PHASE 2 TRIAL OF WR6026, AN ORALLY ADMINISTERED 8-AMINOQUINOLINE, IN THE TREATMENT OF VISCERAL LEISHMANIASIS CAUSED BY LEISHMANIA CHAGASI


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Abstract. There are no recognized orally administered treatments for any of the leishmaniases. The 8-aminoquinoline WR6026 is an orally administered analog of primaquine that cured 50% of patients with kala-azar in Kenya at a dose of 1 mg/kg/day for 28 days. A further phase 2, open-label, dose-escalating safety and efficacy study was performed for kala-azar in Brazil. Cure rates for Brazilian patients treated for 28 days were as follows: 1 mg/kg/day: 0 of 4 (0%); 1.5 mg/kg/day: 1 of 6 (17%); 2.0 mg/kg/day: 4 of 6 (67%); 2.5 mg/kg/day: 1 of 5 (20%); and 3.25 mg/kg/day: 0 of 1 (0%). Nephrotoxicity that was not anticipated from preclinical animal studies or from phase 1 studies was seen at 2.5 mg/kg/day in 2 patients and in the single patient administered 3.25 mg/kg/day. WR6026 demonstrated the unusual clinical features of lack of increased efficacy against Brazilian kala-azar with increased dosing above 2 mg/kg/day and toxicity that was not present in previous investigations.

INTRODUCTION

Visceral leishmaniasis (VL), a characteristically fatal infection of the reticuloendothelial system (liver, spleen, and bone marrow) endemic in Brazil, is caused by Leishmania chagasi, a protozoan of the Leishmania donovani complex. Although > 90% of the cases can be successfully treated, all presently marketed agents are parenteral. The lack of orally administered effective agents for VL prompted the clinical development of WR6026, a primaquine analog found to be highly active in animal testing.1–3

The only previous phase 2 study of the efficacy of WR6026 was performed in Kenya.4 In that study, 16 patients with VL underwent treatment with WR6026 at doses ranging from 0.75–1.0 mg/kg/day for 2 weeks (8 patients) or 1 mg/kg/day for 4 weeks (8 patients). The results indicated one cure (12%) in the 2-week group and 4 cures (50%) in the group that received 1 mg/kg/day for 4 weeks. Adverse effects included headaches in 4 patients and mild abdominal complaints in 2 patients. Elevations in methemoglobin levels, the main side effect of the drug, were low (2.6% in the group dosed at 1 mg/kg/day for 4 weeks).

Because neither sufficient efficacy nor significant toxicity was found in the Kenyan study, a second phase 2 study was undertaken in Brazil. The aim of the study was to escalate the dose of WR6026; it was given daily for 28 days until either 90% efficacy or toxicity resulted.

PATIENTS AND METHODS

Study design. This study was an open-label, dose-escalating safety and efficacy trial of WR6026 in the treatment of VL caused by L. chagasi.

The study was performed between October 1996 and July 1998 in the Clinical Research Center of the Unit of Infectious Diseases at the Biomedical Center of the Federal University of Espírito Santo, Vitória, Brazil. Volunteer patients were enrolled in 6 member cohorts. Participants were patients aged 6–50 years with signs and symptoms consistent with VL, acquired in the states of Espírito Santo, Bahia, and Minas Gerais.

Inclusion and exclusion criteria. The inclusion criteria were a clinical diagnosis of VL with parasitological demonstration of Leishmania amastigotes on smear or promastigotes in culture.

The exclusion criteria included clinical contraindication to splenic aspiration, any history of anti-Leishmania therapy, evidence of serious underlying disease (cardiac, renal, hepatic, or pulmonary), including serious infection other than VL, acquired immunodeficiency syndrome or antibody to human immunodeficiency virus (HIV), severe protein and/or caloric malnutrition (kwashiorkor or marasmus), glucose-6 phosphate dehydrogenase deficiency, pregnancy, hemoglobin concentration < 5 g/100 mL, white blood cell count < 1,000, platelet count < 30,000/mm³, and a significant (> 3 times upper limit of normal) deviation in serum chemistries such as blood urea nitrogen, creatinine, alanine aminotransferase, and aspartate aminotransferase.

