Sodium Status Influences Chronic Amphotericin B Nephrotoxicity in Rats

AKIHIRO OHNISHI, TOMOK OHNISHI, WILLIAM STEVENHEAD, RICHARD D. ROBINSON, ALAN GLICK, DENNIS M. O’DAY, RAMZI SABA, EDWIN K. JACKSON, AND ROBERT A. BRANCH*

Departments of Pharmacology, Medicine, Pathology, and Ophthalmology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

Received 1 December 1988/Accepted 1 May 1989

The nephrotoxic potential of amphotericin B (5 mg/kg per day intraperitoneally for 3 weeks) has been investigated in salt-depleted, normal-salt, and salt-loaded rats. In salt-depleted rats, amphotericin B decreased creatinine clearance linearly with time, with an 85% reduction by week 3. In contrast, in normal-salt rats creatinine clearance was decreased but to a lesser extent at week 2 and 3, and in salt-loaded rats creatinine clearance did not change for 2 weeks and was decreased by 43% at week 3. All rats in the sodium-depleted group had histopathological evidence of patchy tubular cytoplasmic degeneration in tubules that was not observed in any normal-salt or salt-loaded rat. Concentrations of amphotericin B in plasma were not significantly different among the three groups at any time during the study. However, at the end of 3 weeks, amphotericin B levels in the kidneys and liver were significantly higher in salt-depleted and normal-salt rats than those in salt-loaded rats, with plasma/kidney ratios of 21, 14, and 8 in salt-depleted, normal-salt, and salt-loaded rats, respectively. In conclusion, reductions in creatinine clearance and renal amphotericin B accumulation after chronic amphotericin B administration were enhanced by salt depletion and attenuated by sodium loading in rats.

Despite recent availability of newer antifungal drugs, amphotericin B remains the broad-spectrum antymycotic antibiotic of choice for the majority of systemic fungal infections. Unfortunately, amphotericin B also induces a variety of adverse effects, including fever, chills, nausea, vomiting, hypokalemia, hypomagnesemia, and phlebitis. The most restrictive adverse effect is its potential for inducing nephrotoxicity, which can occur in up to 80% of patients (6). The mechanism by which amphotericin B induces nephrotoxicity has not been clearly defined, although it is likely that this drug induces primary effects on the kidney, which can be modified by secondary renal responses. The initial renal toxic event may be due to the ability of amphotericin B to bind the sterols in membranes and alter membrane permeability (1, 2), possibly acting as an ionophore. It is unlikely, however, that this is the sole factor that determines the extent of change in renal function, since other factors have been shown to modify the renal response. The most notable of these is the salt status at the time of drug administration.

There are observations in humans indicating that nephrotoxicity due to amphotericin B is enhanced when salt-depleted patients receive this drug and that salt supplementation can minimize this response (5, 15). It has also been shown that amphotericin B induces acute renal vasoconstriction in both dogs and rats (7, 11, 14) and that this response can be attenuated by salt loading (11). No direct evidence is available concerning whether salt loading is associated with maintenance of renal function during chronic administration in experimental animals. In one study, chronic supplements with sodium bicarbonate were shown to provide protection from renal failure (13); however, it was not ascertained whether the sodium ion or the bicarbonate ion was responsible for the protection.

The objective of the present experiment was to develop a chronic model of amphotericin B nephrotoxicity in rats and to use this model to investigate the long-term relationship of changes in salt status to changes in renal function and drug uptake into the kidney.

MATERIALS AND METHODS

Experimental protocol. Seven-week-old male Sprague-Dawley rats (SASCO Corp.) weighing approximately 200 g were randomized to one of the following three dietary groups: salt-depleted rats, normal-salt-intake rats, and salt-loaded rats. Salt-depleted rats received an initial dose of furosemide (2 mg/kg intraperitoneally [i.p.]) and were subsequently maintained on sodium-free drinking water and low-sodium purified diet (sodium composition, 0.05% NaCl; Ralston Purina Co., St. Louis, Mo). Normal-salt rats received sodium-free drinking water and regular rat chow (sodium composition, >0.39%, Wayne Lab Blox). Sodium-loaded rats received deoxytocosterone acetate (10 mg subcutaneously once weekly) together with 0.1% saline in drinking water and normal rat chow. Each group of rats was maintained on its respective diet for at least 1 week before the experiment. Prior experiments confirmed that urinary excretion of sodium was stable within 5 to 7 days of initiating each regimen.

