The effect of amphotericin B on the BUN 
and serum creatinine levels in fed and fasted rats 72 h after the 
injection is presented in Fig. 2. In comparison to their time-
matched controls, the BUN levels increased only when fed and 
fasted rats were treated at 0700 h (Fig. 2A). At 1900 h, injec-
tion of the antifungal agent did not induce any significant 
change in the BUN levels in either group of rats. Fasting 
reduced significantly \((P < 0.05)\) the concentration of amphotericin B in the renal cortex at both times of the 
injection. Furthermore, no significant difference in the 
accumulation of the drug in the renal cortex in fed and fasted 
rats treated at 0700 or 1900 h was found.

Figure 3 presents the amphotericin B levels in the renal 
cortex 72 h after a single amphotericin B injection to fed and 
fasted rats at 0700 or 1900 h. Fasting reduced significantly 
\((P < 0.05)\) the concentration of amphotericin B. Figure 3A 
shows that injection of amphotericin B to fed rats at 0700 h 
produced serum creatinine levels that were significantly 
\((P < 0.05)\) higher than those in the sera of their time-matched 
controls. A temporal variation was found in fed rats because 
the effect of the antifungal agent on the serum creatinine 
level was larger at 0700 h than at 1900 h. Fasting abolished the toxicity of the drug on the 
kidney because serum creatinine levels remained within the 
normal range; no time-dependent variations in serum creati-
zeine levels were observed in these animals.

**DISCUSSION**

The present study indicates the effects of a short fasting 
period on the temporal variations in the nephrotoxicity of 
amphotericin B. The excretion of NAG and \( \beta \)-GAL in urine 
and the serum creatinine and BUN levels were found to be 
significantly higher \((P < 0.05)\) than the activities found in the urine of rats injected at 1900 h. No significant difference in the level of excretion of \( \beta \)-GAL in urine collected from 48 to 72 h after amphotericin B administration was found (data not shown).

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injection. Furthermore, no significant difference in the 
accumulation of the drug in the renal cortex in fed and fasted 
rats treated at 0700 or 1900 h was found.

**TG, cholesterol, and HDL levels in plasma.** Table 1 presents 
the HDL, cholesterol, and TG levels in fed and fasted rat 
plasma taken a few minutes before amphotericin B injection. 
Fasting or time of day did not have any significant effect on 
serum HDL levels. However, the cholesterol levels of fasting 
rats were significantly \((P < 0.05)\) lower at 1900 h than at 
0700 h. Finally, fasting reduced significantly \((P < 0.05)\) the 
plasma TG levels at both times of day.
TABLE 1. HDL, cholesterol, and TG levels in plasma of fed and fasted rats

<table>
<thead>
<tr>
<th>Lipid in serum</th>
<th>Nutritional status</th>
<th>Time of determination (h)</th>
<th>Level (mmol/liter) in seruma</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL</td>
<td>Fed</td>
<td>0700</td>
<td>1.49 ± 0.24</td>
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<tr>
<td></td>
<td></td>
<td>1900</td>
<td>1.38 ± 0.19</td>
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<tr>
<td></td>
<td>Fasted</td>
<td>0700</td>
<td>1.55 ± 0.35</td>
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<tr>
<td></td>
<td></td>
<td>1900</td>
<td>1.37 ± 0.18</td>
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<tr>
<td>Cholesterol</td>
<td>Fed</td>
<td>0700</td>
<td>1.90 ± 0.26</td>
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<td></td>
<td></td>
<td>1900</td>
<td>1.64 ± 0.20</td>
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<tr>
<td></td>
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<td>0700</td>
<td>1.88 ± 0.40</td>
</tr>
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<td>1.57 ± 0.24b</td>
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<td>TGs</td>
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<td>0700</td>
<td>1.87 ± 0.49</td>
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<tr>
<td></td>
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<td>1900</td>
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</tr>
<tr>
<td></td>
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<td>0700</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>1900</td>
<td>0.68 ± 0.16e</td>
</tr>
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</table>

a A first group of rats had free access to food and water throughout the entire experiment, while the second group was fasted for 36 h (12 h before and 24 h after amphotericin B injection) but had water available ad libitum.
b Plasma was collected a few minutes before amphotericin B injection. Values are means ± standard deviations.
c Significantly lower than that for fasted rats at 0700 h (P < 0.05).
d Significantly lower than that for time-matched fed rats (P < 0.05).