Consent. Informed consent was obtained from all patients or parents of minors. This study was approved by the institutional review board at the Federal University of Espírito Santo and by the Human Subjects Research Review Board of the U.S. Army Surgeon General.

Parasitologic procedures. Splenic aspiration was performed in cases of spleenomegaly in which the clinical presentation was consistent with VL. Demonstration of Leishmania was accomplished either by visualization of amastigotes on Giemsa- or Diff-Quik– (American Scientific Products, McGraw, IL) stained splenic aspirates, or by culture in diphagic blood agar medium with an overlay of 0.1 mL Schneider Drosophila medium (Gibco BRL, Grand Island, NY), supplemented with 20% heat-inactivated fetal calf serum and 100 µg/mL of gentamicin.3 Splenic aspirates were graded according to the number of amastigotes per high-power field (hpf) as follows: 0 = no parasites/1,000 hpf; 1 = 1–10 parasites/1,000 hpf; 2 = 1–10 parasites/100 hpf; 3 = 1–10 parasites/10 hpf; 4 = 1–10 parasites/1 hpf; 5 = 10–100 parasites/1 hpf; and 6 = more than 100 parasites/1 hpf. Additionally, cultures were incubated for 14 days, and if no promastigotes were seen, they were considered negative and discarded.
Hematologic and serum chemistry

Physical examination

analyzed by high-performance liquid chromatography.

levels of WR6026 and its identified metabolites. Plasma was

periods.

trogen, creatinine, aspartate aminotransferase, and alanine

effects, including nausea, abdominal discomfort, and head-

questioned about symptoms suggesting possible drug side

ment, lack of progression of initial improvement to initial

followed by initial cure, and no relapse (splenomegaly with

organ aspiration and clinical examinations in order to deter-

decrease was seen in parasites on smear and clinical im-

were seen by smear or culture of splenic aspirate, or bone

were seen by smear or culture of splenic aspirate, or bone

Drug administration. WR6026 2HCl powder (lot JF7-

Drug was administered with water

between 1.0 mg/kg/day, 2.5 mg/kg/day, and 3.25 mg/kg/day,

124-2) was given in capsule form (5, 15, or 30 mg) as a

Cohorts 1, 2, 3, 4, and 5 were administered

1.0 mg/kg/day, 1.5 mg/kg/day, 2.0 mg/kg/day, and 2.5 mg/kg/day, respectively, for 28 days. Drug was administered with water

1 hr before breakfast and under supervision.

Determination of efficacy. Patients were classified ac-

According to the following categories:

Initial cure (at end of treatment on Day 28). No parasites

were seen by smear or culture of splenic aspirate, or bone

marrow aspirate if the spleen was too small for aspiration.

Initial improvement (at end of treatment). At least a 2-log

decrease was seen in parasites on smear and clinical im-

provement, primarily on the basis of decrease in spleen size.

Patients with initial improvement were followed with further

organ aspiration and clinical examinations in order to deter-

mine if parasites and splenomegaly completely remitted, in

which case the patient was characterized as an initial cure.

Final cure. Initial cure was seen, or initial improvement

followed by initial cure, and no relapse (splenomegaly with

a positive splenic aspirate) by the end of 12 months of

follow-up.

Treatment failure. Lack of initial cure or initial improve-

ment, lack of progression of initial improvement to initial
cure, or relapse after initial cure was seen.

Determination of drug toxicity. Each day, patients were

questioned about symptoms suggesting possible drug side
effects, including nausea, abdominal discomfort, and head-

ache. In addition, vital signs were recorded and examined.

Laboratory tests to monitor methemoglobin, blood urea ni-

rogen, creatinine, aspartate aminotransferase, and alanine

aminotransferase concentrations were repeated weekly dur-

ing the 4-week treatment period and during the follow-up

periods.

Drug concentrations. Plasma was drawn initially and

weekly just before drug dosing for determination of trough

levels of WR6026 and its identified metabolites. Plasma was

analyzed by high-performance liquid chromatography.6

RESULTS

Patient characteristics. The enrolled patients were pri-

arily young adults who presented with the characteristic

picture of moderate kala-azar: splenomegaly of ~ 8 cm be-

low the left costal margin, moderate decreases in the formed

elements of the blood, and parasitemia of ~ 3.5 log units

(Table 1).

