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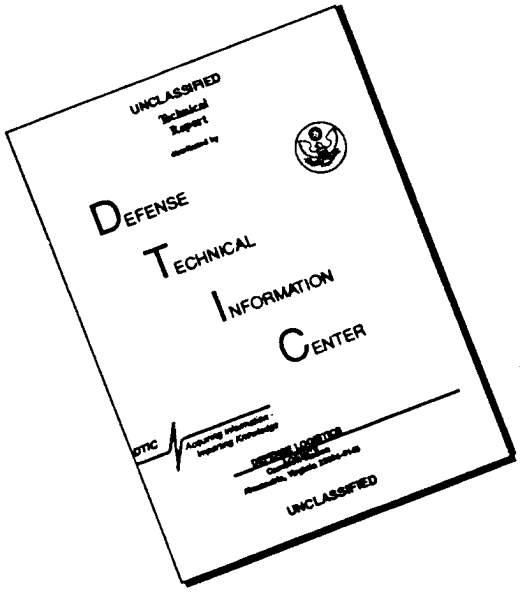

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Under this Cooperative Agreement, the efficacy of an oral 8-aminoquinoline (8-[[6-(diethylamino)hexyl]amino]-6-methoxy-4-methylquinoline)(WR6026) in the treatment of 16 patients with kala azar was evaluated. The first 8 patients received therapy for 2 weeks at a dosage of 0.75-1.00 mg/(kg.day); 1 patient was cured, and in the other 7, a 1-logarithm decrease in the number of splenic parasites and clinical improvement were noted. The next 8 patients received therapy for 4 weeks at the same daily dosage (1 mg/[kg.day]); 4 were cured, and of the other 4 patients, 1- to 2-log decreases in the number of parasites and clinical improvement were noted. The therapy was associated with minimal toxicity; adverse effects included gastrointestinal distress, headache, and methemoglobinemia. The fact that one-half of the patients were cured indicates that future trials with longer regimens and higher dosages are warranted and should include patients for whom existing treatment methods have failed.

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Report on Cooperative Agreement Between the
U.S. Army Medical Research and Materiel Command and the
Kenya Medical Research Institute
DAMD17-91-Z-1018

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840, Tel: (02)722541, Fax: (02)720030, Tlx. 25696 KEMRI, NAIROBI, Kenya.

May 24, 1996

REPORT ON COOPERATIVE AGREEMENT BETWEEN THE U.S ARMY MEDICAL RESEARCH AND MATERIAL COMMAND AND THE KENYA MEDICAL RESEARCH INSTITUTE DAMD17-91-Z-1018

Over the past 25 years the U.S. Army, through the Walter Reed Army Institute of Research, has collaborated in numerous medical research projects in Kenya. The primary emphasis of this work has always been the study of various human tropical diseases. Under this specific Cooperative Agreement (DAMD17-91-Z-1018) efforts were specifically directed at evaluating the safety, tolerability, and efficacy of a novel 8-aminoquinoline (WR 6026) against endemic visceral leishmaniasis (kala azar, *Leishmania donovani*) in patients recruited from the Baringo District, Rift Valley, North-Central Kenya.

The clinical trial conducted under this cooperative agreement demonstrated that WR 6026 was relatively well tolerated at the doses evaluated, and effective in curing one-half of the enrolled patients.

As future developmental work continues with WR 6026 to better define dosage levels and treatment duration with regards to treatment of New World Leishmaniasis (work currently being conducted in Brazil), we would propose to further expand our understanding of the utility of this compound for definitive therapy of Old World Leishmaniasis. It is hoped that the future assignment of a U.S. Army clinical investigator to our Institute interested in the treatment of leishmaniasis will help to further advance our work in this area of tropical infectious diseases.

Davy K. KOECH, PhD, SS, OGW
DIRECTOR
KENYA MEDICAL RESEARCH INSTITUTE

- Summary, Clinical Evaluation of WR 6026

Under this Cooperative Agreement, DAMD17-91-Z-1018, the safety, tolerability, and efficacy of the oral 8-aminoquinoline 8-[[6-(diethylamino)hexyl]amino]-6-methoxy-4-methylquinoline (also known as WR6026) was evaluated in the treatment of 16 patients with kala azar.