Rats received amphotericin B (5 mg/kg i.p.) daily for 3 weeks. This dose was selected on the basis of information obtained in preliminary experiments, which indicated that 3 weeks of administration decreases renal function without causing death in control animals. The attrition rate in these studies was negligible. A further group of salt-depleted rats received 1 ml of vehicle instead of amphotericin B daily for 3 weeks. Amphotericin B or vehicle was injected into the peritoneal cavity via a permanently implanted tygon catheter connected to a rubber end that was brought subcutaneously...
to the nape of the neck, where it was exteriorized. This catheter was positioned under pentobarbital anesthesia 2 days before the experiment was started.

Endpoint measurements were obtained before therapy and at 7, 14, and 21 days of administration of amphotericin B or vehicle. Two days before each series of measurements, rats were placed in metabolic cages (Nalge Co., Rochester, N.Y.) to permit acclimatization.

On the day of measurement, a 24-h urine sample was collected for measurement of creatinine, sodium, and potassium concentrations in urine during an amphotericin B dose interval. After collection of urine and just before the next dose of amphotericin B, 0.8 ml of blood was obtained from the tail vein of the rat for measurement of creatinine and amphotericin B concentrations in plasma. Blood withdrawn for amphotericin B measurement was immediately placed on ice and centrifuged at 4°C, and plasma was stored at −70°C until subsequent analysis. In a further series of experiments, salt-depleted rats receiving amphotericin B or vehicle and normal-salt and salt-loaded rats had their glomerular filtration rate (GFR) measured on day 21 of the experiment by using 59mTc-labeled diethylene triamine pentaacetic acid (DTPA) (8). [59mTc]DTPA (100 μCi) was injected into a catheter that had been placed in the carotid artery under light ether anesthesia. Blood samples were drawn at 75, 90, 105, and 120 min, and a sample of infusate and plasma was counted on a gamma spectrometer. The GFR was estimated from the plasma clearance of [59mTc]DTPA from GFR = (dose × k0)/Cp0, where Cp0 is the extrapolated plasma concentration at time zero and k0 is the rate constant of elimination. This approach assumes a one-compartment model, with DTPEA having an elimination better characterized by a two-compartment or multicompartment model, this analysis would provide an underestimate of GFR, but comparisons between groups should still provide valid comparative information.

At the end of the study, the kidneys and liver were removed for measurement of levels of amphotericin B and for evaluation of renal histology.

Creatinine concentrations in plasma and urine were measured in triplicate on a Beckman creatinine autoanalyzer. Sodium and potassium concentrations were measured by flame photometry. Plasma samples were serially diluted in saline, whereas renal and liver tissues were homogenized in four times their volume in isotonic saline before serial dilution. Amphotericin B was assayed by a modified standard radial diffusion bioassay (20) with appropriate standard curves run in blank plasma, renal tissue homogenate, or liver tissue homogenate. Serial dilution of standards was routinely done with saline after it had been established that values were similar to those with serial dilution in blank plasma.

**Statistics.** Data are presented as the means ± standard errors of the means. The relationships between the variables, percentage change in weight, urinary sodium excretion, potassium excretion, and creatinine clearance with time were evaluated between different groups by two-way analysis of variance, followed by a multiple comparison analysis with the Student-Newman test and the Number Cruncher Statistical System (J. Hintze, Kaysville, Utah). Factor A was the effect of amphotericin B over time, and factor B was comparison between treatment groups. Statistical comparisons of values at different time points were performed by using the Student t test. Regression was calculated by least-square regression analysis. The minimum level of statistical significance was considered to be P < 0.05.