Efficacy. Table 2 summarizes the results of treatment with

WR6026 in the 5 cohorts. In Cohort 1 (1 mg/kg/day), one

patient showed diminution of white blood cells to < 1,000/

mm3, which was ascribed to advancing disease. The other 3

patients were also classified as initial failures. The respective

reasons were that the splenic parasite count changed from

4+ before therapy to 2+ after therapy but the size of the

spleen did not change; the parasite count decreased from 2+

to 1++; and the parasite count did not decrease from 2+. The

final cure rate for this cohort was 0%.

In Cohort 2 (1.5 mg/kg/day), one patient was cured. Three

patients were initial failures in that the pre- and posttherapy

parasite counts were 4+ versus 4+, 4+ versus 3+, and 3+

versus 3+, respectively. One patient was classified as an

initial failure because the parasite count decreased from 4+ to

2++; but the spleen did not diminish in size. The remaining

patient initially improved (parasite counts decreased from

4+ to 2++; with a spleen size that was 50% of initial size).

However, there was no further improvement in spleen size on

a follow-up examination at Day 42, and this patient was

ultimately classified as a failure. The final cure rate for this

cohort was 1 (17%) of 6 patients.

In Cohort 3 (2.0 mg/kg/day), 4 patients demonstrated ini-
tial cure and did not relapse. Two patients, both of whom

had clinical evidence of varicella infection during the period

of WR6026 treatment, showed initial improvement on the

basis of splenic amastigote counts (3+ to 1+ in the first

patient; 2+ to 0, but promastigotes grew upon culture, in the

second patient), and spleen sizes that had slightly diminished

(11 to 8 cm in the first patient, and 16 to 14 cm in the second

patient). However, there was no further clinical improvement

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cohort 1 (n = 4)</th>
<th>Cohort 2 (n = 6)</th>
<th>Cohort 3 (n = 6)</th>
<th>Cohort 4 (n = 5)</th>
<th>Cohort 5 (n = 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, M/F</td>
<td>4/0</td>
<td>5/1</td>
<td>5/1</td>
<td>3/2</td>
<td>1/0</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>19.0 ± 2.5</td>
<td>32.8 ± 12.9</td>
<td>23.8 ± 12.4</td>
<td>23.8 ± 8.8</td>
<td>22</td>
</tr>
<tr>
<td>WR6026 daily dose (mg/kg)</td>
<td>1.0</td>
<td>1.5</td>
<td>2.0</td>
<td>2.5</td>
<td>3.25</td>
</tr>
<tr>
<td>Duration of disease (months)</td>
<td>4.7 ± 2.9</td>
<td>3.5 ± 1.2</td>
<td>6.2 ± 4.2</td>
<td>6.6 ± 9.8</td>
<td>1</td>
</tr>
<tr>
<td>Amastigotes (grade on splenic aspiration)</td>
<td>3.0 ± 1.2</td>
<td>3.5 ± 0.8</td>
<td>2.8 ± 0.75</td>
<td>4.0 ± 1.2</td>
<td>1</td>
</tr>
<tr>
<td>Physical examination</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Axillary temperature (°C)</td>
<td>38.5 ± 1.2</td>
<td>38.6 ± 1.3</td>
<td>38.5 ± 1.3</td>
<td>38.5 ± 0.5</td>
<td>38.7</td>
</tr>
<tr>
<td>Spleen size (cm)</td>
<td>8.2 ± 3.3</td>
<td>9.2 ± 3.2</td>
<td>10.5 ± 3.9</td>
<td>6.9 ± 2.7</td>
<td>5</td>
</tr>
<tr>
<td>Hematologic and serum chemistry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methemoglobin</td>
<td>0.46 ± 0.18</td>
<td>0.7 ± 0.37</td>
<td>0.7 ± 0.1</td>
<td>0.5 ± 0.18</td>
<td>0.2</td>
</tr>
<tr>
<td>Hemoglobin g/dl (normal 12–17)</td>
<td>9.2 ± 0.93</td>
<td>7.7 ± 1.5</td>
<td>8.7 ± 0.9</td>
<td>8.4 ± 1.6</td>
<td>8.8</td>
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<tr>
<td>White blood cells per 1,000/mm3 (normal 5–10)</td>
<td>2.9 ± 0.76</td>
<td>2.3 ± 0.89</td>
<td>2.2 ± 0.91</td>
<td>2.8 ± 1</td>
<td>2.8</td>
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<tr>
<td>Platelets per 1,000/mm3 (normal 200–400)</td>
<td>157 ± 58</td>
<td>95 ± 39</td>
<td>100 ± 32</td>
<td>142 ± 45</td>
<td>111</td>
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<tr>
<td>Albumin, g/dL (normal 3.5–5.5)</td>
<td>2.8 ± 0.52</td>
<td>2.9 ± 0.81</td>
<td>2.7 ± 0.5</td>
<td>2.7 ± 1.1</td>
<td>2.9</td>
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<tr>
<td>Gamma globulin, mg/dL (normal 0.5–1.6)</td>
<td>3.04 ± 1.2</td>
<td>2.19 ± 0.8</td>
<td>4.0 ± 1.5</td>
<td>3.7 ± 1.4</td>
<td>3.2</td>
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<tr>
<td>Blood urea nitrogen, mg/dL (normal 10–20)</td>
<td>13.2 ± 1.7</td>
<td>13.8 ± 3.6</td>
<td>12.8 ± 2.7</td>
<td>9.0 ± 3.4</td>
<td>12</td>
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<tr>
<td>Creatinine, mg/dL</td>
<td>0.7 ± 0.2</td>
<td>0.7 ± 0.14</td>
<td>0.8 ± 0.3</td>
<td>0.7 ± 0.1</td>
<td>1</td>
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<tr>
<td>Aspartate aminotransferase, U/L (normal 4–32)</td>
<td>34.0 ± 10.5</td>
<td>34.6 ± 22.6</td>
<td>53.8 ± 31.8</td>
<td>33.0 ± 18.3</td>
<td>86</td>
</tr>
</tbody>
</table>