Patients were recruited from the Baringo District of the Rift Valley, north-central Kenya, a region endemic for visceral leishmaniasis. Patients with splenomegaly or who had positive direct agglutination titers were referred to the Clinical Research Centre of the Kenya Medical Research Institute, Nairobi. Informed consent was obtained from the patients, medical histories were obtained and physical examinations, with particular attention to weight, spleen size, and liver span, performed. Clinical laboratory investigations included determination of hemoglobin concentration, packed cell volume, white blood cell count with differential, platelet counts, prothrombin time, antibodies to human immunodeficiency virus type 1 by ELISA, blood type, and levels of glucose-6-phosphate dehydrogenase, methemoglobin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, bilirubin, lactate dehydrogenase, total protein, and fasting levels of triglycerides. Electrocardiograms and x-rays films of the chest were also obtained. Splenic aspiration was performed with aspirates stained for amastigotes and cultured for promastigotes. Complete inclusion and exclusion criteria are detailed in the accompanying published manuscript (see page 1035 of the manuscript, first paragraph second column).

A total of 16 patients were enrolled and completed evaluation. The first 8 patients received therapy for 2 weeks at a dosage of 0.75-1.00 mg/(kg.day); 1 patient was cured, and in the other 7, a 1-logarithm decrease in the number of splenic parasites and clinical improvement were noted. The next 8 patients received therapy for 4 weeks at the same daily dosage (1 mg/[kg.day]); 4 were cured, and of the other 4 patients, 1- to 2-log decreases in the number of parasites and clinical improvement were noted. The therapy was associated with minimal toxicity; adverse effects included gastrointestinal distress, headache, and methemoglobinemia. The fact that one-half of the patients were cured indicates that future trials with longer regimens and higher dosages are warranted and should include patients for whom existing treatment methods have failed.

Phase 2 Efficacy Trial of an Oral 8-Aminoquinoline (WR6026) for Treatment of Visceral Leishmaniasis

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From the Clinical and Biomedical Sciences Research Centres of the Kenya Medical Research Institute and the U.S. Army Medical Research Unit–Kenya, Nairobi, Kenya; Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C.; and Unit of Infectious and Tropical Diseases, Academic Medical Center, Amsterdam, the Netherlands

The efficacy of an oral 8-aminoquinoline (8-[[6-(diethylamino)hexyl]amino]-6-methoxy-4-methylquinoline) (WR6026) in the treatment of 16 patients with kala azar was evaluated. The first 8 patients received therapy for 2 weeks at a dosage of 0.75–1.00 mg/(kg · d); 1 patient was cured, and in regard to the other 7, a 1-logarithm decrease in the number of splenic parasites and clinical improvement were noted. The next 8 patients received therapy for 4 weeks at the same daily dosage (1 mg/[kg · d]); 4 were cured, and for the other 4, 1- to 2-log decreases in the number of parasites and clinical improvement (in regard to weight, liver and spleen size, hemoglobin level, and leukocyte count) were noted. The therapy was associated with minimal toxicity; adverse effects included gastrointestinal distress, headache, and methemoglobinemia. The fact that one-half of the patients were cured indicates that future trials with longer regimens and higher dosages are warranted and should include patients for whom existing treatment methods have failed.

Leishmania donovani causes visceral leishmaniasis (kala-azar) [1, 2]. The disease is endemic in the Rift Valley of Kenya, where it occurs commonly in children [3], and untreated cases are associated with a mortality rate of 77% [4]. Pentavalent antimonial agents, diamidine compounds, and amphotericin were developed in 1920, 1939, and 1956, respectively, and were proved to be effective against human visceral leishmaniasis in 1935, 1939, and 1963 [5, 6]. However, because of the drawbacks of treatment with such drugs (i.e., therapeutic failure [7, 8], toxicity, expense, and parenteral administration), an oral antileishmanial agent has been sought. Ketoconzaole was not effective against kala-azar in Kenya (J. B. O. Were, unpublished observation).