**RESULTS**

Amphotericin B (5 mg/kg per day i.p.) was given to three groups of rats receiving different sodium intake with either high, normal, or low salt intake for 3 weeks. The influence of

### TABLE 1. Changes in weight during 3 weeks of administration of amphotericin B (5 mg/kg i.p. daily) or vehicle to rats on different sodium diets

<table>
<thead>
<tr>
<th>Salt balance</th>
<th>Treatment</th>
<th>Baseline wt (g)</th>
<th>% Change from baseline on day:</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>7</td>
<td>14</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Depleted</td>
<td>Vehicle</td>
<td>197 ± 3</td>
<td>5.3 ± 0.9</td>
<td>7.4 ± 1.2</td>
<td>10.7 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Depleted</td>
<td>Amphotericin B</td>
<td>203 ± 3</td>
<td>−5.5 ± 1.3a</td>
<td>−9.3 ± 2.3b</td>
<td>−18.6 ± 2.5b</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>Amphotericin B</td>
<td>200 ± 3</td>
<td>3.0 ± 1.4</td>
<td>7.5 ± 2.7</td>
<td>11.4 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>Loaded</td>
<td>Amphotericin B</td>
<td>207 ± 4</td>
<td>9.8 ± 5.6</td>
<td>13.4 ± 4.4</td>
<td>16.7 ± 6.1</td>
<td></td>
</tr>
</tbody>
</table>

a Baseline data are presented as absolute values; changes at days 7, 14, and 21 for weight are presented as percentage changes from the baseline value (n = 7 in each group).

b P < 0.05 compared with control salt-depleted rats.

### TABLE 2. Urinary excretion of sodium and potassium, and creatinine clearance during 3 weeks of administration of amphotericin B (5 mg/kg i.p. daily) or vehicle to rats on different sodium intake (n = 7 in each group)

<table>
<thead>
<tr>
<th>Substance excreted</th>
<th>Salt balance</th>
<th>Treatment</th>
<th>Amt of Na or K excreted (mEq/day)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Baseline</td>
<td>Day 7</td>
<td>Day 14</td>
<td>Day 21</td>
</tr>
<tr>
<td>Sodium</td>
<td>Depleted</td>
<td>Vehicle</td>
<td>0.05 ± 0.02</td>
<td>0.07 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Depleted</td>
<td>Amphotericin B</td>
<td>0.07 ± 0.02</td>
<td>0.54 ± 0.02a</td>
<td>0.29 ± 0.05a</td>
<td>0.28 ± 0.05a</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>Amphotericin B</td>
<td>1.56 ± 0.23</td>
<td>3.12 ± 0.84a</td>
<td>2.37 ± 0.54a</td>
<td>1.63 ± 0.97a</td>
</tr>
<tr>
<td></td>
<td>Loaded</td>
<td>Amphotericin B</td>
<td>27.1 ± 5.50</td>
<td>55.8 ± 6.09a</td>
<td>63.2 ± 9.55a</td>
<td>57.8 ± 12.1a</td>
</tr>
<tr>
<td>Potassium</td>
<td>Depleted</td>
<td>Vehicle</td>
<td>6.33 ± 0.75</td>
<td>8.05 ± 1.21</td>
<td>5.24 ± 0.57</td>
<td>3.58 ± 0.97</td>
</tr>
<tr>
<td></td>
<td>Depleted</td>
<td>Amphotericin B</td>
<td>4.03 ± 0.85</td>
<td>4.81 ± 0.21</td>
<td>3.49 ± 0.46</td>
<td>3.59 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>Amphotericin B</td>
<td>5.24 ± 0.91</td>
<td>7.32 ± 1.13</td>
<td>4.35 ± 1.32</td>
<td>5.77 ± 0.43</td>
</tr>
<tr>
<td></td>
<td>Loaded</td>
<td>Amphotericin B</td>
<td>8.80 ± 2.53</td>
<td>11.3 ± 1.37</td>
<td>9.24 ± 1.16</td>
<td>13.0 ± 4.41</td>
</tr>
</tbody>
</table>

a P < 0.05 compared with the baseline value in the same group.
amphotericin B on changes in weight, urinary sodium excretion, urinary potassium excretion, and creatinine clearance was compared with that in a control group of salt-depleted rats receiving vehicle (Tables 1 and 2, Fig. 1). Baseline weight, urinary potassium excretion, and creatinine clearance were similar in all four groups, whereas sodium excretion reflected the marked differences in sodium intake.