* Data are presented as mean ± SD, with exception of Cohort 5.
### TABLE 2
Results of therapy of patients with visceral leishmaniasis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cohort 1</th>
<th>Cohort 2</th>
<th>Cohort 3</th>
<th>Cohort 4</th>
<th>Cohort 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 mg/kg/day</td>
<td>1.5 mg/kg/day</td>
<td>2.0 mg/kg/day</td>
<td>2.5 mg/kg/day</td>
<td>3.25 mg/kg/day</td>
</tr>
<tr>
<td></td>
<td>(n = 4)</td>
<td>(n = 6)</td>
<td>(n = 6)</td>
<td>(n = 5)</td>
<td>(n = 1)</td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initially cured patients</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Initially improved patients</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>Final cure (%)</td>
<td>0/4 = 0%</td>
<td>1/6 = 17%</td>
<td>4/6 = 67%</td>
<td>1/5 = 20%</td>
<td>0/1 = 0%</td>
</tr>
<tr>
<td>WR6026 on Day 7</td>
<td>37 ± 23</td>
<td>302 ± 341</td>
<td>199 ± 105</td>
<td>526 ± 716</td>
<td>ND</td>
</tr>
<tr>
<td>WR6026 on Day 28</td>
<td>21 ± 12</td>
<td>145 ± 253</td>
<td>114 ± 90</td>
<td>414 ± 614</td>
<td>ND</td>
</tr>
<tr>
<td>WR211789 on Day 7</td>
<td>39 ± 22</td>
<td>132 ± 116</td>
<td>97 ± 32</td>
<td>197 ± 105</td>
<td>ND</td>
</tr>
<tr>
<td>WR211789 on Day 28</td>
<td>33 ± 23</td>
<td>82 ± 72</td>
<td>69 ± 41</td>
<td>212 ± 211</td>
<td>ND</td>
</tr>
<tr>
<td>Physical examination at Day 28</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Axillary temperature (°C)</td>
<td>37.5 ± 0.3 (37.2–38)</td>
<td>37.3 ± 1.1 (36–39.4)</td>
<td>37.4 ± 1 (36.4–39.5)</td>
<td>36.4 ± 0.2 (36.1–36.7)</td>
<td>38.7</td>
</tr>
<tr>
<td>Spleen size (cm)</td>
<td>5.4 ± 2.8 (3–8.5)</td>
<td>7.1 ± 5.1 (0–13)</td>
<td>4.9 ± 6 (0–14)</td>
<td>3.7 ± 3.1 (0–7)</td>
<td>5</td>
</tr>
<tr>
<td>Methemoglobin (%) on Day 7</td>
<td>3.3 ± 4.2 (0.7–9.6)</td>
<td>3.1 ± 1.5 (1.4–5.4)</td>