The agent 8-[[6-(diethylamiño)hexyl]amino]-6-methoxy-4-methylquinoline (SN-11,191) was developed during the Second World War as a potential antimalarial drug [9]. The compound (WR6026) was demonstrated to be effective against *L. donovani* in hamsters [10]—more so than stibogluconate [11] or meglumine antimonate [12], and more so orally than subcutaneously. In one study it reduced the number of hepatic amastigotes [13], and in another it was effective in mice infected with *L. donovani* (median effective dosage, 0.72 mg/[kg · d] for 5 days) [14]. It has suppressed amastigotes in the opossum, without preventing death [15], but it had an unclear effect on *Leishmania tropica* (later reclassified as *Leishmania major*) in mice [16]. At a dosage of 0.20–3.25 mg/(kg · d) for 5 days (administered intravenously), it cured *L. donovani*–infected dogs [17]. In vitro studies have shown that the 8-aminoquinoline is effective against *L. major* [18] and *L. tropica* [19, 20].

The mechanism of action of this aminoquinolone against leishmanias is unclear. Changes in the amastigote's outer membrane in the flagellar pocket, mitochondria, kinetoplast [21], and cytoplasm have been induced [22]. Investigators have described the metabolism [23, 24], pharmacological characteristics [25], and efficacy [26–30] of the compound, and the metabolites have been said to be active [31]. Its adverse effects include methemoglobinemia and transient elevations of hepatic transaminase levels [32]. In a phase-1 trial it was absorbed orally, and the following findings were

Received 16 November 1993; revised 7 April 1994.

Informed consent was obtained from the patients or their parents or guardians, and the policies of the Kenya National AIDS Committee and the Kenya Ministry of Health were followed.

This study was approved by the Ad Hoc Scientific Committee of the Office of Research Management, Walter Reed Army Institute of Research (protocol no. 316); the Scientific Steering Committee of the Kenya Medical Research Institute (protocol no. 130); the National Ethical Review Committee of Kenya; and the Human Subjects Research Review Board of the Office of the Surgeon General of the U.S. Army (protocol no. A-5369).

The assertions, opinions, intellectual content, and wording are the private views and responsibility of the principal author and coauthors and are not to be construed as official or as reflecting the views of the U.S. Department of the Army or the U.S. Department of Defense.

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n peak serum level at 4 hours, a mean elimination ha... ...e of 10.5 hours (range, 3.6–14.5 hours), and a four-fold difference in areas under the plasma concentration curves, without adverse symptoms or signs and without significant changes in concentrations of methemoglobin or in hematologic, chemical, or electrocardiographic findings [33]. Other investigators have found this 8-aminoquinolone to be safe at a dosage of 1.0 mg/(kg · d) for 14 days [34].

The purpose of this study was to determine the efficacy and toxicity of this 8-aminoquinoline (within a dosage range of 0.25–1.0 mg/[kg · d]) for the treatment of visceral leishmaniasis in humans.

Patients and Methods

As part of an ongoing program for the clinical evaluation of potential antileishmanial treatments, patients were recruited from the Baringo District of the Rift Valley, in north-central Kenya, about 300 km north of Nairobi. The first reported cases of visceral leishmaniasis occurred in this area in 1954 [3, 4]. The epidemiology of leishmaniasis in this region h... ...n described [35, 36], and the annual incidence rate of t... ...ase there has been estimated as 27 cases per 1,000 residents [37]. It occurs primarily in children aged 5–14 years and is seldom seen in persons aged >30 years [4, 38]. ...s with splenomegaly or for whom direct agglutinatio... ...s were positive were referred to the Clinical Research Centre of the Kenya Medical Research Institute in Nairobi; informed consent and medical histories were obtained and physical examinations and laboratory tests were performed routinely for all admitted patients. If the clinical condition or histopathologic findings suggested visceral leishmaniasis, the patient was enrolled in the study and screened. The physical examination included determination of weight, spleen size (from the costal margin in the left anterior axillary line to the spleen tip), and liver span (in the right midclavicular line, from the costal margin to the liver edge).

Clinical laboratory investigations were performed for determination of hemoglobin concentration; packed cell volume; white cell, differential blood cell, and platelet counts; prothrombin time; presence of antibodies to human immunodeficiency virus type 1 (HIV-1) via ELISA; blood type; and levels of glucose-6 phosphate dehydrogenase, methemoglobin, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, lactate dehydrogenase (LDH), and total protein and fasting levels of triglycerides. Electrocardiograms and roentgenograms of the chest a... ...e obtained. Splenic aspiration was performed in c... ... splenomegaly in which the clinical presentation was consistent with visceral leishmaniasis [1, 39, 40]; aspirates wer... ...ined for amastigotes and cultured for promastigotes [4... ...Splenic aspirates were graded according to the number o... parasites per high-power field (hpf): 0 = no parasites/1,000 hpf; 1 = 1–10/1,000 hpf; 2 = 1–10/100 hpf; 3 = 1–10/10 hpf; 4 = 1–10/1 hpf; 5 = 10–100/1 hpf; and 6 = >100/1 hpf [1]. Cultures were incubated for 14 days. If no promastigotes were seen, the culture was considered negative and discarded.

