INTRODUCTION

Amphotericin B deoxycholate (AB) is a polyene antifungal agent licensed in 1959. AB shows a broad spectrum of activity against agents of systemic fungal infections, such as Cryptococcus neoforms, Candida spp., Blastomyces dermatitidis, Coccidioides immitis, Aspergillus spp., and susceptible species of Zygomycosis. It is fungicidal and, resistance develops infrequently. For these reasons AB remains the first choice to manage many systemic fungal infections. Other indications of AB include visceral leishmaniasis, where it is considered a second-line drug, and for mucosal leishmaniasis caused by Leishmania braziliensis v. braziliensis, particularly for patients refractory to pentavalent antimonials, and when extensive mucosal involvement is observed.

Toxicity is commonly observed in patients treated with AB. Infusion-related toxicity is characterized by fever, chills, headache, nausea, arthralgia, and myalgia. These symptoms are associated with the speed of the infusion, and can be prevented by slowing the infusion rate, adding a nonsteroidal anti-inflammatory drug, or using hydrocortisone prior or during the infusion. While these infusion related side effects are usually mild and easy to treat, severe and life-threatening events including hypokalemia, acute renal failure, and hypomagnesemia may arise. These more serious events are dose related, and often lead to temporary discontinuation of AB treatment, jeopardizing the efficacy of the regimen, particularly in immunocompromised patients. AB causes glomerular and tubulo-interstitial nephrotoxicity. The former is probably due to vasoconstriction of the afferent glomerular arteriole, and is manifested by a reduction in the glomerular filtration rate, leading to acute renal failure. The latter is presumably due to direct damage of AB on the renal tubule leading to hypokalemia, hypomagnesemia, and renal tubular acidosis.

Salt loading with intravenous saline solution is recommended to avert the renal toxicity of AB.

Three lipid formulations of AB have been licensed more recently: AB lipid complex (Abelcet [Enzon, Bridgewater, NJ]), liposomal AB (AmBisome [Astellas Pharma, Deerfield, IL]), and AB cholesteryl sulfate complex (Amphotec [Three Rivers Pharmaceuticals, Cranberry Township, PA]). These compounds have similar efficacy than AB, but induce less renal toxicity, although not all of them are free from causing infusion related and hypersensitivity reactions. The new lipid formulations of AB and other new antifungals, including new azoles and echinocandins, are promising compounds that may replace AB in the future as first-line treatment of systemic fungal infections. Due to the high cost of these new antifungal medications, and the fact that many of them have not been licensed in the developing world yet, it is expected that the pattern of use of AB in these countries will not change in the near future. Thus, identifying simple measures to prevent AB-induced nephrotoxicity are needed. We evaluated the use of an oral rehydration solution (ORS) recommended to manage diarrheal diseases, compared with intravenous saline solution to prevent AB-induced nephrotoxicity in patients with extensive mucosal leishmaniasis.

PATIENTS AND METHODS

Study design and enrollment criteria. The study followed a randomized and open design, and was conducted at the Hospital de Guá in the city of Cuzco, Peru, between April 1994 and January 1995. Ethical approval from Universidad Peruana Cayetano Heredia’s Institutional Review Board was obtained. Patients gave written consent to participate in the study.

Eligible patients were adults between 18 and 60 years of age with clinically suspected muco-cutaneous leishmaniasis presumably caused by Leishmania braziliensis v. braziliensis with indication to receive AB, either because they had failed to conventional treatment with two regimens of pentavalent antimonials, or because they had extensive muco-cutaneous disease with laryngeal involvement. Attempts to microbio-
logically confirm the diagnosis were made using a Giemsa stain of an aspirate from a mucosal site, culture of an aspirate or tissue obtained by biopsy, or by a specific PCR applied to a tissue sample. Patients with history of allergy to AB or who had received AB in the week before recruitment were excluded, as well as pregnant or nursing women, patients with severe underlying medical conditions including renal disease, cardiac disease, chronic liver disease, alcohol abuse, tuberculosis, and HIV infection. Patients receiving other nephrotoxic drugs, such as aminoglycosides, antivirals, nonsteroidal anti-inflammatory drugs, and cyclosporine were also excluded. We also excluded patients who had baseline creatinine values above 1.5 mg/dl, hemoglobin levels below 10 gr/dl, and serum albumin concentration below 3 gr/dl.