<td>4.3 ± 4.5 (1.2–13.3)</td>
<td>5.4 ± 4.1 (1.1–7.2)</td>
<td>3.5</td>
</tr>
<tr>
<td>Methemoglobin (%) on Day 28</td>
<td>2.6 ± 3.1 (0.7–7.2)</td>
<td>4.1 ± 2.3 (1.6–6.9)</td>
<td>5.4 ± 2.6 (2.8–9.1)</td>
<td>5.7 ± 2.3 (2.1–8.3)</td>
<td>5.6</td>
</tr>
<tr>
<td>Hemoglobin, g/dL (normal 12–17) on Day 7</td>
<td>9.45 ± 1.5 (8–11.2)</td>
<td>9.3 ± 1.1 (7.1–10.3)</td>
<td>10.2 ± 1.7 (8.8–13.3)</td>
<td>10.2 ± 2.1 (7.6–12.5)</td>
<td>10.2</td>
</tr>
<tr>
<td>Hemoglobin, g/dL (normal 12–17) on Day 28</td>
<td>3.3 ± 1.3 (1–5.5)</td>
<td>3.8 ± 2.6 (1.3–8.2)</td>
<td>3.1 ± 1.2 (2–4.8)</td>
<td>3.5 ± 1.4 (1.7–5.3)</td>
<td>4.9</td>
</tr>
<tr>
<td>White blood cells per 1,000/mm³ (normal 5–10) on Day 7</td>
<td>198 ± 33 (150–228)</td>
<td>187 ± 115 (86–406)</td>
<td>149 ± 66 (100–126)</td>
<td>191 ± 64 (116–292)</td>
<td>252</td>
</tr>
<tr>
<td>White blood cells per 1,000/mm³ (normal 5–10) on Day 28</td>
<td>3.5 ± 0.2 (3.3–3.7)</td>
<td>4.1 ± 0.9 (3.2–5.4)</td>
<td>3.9 ± 0.6 (3.1–5)</td>
<td>3.5 ± 1.3 (2–4.8)</td>
<td>2.2</td>
</tr>
<tr>
<td>Platelets per 1,000/mm³ (normal 200–400) on Day 7</td>
<td>3.2 ± 0.9 (2.3–4.5)</td>
<td>2.3 ± 1.3 (1.1–4)</td>
<td>4.3 ± 1.7 (1.4–6.2)</td>
<td>3.5 ± 2.7 (1.3–8.1)</td>
<td>1.6</td>
</tr>
<tr>
<td>Platelets per 1,000/mm³ (normal 200–400) on Day 28</td>
<td>10 ± 0</td>
<td>12.3 ± 2.2 (10–15)</td>
<td>10 ± 2.1 (9–13)</td>
<td>10.6 ± 2.8 (7–15)</td>
<td>19</td>
</tr>
<tr>
<td>Aspartate aminotransferase, U/L (normal 4–32) on Day 7</td>
<td>0.7 ± 0.1 (0.7–0.9)</td>
<td>1.01 ± 0.1 (0.8–1.2)</td>
<td>0.9 ± 0.4 (0.5–1.6)</td>
<td>0.8 ± 0.2 (0.5–0.9)</td>
<td>1.6</td>
</tr>
<tr>
<td>Aspartate aminotransferase, U/L (normal 4–32) on Day 28</td>
<td>28.5 ± 9.4 (17–40)</td>
<td>33.3 ± 14.8 (15–50)</td>
<td>40.4 ± 28.4 (20–89)</td>
<td>35.8 ± 14.6 (21–59)</td>
<td>41</td>
</tr>
</tbody>
</table>