effect of fasting on amphotericin B nephrotoxicity

using determination of cellular regeneration, BUN and serum creatinine levels, and the level of accumulation of amphotericin B in the renal cortex (17). In agreement with the present data obtained for fed rats, our previous study indicates that the highest level of toxicity was obtained when amphotericin B was injected at 0700 h (i.e., at the beginning of the resting period) compared with that when amphotericin B was injected at some other time of day.

The mechanisms of the temporal variation in the nephrotoxicity of amphotericin B are still unknown. In agreement with our previous work (17), a first hypothesis could be that the highest level of toxicity of the antifungal agent observed at 0700 h is due to temporal changes in the level of accumulation of drug in the kidney and in its pharmacokinetics. Unfortunately, these data could not be reproduced in the present study. This discrepancy could be explained by methodological differences because the effect of chronic administration of the amphotericin B was studied in our previous work (17), while determination of the effect of fasting forced us to use a single acutely administered large dose in the present work. Could the time-dependent variations in the nephrotoxicity of amphotericin B be explained by temporal changes in the pharmacokinetics of the drug? To date, no data support this aspect of the hypothesis. However, the temporal changes in pharmacokinetics may not explain the data because amphotericin B has a long elimination half-life in human serum (15 days; only 3% of the total dose is excreted by the kidneys) (1) and in rats (16 to 18 h) (14). In addition, no data in the literature describe the effect of fasting on the pharmacokinetics of amphotericin B in serum and tissue. Thus, further studies are needed to confirm or refute this first working hypothesis.

Another possibility could be that the effect of diet on amphotericin B is due to an interaction between the antifungal agent and cholesterol, proteins, or lipoproteins in the blood (5, 9). Studies done with experimental animals suggested that the renal toxicity of amphotericin B is either increased when LDL-associated amphotericin B was given to hypercholesterolemic rabbits (15) or decreased in the presence of increased levels of TGs in serum (8, 27) or when the interaction between amphotericin B-LDL is inhibited (3). Studies done with humans suggested that low cholesterol levels in serum are associated with a lower level of renal toxicity of amphotericin B (7, 24) but that a higher incidence of toxicity was observed in patients with high serum LDL-cholesterol levels (30, 32). Experiments done in cell culture suggest that an increase in the level of association between amphotericin B with LDL enhances the toxicity of amphotericin B to kidney cells (31, 32) since the cellular uptake of amphotericin B seems to be mediated through the LDL receptors (16).

Our study shows significant differences in the renal toxicity of amphotericin B in fed animals injected at 0700 and 1900 h in the presence of similar levels of TGs and cholesterol in serum. However, these differences were significantly attenuated by fasting, which induced significantly lower levels of TGs in serum but similar levels of cholesterol. In other words, the protective effect of fasting seems to correlate with the low serum TG levels. By contrast, Chavanet et al. (8) found that serum TG levels correlated significantly with the 50% lethal dose of amphotericin B in mice. It is thus clear that the relationship between amphotericin B nephrotoxicity and the levels of TGs in serum as well as the levels of other serum components should be investigated further.

A last hypothesis could be that the susceptibility of the kidney to amphotericin B administration varied as a function of time of day because of temporal changes in the level of binding of amphotericin B with its receptor sites. Binding would be increased when the animals are eating, and this would explain why the toxicity was the greatest at the end of the activity period (i.e., at 0700 h). Fasting would reduce the level of binding to the receptor sites, and this would explain why the nephrotoxicity was least at the end of the resting period because rodents are not eating much in their sleeping period. Further research at the receptor level is needed to prove or disprove this working hypothesis.

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REFERENCES