Randomization and treatment. Eligible patients were randomly allocated to the two study groups using a computer-generated random table. Patients received either intravenous normal saline solution (SS group) or oral rehydration solution (Laboratorios Luza, Lima, Peru) (ORS group). Patients in the SS group received 1 L of the solution 60 minutes before starting the infusion of AB (sodium 153 mEq/L, chloride 153 mEq/L, osmolarity 306 mosm/L). Patients in the ORS group received 1 L of a solution containing: 90 mEq/L of sodium, 104 mEq/L of chloride, 22 mEq/L of bicarbonate, and 12 mEq/L of potassium, osmolarity 290 mosm/L, 60 minutes before starting the infusion of AB, and 2 L throughout the rest of the day, for a total of 3 L per day. Patients in both groups received AB (Fungizone, Bristol Myers Squibb, Bedfordview, NJ) for the treatment of leishmaniasis, at a daily dose of 0.6 mg/kg until attaining a cumulative dose of 25.2 mg/kg. AB was reconstituted with 5% dextrose in water, to attain a concentration of 0.1 mg/mL, and was infused over 2 hours. Patients were allowed to drink plain water as needed. No other solution containing electrolytes was permitted. Patients received a standard diet provided by the hospital. A complete medical history and physical examination was performed at baseline and daily while in the study. Patients remained hospitalized until finishing the planned cumulative dose of AB.

Outcome measurements. The primary outcome of the study was the effect of the two interventions on renal function while receiving AB therapy. Renal function was evaluated with periodic measurements of serum creatinine, urea, creatinine clearance (collecting 24-hour urine), and electrolytes, including serum sodium and potassium at baseline, and on treatment days 8, 16, 24, 32, and 42. Baseline measurements also included weight, height, hematology (hemoglobin, total white blood cells and differential), serum albumin, serum transaminases (ALT and AST), 12-lead electrocardiogram, and chest radiograph. Patients who had elevations of serum creatinine above 2 mg/dl at any time were temporarily discontinued from receiving AB therapy, until the value returned to levels equal or below 1.5 mg/dl. Patients who developed hypokalemia with serum levels below 3.0 mEq/L or those who developed symptoms were also temporarily discontinued of the AB infusion; potassium supplementation was provided until the potassium value returned to normal. Patients were permanently discontinued of AB therapy if the elevation in serum creatinine did not return to normal after a week, if two temporal interruptions of AB therapy occurred during the study period, or if persistent hypokalemia ensued despite potassium and magnesium replacement. Serum samples were stored at −20°C, and transported to the Ne-phrology Laboratory at Universidad Peruana Cayetano Heredia in Lima, to be processed.

Statistical analysis. The sample size was estimated in 48 subjects, 24 participants in each study group, to detect a difference in the incidence of hypokalemia of 40% between study groups with an alpha error of 0.05 and a beta error of 0.2. The χ² test with continuity correction or Fisher exact test was used to contrast categorical variables. One-way analysis of variance or Kruskal-Wallis test were used to compare continuous variables with multiple evaluations over time. All tests were two-tailed. Multiple analyses of variance was used to test for interactions between renal tests (creatinine, creatinine clearance, serum urea, and electrolytes) and cumulative doses of AB, patient weight, and study groups. Data were entered and analyzed using EPI-Info statistical software (version 6.0, CDC, Atlanta).

RESULTS

Patient characteristics. Forty-eight patients were included in the study, 23 in the SS group and 25 in the ORS group. The comparison of baseline parameters is shown in Table 1. Study groups were comparable in all variables tested. All patients were male. Parasitological confirmation was achieved in 92% (44 of 48) of the patients. The remaining four patients had clinical diagnosis only, biopsies of mucosal lesions ruled out other diagnosis, and all of them responded well to AB. Baseline values for serum potassium were unexpectedly high but comparable in both treatment groups.