* Data are presented as mean ± SD (range), with exception of cohort 5. NA = not applicable; ND = not done.
by Day 42: the spleen size enlarged to 10 cm in the first patient and to 16 cm in the second patient. The final cure rate for this cohort was 67%.

In Cohort 4 (2.5 mg/kg/day), 3 patients showed initial cure, with the splenic parasite count diminishing from 4+ to 0, 4+ to 0, and 2+ to 0, but 2 of these patients relapsed, one at 3 months and one at 6 months. The other 2 patients were initial failures in that parasite counts decreased from 5+ to 4+ and from 4+ to 3+. The final cure rate for this cohort was only 20%.

Only 1 patient was entered into Cohort 5 (3.25 mg/kg day). As a result of renal toxicity, his treatment was interrupted on Day 24. Although he was classified as an initial cure, he relapsed 10 months after therapy.

Patients who failed to respond to WR6026 were then successfully treated with meglumine antimonate or with amphotericin B.

**Toxicity.** Clinical side effects often ascribed to drugs (e.g., gastrointestinal upset or headache) were found in < 5% of patients in this open-label trial. Methemoglobinemia is a common side effect of 8-aminquinoline administration. In this study, blood methemoglobin levels reached steady state at 7 days and was < 6% in patients receiving — 3 mg WR6026/kg/day. There was no significant relationship (simple regression: \( R^2 = 0.05, P = 0.36 \)) between Day 7 blood methemoglobin levels and daily dose (Table 2). There was also no correlation between Day 7 blood methemoglobin levels and cure (\( P = 0.75, t\)-test). Liver enzymes and triglycerides did not appreciably rise (Table 2).

An unexpected side effect was nephropathy in 3 patients. Two patients in Cohort 3, neither of whom had preexisting antibodies to varicella, manifested primary varicella infection. The first patient with varicella demonstrated vesicles 4 days after beginning WR6026 therapy. Day 7 trough levels of WR6026 were 267 ng/mL, only somewhat above the mean value for Cohort 3. Forty-four days later (20 days after WR6026 therapy had finished), his creatinine was 1.8 mg/dL (normal, 0.6–1.2 mg/dL), an increase from 0.9 mg/dL before therapy. Renal biopsy was performed and therapy with steroids was begun. The biopsy revealed interstitial nephritis and areas of acute tubular necrosis in regeneration.

The second patient developed cutaneous vesicles 2 days after finishing WR6026 therapy. Day 7 trough levels of WR6026 had been 160 ng/mL, only slightly below the mean value for Cohort 3. Twelve days after vesicles first appeared (14 days after finishing WR6026), his creatinine was 3.3 mg/dL, an increase from 1.3 mg/dL pretherapy levels. Twenty-seven days later (41 days after finishing his regimen of WR6026), the serum creatinine value reached 5.6 mg/dL. The patient underwent kidney biopsy; analysis of the biopsy again revealed interstitial nephritis and areas of acute tubular necrosis in regeneration. Immunohistochemical examination of the biopsy was negative for *Leishmania*, adenoovirus, herpes simplex 1 and 2, and cytomegalovirus. The biopsy was also negative for varicella DNA via in situ hybridization and polymerase chain reaction. As noted above, both patients in Cohort 3 with kidney dysfunction were additionally classified as therapeutic failures. After their renal biopsies, both patients were treated with lipid-encapsulated amphotericin B for VL and steroids for kidney dysfunction. Further splenic aspirates were negative for *Leishmania*, and kidney function ultimately normalized.

Because the patients with nephropathy in Cohort 3 also had primary varicella, it was thought that the combination of kala-azar, WR6026, and varicella was needed to produce this side effect. After control measures for varicella were instituted, WR6026 dose escalation continued, and one patient in Cohort 5 (3.25 mg/kg/day) was entered. When this patient demonstrated a rise in creatinine to 2 mg/dL on Day 24 with a creatinine clearance of 29 mL/min, drug administration was stopped and the study was terminated. Kidney function returned to normal 18 days later.

**Plasma levels of WR6026.** Plasma levels of WR6026 and a major metabolite (WR211789) were in the majority of patients higher on Day 7 than on Day 28. Although Week 1 trough levels of WR6026 showed an absolute increase between Cohort 1 and Cohort 2 (Table 2), there was no significant correlation (simple regression: \( R^2 = 0.12, P = 0.13 \)) between drug levels and administered dose when Cohorts 1 to 4 were considered.