In the control group, daily i.p. administration of vehicle did not result in any change in creatinine clearance, urinary sodium excretion, or urinary potassium excretion, whereas weight increased with time.

In the salt-loaded group of rats receiving deoxycholic acid and 0.1% salt in their drinking water, baseline daily sodium excretion was 17-fold greater than that in rats with normal salt intake. In these salt-loaded rats, amphotericin B did not change creatinine clearance over the first 14 days, despite a twofold increase in urinary sodium excretion. However, analysis of variance comparison between this group and the control group showed a significant difference in creatinine clearance over the 21-day experiment; creatinine clearance was significantly reduced by 43% by week 3.

In the normal-salt group of rats, amphotericin B induced significant changes in sodium excretion and creatinine clearance over the 21 days of the experiment. Urinary sodium excretion was increased twofold and creatinine clearance was decreased by 30% by day 7. Both of these parameters remained stable and significantly different from baseline values between days 7 and 21. By day 21, creatinine clearance was decreased by 55%.

In salt-depleted rats, amphotericin B-induced changes were enhanced in comparison to those in rats with normal salt intake. There was an approximately sevenfold increase in the urinary sodium excretion rate, although this was still substantially less than that in the rats on a normal salt diet. The reduction in creatinine clearance at day 7 was similar to that in rats on a normal-salt diet on day 7 but was significantly greater on days 14 and 21. There was an 83% reduction in creatinine clearance by day 21.

Since creatinine clearance may underestimate changes in GFR, a series of studies in further groups of rats was undertaken to measure GFR after 21 days of treatment by using the plasma clearance of [99Tc]DTPA in the ether-anesthetized rat model (Fig. 2). Amphotericin B significantly reduced the GFR with each dietary intake of salt. The extent of reduction of 66, 82, and 87% for high-salt, normal-salt, and low-salt diets, respectively, were greater than the changes in creatinine clearance but had the same sequence in rank order with the three diets.

In the initial series of studies, amphotericin B levels in plasma drawn at the dosage interval on days 7, 14, and 21 were not significantly different among the groups of rats on different salt diets (Table 3). Concentrations in kidney and liver tissues were approximately 1 order of magnitude greater than levels in plasma at the end of the study. In contrast to levels in plasma, levels of amphotericin B in both kidneys and liver were significantly lower in the salt-loaded

---

**TABLE 3.** Influence of salt status on levels of amphotericin B in plasma and tissues (n = 7 in each group)

<table>
<thead>
<tr>
<th>Salt balance</th>
<th>Amphotericin B (μg/ml) on day:</th>
<th></th>
<th>Amphotericin B (μg/g) in tissue on day 21</th>
<th>Kidney</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7</td>
<td>14</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depleted</td>
<td>0.18 ± 0.03 (0.06-0.24)*</td>
<td>0.18 ± 0.04 (0.07-0.36)</td>
<td>0.24 ± 0.03 (0.12-0.34)</td>
<td>4.13 ± 0.62 (1.80-7.16)</td>
<td>10.11 ± 2.15 (6.90-16.45)</td>
</tr>
<tr>
<td>Normal</td>
<td>0.25 ± 0.03 (0.08-0.55)</td>
<td>0.23 ± 0.04 (0.12-0.52)</td>
<td>0.28 ± 0.05 (0.18-0.51)</td>
<td>3.84 ± 0.35 (2.63-4.85)</td>
<td>9.81 ± 0.82 (6.70-11.98)</td>
</tr>
<tr>
<td>Loaded</td>
<td>0.22 ± 0.08 (0.08-0.65)</td>
<td>0.24 ± 0.07 (0.10-0.65)</td>
<td>0.30 ± 0.07 (0.12-0.65)</td>
<td>2.41 ± 0.32 (1.28-3.62)</td>
<td>4.68 ± 0.46 (3.69-5.47)</td>
</tr>
</tbody>
</table>

* Values within parentheses are ranges.