Outcome evaluations. The variation of serum creatinine, urea, and sodium values by study groups and by cumulative doses of AB is shown in Table 2. No statistical difference was observed in creatinine values over the study period. As a consequence, the creatinine clearance also showed no variation by study groups (data not shown). Serum urea values showed a statistical significant increase in treatment days 8 and 16 in the SS group compared with the ORS group (P < 0.03 for both comparisons), but this difference disappeared thereafter. Serum sodium values differed by study groups only on treatment day 16; hyponatremia was detected in the SS group compared with the ORS group (P = 0.039). Serum potassium values showed a marked difference between the study groups over time, as shown in Figure 1. Study groups

### Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SS group* (n = 23)</th>
<th>ORS group* (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years, mean ± SD</td>
<td>35.8 ± 11.4</td>
<td>38.8 ± 10.6</td>
</tr>
<tr>
<td>Weight in kg, mean ± SD</td>
<td>53.1 ± 7.0</td>
<td>53.7 ± 3.2</td>
</tr>
<tr>
<td>Duration in months of mucosal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>involvement, mean ± SD</td>
<td>78.4 ± 65.2</td>
<td>67.5 ± 49.0</td>
</tr>
<tr>
<td>Previous treatment with penicillin</td>
<td>18 (78)</td>
<td>17 (68)</td>
</tr>
<tr>
<td>antimonials, n (%)</td>
<td>22 (96)</td>
<td>22 (88)</td>
</tr>
<tr>
<td>Laryngeal involvement, n (%)</td>
<td>15.6 ± 1.4</td>
<td>15.4 ± 1.4</td>
</tr>
<tr>
<td>Hemoglobin, g/dl, mean ± SD</td>
<td>7.7 ± 1.1</td>
<td>7.6 ± 1.5</td>
</tr>
<tr>
<td>White blood cells, cells/l, mean ± SD</td>
<td>25.2 ± 6.7</td>
<td>22.2 ± 7.3</td>
</tr>
<tr>
<td>Creatinine, mg/dl, mean ± SD</td>
<td>0.7 ± 0.2</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Creatinine clearance, mil/min, mean ± SD</td>
<td>73.6 ± 14.0</td>
<td>73.0 ± 19.5</td>
</tr>
<tr>
<td>Serum sodium, mEq/L, mean ± SD</td>
<td>140.0 ± 14.5</td>
<td>136.1 ± 13.8</td>
</tr>
<tr>
<td>Serum potassium, mEq/L, mean ± SD</td>
<td>5.6 ± 1.7</td>
<td>6.1 ± 1.7</td>
</tr>
</tbody>
</table>

* SS = saline solution; ORS = oral rehydration solution.
differed on treatment days 16, 24, and 42 ($P < 0.03$). The multivariate analysis showed that potassium values depended significantly on the study groups and cumulative doses of AB. The regression model predicted that, on average, serum potassium values in the ORS group were 0.678 mEq/L higher than in the SS group, adjusting for hospitalization day, and age ($P = 0.003$). Hypokalemia was more frequently observed in the SS group (14 of 23, 61%) than in the ORS group (6 of 25, 24%), $P = 0.0096$, and accounted for more temporal discontinuations of AB treatment: five in the SS group and none in the ORS group, $P = 0.014$. Treatment with AB was temporarily discontinued in seven patients in the SS group and in one patient in the ORS group, $P = 0.02$. No difference in the rate of definitive discontinuation of AB therapy was observed: two patients in the SS group versus one patient in the ORS group, $P = 0.58$. Patients reported a variety of side effects, including headache, fever, chills, general malaise, anorexia, myalgia, arthralgia and abdominal pain; no difference in the rate of these events was observed between study groups.

**DISCUSSION**

This study shows that ORS is as effective as SS in preventing the glomerular damage of AB, but as expected, it is superior to SS in preventing tubulo-interstitial damage. As a consequence, hypokalemia was more frequently observed among patients treated with SS than in ORS-treated patients, and more patients in the SS group temporarily discontinued the treatment due to hypokalemia than did patients in the ORS group. These results are remarkable, and have important implications for the use of AB in developing and developed countries.

Renal toxicity of AB is reported to occur at variable rates. Different definitions of nephrotoxicity, diverse patient populations, and dissimilar settings account for the observed variability. The exact mechanisms by which AB causes nephrotoxicity are still not well understood, but it is known that patients who develop renal toxicity remain longer in hospitals, and some studies have shown higher mortality rates in these patients. Thus, preventing renal toxicity may have a positive impact on hospital costs, length of stay, and patient survival.