Because Week 1 trough levels of WR6026 did not correlate with cure (\( P = 0.40, t\)-test), there was not a correlation between drug exposure and the pharmacodynamic parameter of efficacy.

**Discussion.**

The need for an orally administered antileishmanial drug, along with encouraging data in the previous Kenyan trial, prompted this study of WR6026 against Brazilian kala-azar. The current study yielded unanticipated results with respect to both efficacy and toxicity. Given that the cure rate in the Kenyan study with a dose of 1 mg/kg/day for 28 days was 50%, drug efficacy in the current study was less than expected. The starting dose of 1 mg/kg/day cured none of 4 patients. In addition, efficacy did not uniformly increase with escalating doses, as best demonstrated by comparing the cure rate of Cohort 3, the members of which received 2.0 mg/kg/day (67%), to the cure rate of members of Cohort 4, who received 2.5 mg/kg/day (20%).

The side effect profile in the current study also deviated from those reported in previous studies. In earlier experimental studies in dogs and in clinical trials with patients with HIV, dose escalation yielded corresponding increases in methemoglobinemia, a result of oxidant action of the drug on hemoglobin, which ultimately became dose-limiting. A mean of 30% methemoglobin was seen in dogs administered 3 mg/kg/day for 4 weeks. Three of 6 patients with HIV who were administered — 2 mg/kg/day for 3 weeks had methemoglobinemia values in excess of 20%.

In the current clinical study, despite a slightly higher peak dose (3 mg/kg/day) and longer duration of treatment (4 weeks) than that used in the HIV study, maximal mean methemoglobin blood levels remained < 6%. The same lot of drug was used for the present study, the HIV study, and the dog study.

Clinically significant renal toxicity was a side effect not reported in the dog or HIV studies. Two of the 3 patients developing nephropathy in the current study experienced coincident varicella infection. Although varicella in some cases has been associated with glomerulonephritis, the damage reported in the renal biopsies we analyzed from patients in
the current study affected the tubules and the interstitium. Nephrotoxicity is not a documented side effect with drugs of the primaquine family, but it is possible that drug toxicity was responsible for the interstitial nephritis. If this were the case, one might expect more cases of this side effect with higher doses.

The pharmacokinetics of WR6026 are poorly understood, and the unanticipated results in our study could be explained by unusual metabolic phenomena. In the dog, radioactive WR6026 is virtually 100% orally bioavailable, but only 4% of the orally administered drug can be recovered as WR6026 due to presumed first-pass metabolism in the liver.\(^{11}\) Approximately 60% of all 8-aminoquinoline species are excreted in dog feces, and 28% is excreted in dog urine. In clinical phase 1 studies, 14% of administered drug was recovered in the urine, with 7% of this (1% of total administered drug) being WR6026 and a lesser amount being the desethyl metabolite, WR211789.\(^{12}\) Determination of WR6026 and WR211789 levels in the blood and urine therefore provides information on perhaps 5% of 8-aminoquinoline species in the body. The majority of 8-aminoquinoline species formed in the body are unknown, and their efficacy versus *Leishmania* and toxicity to mammalian cells are equally unknown. It can be hypothesized that marked differences in metabolism, and therefore in efficacy and toxicity, could occur in different hosts, in different disease states, and (due to oxidation) with different doses.

The future for WR6026 as an antileishmanial agent depends on the demonstration of efficacy without undue toxicity in further trials. If *Leishmania* species other than *L. chagasi* are more sensitive to WR6026, or if patients from different regions metabolize drugs differently, or if more patients are examined, the relatively discouraging results from this trial might be contradicted. Ultimately, the administrators are examined, the relatively discouraging results from this trial might be contradicted. Ultimately, the administrative bility of WR6026 via the oral route and its therapeutic index in this trial might be contradicted. Ultimately, the administration of WR6026 via the oral route and its therapeutic index will be compared with other agents for VL. Pentavalent antimony, a parenteral agent, is standard therapy with ~90% efficacy, except where resistance has emerged, and mild to moderate toxicity.\(^{13}\) Amphotericin B, another parenteral agent, is more effective (>95% efficacy), but it is also more toxic,\(^{14}\) except in the new formulations, which are expensive.\(^{15}\) Miltefosine is an orally administered agent in clinical trials that has to date displayed high efficacy but also a range of toxicities.\(^{16}\)

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