* P < 0.05 compared with the normal-salt group.
rats than in either the normal-salt or salt-depleted rats. Furthermore, there was a significant positive correlation between renal concentration of amphotericin B and the change in creatinine clearance when the three dietary groups of rats were considered together (Fig. 3).

Histopathological sections from the kidneys of each rat at the end of the experiment were evaluated by an observer who was blinded to the dietary group (Table 4). Occasional tubule dilation and interstitial hemorrhage were observed in some rats of each group. However, patchy tubular cytoplasmic degeneration was also observed in each rat in the salt-depleted group but was not observed in any rat in the normal-salt or high-salt group. It was notable that the extent of histopathological changes in the kidney was minimal considering the extent of reduction in creatinine clearance and that the changes observed were confined to the tubules. There was no light-microscopic evidence of glomerular injury in any rat.

**DISCUSSION**

The present study confirms that reductions in renal function associated with chronic amphotericin B therapy can be influenced by variables in the recipient of the drug as well as by the dosage regimen being administered. Specifically, studies in rats now confirm for the first time that differences in sodium chloride intake at the time of the drug administration influence the extent of change in GFR, with salt depletion enhancing the response and salt loading minimizing but not eliminating the adverse response. These observations are consistent with observations in humans and suggest that this model might be useful for further investigation of the mechanisms involved in altering renal function. A further new observation is that levels of amphotericin B in both kidney and liver tissues were greatest in salt-depleted rats, which had the greatest change in renal function, despite no change in levels of amphotericin B in plasma. The reason for drug accumulation under these circumstances merits further investigation.

Evidence to support an important role for salt status in determining renal response in humans was reported shortly after the release of amphotericin B when Butler and colleagues (6) described their experience in a patient in whom a salt diet of 9 mEq per day exacerbated renal impairment during amphotericin B therapy. The change in renal function was reversed by salt supplementation. Further case reports have confirmed that the occurrence of sodium depletion during amphotericin B results in reductions in renal function which can be reversed by salt loading even while continuing amphotericin B therapy (15). Additional evidence to support the concept that salt status is important in determining the renal response to amphotericin B is the observation that patients receiving an obligatory sodium load due to concomitant intravenous sodium ticarcillin therapy had only a 12% incidence of impaired renal function compared with a 67% incidence in patients not receiving the salt supplement (5). This observation raises the possibility that salt supplementation can confer renal protection. Support for this idea has been obtained by the observation that only 10% of a series of patients who received 150 mEq of sodium chloride intravenously daily with their amphotericin B therapy developed nephrotoxicity (5).

Salt status is also known to influence the acute renal vasoconstrictor response to amphotericin B (7, 11). A postulated mechanism is via activation of tubuloglomerular feedback. This renal reflex can be activated by increased sodium chloride reabsorption at the macula densa (19) and inhibited by loop diuretics (22) and appears to be mediated by adenosine (18). Observations that the acute renal vascular response to amphotericin B can be attenuated by furosemide and aminophylline (11, 14, 15) are consistent with tubuloglomerular feedback mediating the response to amphotericin B. Furthermore, micropuncture studies in the rat confirm that amphotericin B can activate single-nephron tubuloglomerular feedback (H. Hermes, K. Leser, and H. Os-wald, 16th Symp. Nephrology, Salzburg, 1983). However, it is possible that additional or alternative mechanisms might be involved, since the calcium channel blockade blocks the acute reduction in renal blood flow but not the reduction in GFR in rats (21).