Several interventions have been proposed to prevent AB-induced nephrotoxicity. First, saline load has shown to prevent AB-induced nephrotoxicity in several open and non-randomized studies. Since these early studies, evidence in support of the protective effect of salt loading and proper electrolyte management on the incidence of AB-induced nephrotoxicity has accumulated over the years from case series and retrospective studies, both in adults and children. The only clinical trial published to date that evaluated the effect of salt loading on the incidence of renal damage induced by AB was conducted by Llanos and others in that study, which followed a randomized and double-blind design, 20 patients with mucocutaneous leishmaniasis received by the intravenous route 1 L of either normal saline, or 5% dextrose in water before the infusion of AB. Glomerular function, measured by changes over time in serum creatinine and creatinine clearance was markedly different between study groups, with a more favorable pattern in the saline group. Interestingly, serum potassium levels decreased over time in both study groups, and potassium supplement was more frequently prescribed in the saline group. This study clearly demonstrated the utility of salt loading in preventing renal damage induced by AB, but also suggested that solutions containing potassium would be more efficacious in preventing both glomerular and tubular damage. Our study adds important information to that study, showing that the two main components of renal toxicity induced by amphotericin B can effectively be prevented with ORS, rather than only preventing glomerular damage with saline load. Preventing hypokalemia and potassium depletion with ORS not only reduce morbidity but also ameliorate hypokalemia-induced tox-
ictivity to renal tubules. Second, extending the duration of AB infusion is another strategy that has been investigated recently. One study evaluated the effect of continuous versus conventional 4-hour infusion of AB on infusion-related toxicity, renal toxicity, and overall mortality in Switzerland. Daily continuous infusion of AB was associated with lesser side effects, lower renal toxicity, and lower mortality than a 4-hour infusion. Although these achievements are notable, infusion-related fever was reported in 25% and hypokalemia was reported in 10% of patients receiving the continuous infusion of AB. In another study, continuous infusion of escalated doses of AB in neutropenic patients did not cause more renal impairment or side effects than conventional 2–6-hour infusion. Extending the duration of the infusion is clearly an alternative, but its implementation is less feasible in developing countries. Third, most of the recent research in preventing AB-induced nephrotoxicity has been directed at producing new lipid formulations rather than evaluating alternative nephroprotective measures.

There are some limitations of our study. First, it was conducted in a randomized but open fashion. Double masking the two interventions was not possible to achieve. Thus, the investigators were aware of the intervention provided to the patients. Although this might have an effect on the evaluation of outcomes, we believe that it was minimal. The main outcome of the study was the effect of the two interventions on the renal function in patients receiving AB. Measurements of renal function relied on objective laboratory tests, not on subjective evaluations of the well being of patients. Additionally, clear guidelines to temporarily or definitely discontinuing the treatment with AB, based on objective laboratory measurements were written before starting the trial. Second, serum potassium values at baseline and during the study were unexpectedly high. Potential problems with sampling, storing, and processing the samples may explain these findings. We believe that this phenomenon occurred randomly, and does not have an effect of the evaluation of outcomes. Third, the patients included in the study received a lower dose of amphotericin B than recommended for invasive fungal infections, all had normal renal function at baseline, disclose good oral tolerance, and were not receiving other nephrotoxic drugs. Therefore, the results of this study should be extrapolated to patients with similar conditions. Finally, the sample size was small enough to show similar protection rates of glomerular function between study groups, and lower incidence of hypokalemia in the ORS-treated group.

In conclusion, ORS is as effective as SS in preventing glomerular damage of AB, but more effective in preventing hypokalemia. Because the pattern of use of AB in developing countries is not expected to change in the near future, using ORS to prevent the renal damage of AB is a simple measure that clinicians may use to reduce the burden of toxicity associated with AB.

Received July 28, 2006. Accepted for publication August 7, 2006.

Acknowledgments: The authors thank Dennis Grasela, Diego Gonzalez, Jaime Espinoza, Juan Miyahira, and Julieta Tupayachí for their support in conducting the study, and Pedro Legua for reviewing the manuscript.

Financial support: This study was supported by core funds of Instituto de Medicina Tropical Alexander von Humboldt. Amphotericin B was provided by Bristol Myers Squibb, Peru. The American Committee on Clinical Tropical Medicine and Travelers’ Health (ACCTMTH) assisted with publication expenses.

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