Despite evidence to suggest that a variety of interventions can influence the acute renal response to amphotericin B, little evidence is available to link these observations to changes that occur during chronic treatment with amphotericin B. In one chronic study in rats, sodium bicarbonate, given to correct renal tubular acidosis caused by amphotericin B, did provide protection compared with control rats.

![Graph](http://aac.asm.org/)  
**FIG. 3.** Relationship of levels of amphotericin B in renal tissue and change in creatinine clearance in rats with various salt intakes which received daily i.p. doses of amphotericin B (5 mg/kg per day) ($r = 0.56, P < 0.05$).

<table>
<thead>
<tr>
<th>Salt balance</th>
<th>Treatment</th>
<th>Tubular degeneration</th>
<th>Dilation in tubules</th>
<th>Interstitial hemorrhage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depleted</td>
<td>Vehicle</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Depleted</td>
<td>Amphotericin B</td>
<td>7</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Normal</td>
<td>Amphotericin B</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Loaded</td>
<td>Amphotericin B</td>
<td>0</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>
The renal sparing effect was attributed to the bicarbonate; however, it could as easily have been due to the concomitant sodium load. This alternative explanation is addressed in the present series of studies, in which there was a clear difference in the extent of change in renal function in rats on different salt diets. In each group of rats, amphotericin B induced an increase in sodium excretion despite reducing GFR, suggesting that a direct renal tubular effect results in a decrease in fractional sodium reabsorption. In rats on a high- or normal-salt diet, there were significant reductions in renal function over the 3 weeks of the experiment, but they were less than those in the salt-depleted group. Furthermore, this latter group was the only group with histopathological evidence of patchy tubular degeneration on conventional light microscopy. These observations suggest those in humans indicating the importance of salt status in determining renal response to amphotericin B. In addition, this study suggests that the rat can be used as a model to investigate mechanisms that cannot be evaluated in humans.

The pharmacokinetics of amphotericin B are still poorly understood (3). It is apparent that it has an extensive volume of distribution and that only a small proportion of drug is eliminated in urine (3, 9). Amphotericin B is a large molecule and is in part eliminated by excretion into bile (10). Impaired elimination in patients with renal failure has not been observed (17), and dosage modifications in patients with renal failure are not advocated (4, 16). It was therefore surprising to find that levels of amphotericin B in tissues were different between salt-depleted and salt-loaded rats in both the kidney and the liver, despite no evidence of accumulation in plasma. The simplest explanation for the association between tissue levels of amphotericin 21 days after the start of daily administration and the percentage change in creatinine clearance (Fig. 3) is that reduced renal function had reduced renal clearance of drug, resulting in accumulation in tissues. This would be consistent with concentration increases in tissues such as liver as well as kidney. However, the two features inconsistent with this explanation are as follows. First, renal clearance is only a minor route of amphotericin B elimination, accounting for less than 10% of total clearance (17). Second, levels in plasma, which were in the measurable range of the assay, were not different among the groups. An alternative explanation is that either salt status or mineralocorticoid administration directly influences tissue uptake of amphotericin B. This would imply that tissue uptake is not solely due to passive distributional processes and that similar mechanisms are involved in both liver and kidney. Uptake could be either dependent on active transport processes or secondary to membrane modification altering the extent of drug binding to membranes. These observations clearly merit further study, and alternative explanations cannot be determined conclusively with the present data.

In conclusion, salt status does influence the extent of change in renal function. We propose that amphotericin B has a direct effect on renal integrity. These priming events have the potential to initiate secondary responses due to activation of renal reflex mechanisms, which determine the ultimate change in renal function. Modification of these secondary responses with down-regulation of renal vasocstrictive responses offers a way to minimize the adverse effect of amphotericin B on renal function. These observations raise the possibility that the therapeutic window of this drug can be widened by the simple maneuver of salt supplementation.

ACKNOWLEDGMENTS

A.O. is a Merck Sharp & Dohme International Fellow in Clinical Pharmacology. D.M.D. is a Senior Scientific Investigator in research to prevent blindness. This work was supported by Public Health Service grants HL 14192 and EY 01621 from the National Institutes of Health.

LITERATURE CITED