There were two criteria for inclusion of patients in the study: an age of between 10 and 50 years and a diagnosis of visceral leishmaniasis (proven by a splenic aspirate graded 2+ to 5+ and a splenic culture positive for *Leishmania* species). Exclusion criteria were use of other antileishmanial drugs; an enlarged spleen reaching the pelvic crest; evidence of another serious disease or of immunodeficiency (e.g., congenital or HIV-related); a diagnosis of kwashiorkor or marasmus; a glucose-6-phosphatase dehydrogenase deficiency; a positive pregnancy test; nursing a child; a hemoglobin concentration of <5 g/dL; a white cell count of <1,000/mm^3; a platelet count of <30,000/mm^3; clinical chemistry values of >2 times normal; and liver chemistry values of >4 times normal. Levels of LDH, AST, and alkaline phosphatase are usually normal in cases of visceral leishmaniasis but have been reported to be 1.5 times the upper limit of normal in otherwise uncomplicated cases [43]. Of 86 children with visceral leishmaniasis in Baghdad, Iraq, 40% had elevated levels of ALT and 91% had elevated levels of AST; levels of LDH were elevated in all 86, but none had elevated creatine phosphokinase levels [44].

WR6026 powder was given in capsules of dihydrochloride salt (5, 15, or 30 mg) as a single daily dose at noon (before lunch) to 16 patients, aged 10–30 years. The first cohort (4 patients) received 0.75 mg/(kg · d) for 14 days, the second cohort (4 patients) received 1.00 mg/(kg · d) for 14 days, and the third cohort (8 patients) was to receive 1.00 mg/(kg · d) for 28 days. One patient in the latter cohort inadvertently received 0.75 mg/(kg · d) for 28 days. Patients were enrolled and treated between 1 October 1990 and 15 June 1992.

Patients were questioned daily about adverse reactions to treatment. Specific physical examinations and measurements as well as clinical laboratory investigations were performed weekly. Parasitological response to treatment was determined by staining and culture of splenic aspirates obtained 2 weeks after the last (14th or 28th) dose of 8-aminoquinoline. Those patients for whom stains or cultures did not prove elimination of parasites were treated with iv stibogluconate (10 mg/[kg · d]) for 10 days [45, 46]. Serum samples were obtained 5–30 minutes before each dosing and 2 hours after each dosing for determination of trough and peak drug levels by high-pressure liquid chromatography [47].

Results

The patients' ages ranged from 10 to 30 years. Their characteristics included abdominal swelling of 1–6 months' duration, splenomegaly (spleen size, 7–23 cm), and parasitic infection (splenic aspirate grade, 4+ to 5+). Patients selected were chronically ill but not acutely or severely so.

Table 1. Treatment and outcome of 16 cases of visceral leishmaniasis with the 8-aminoquinoline WR6026.

Patient no.	Patient age (y)/weight (kg)	8-Aminoquinoline Dosage mg/kg·d	8-Aminoquinoline Dosage mg/d	8-Aminoquinoline Duration (d)	Splenic aspirate parasite grade*/culture results Pretreatment	Splenic aspirate parasite grade*/culture results Post-treatment	Response to therapy	Antimony Administered subsequently	Antimony Response	Duration of follow-up (d)
1	12/25	0.75	20	14	4+/Pos	0/Cont	Cure	N	NA	416
WD†	21/51	0.75	40	5	5+/Pos	4+/Cont	Imp	Y	Cure	432
2	27/52	0.75	40	14	5+/Pos	4+/Pos	Imp	Y	Cure	364
3	16/40	0.75	30	14	4+/Pos	3+/Pos	Imp	Y	Cure	312
4	12/32	0.75	24	14	4+/Pos	3+/Pos	Imp	Y	Cure	423
5	15/48	1.00	50	14	5+/Pos	4+/Pos	Imp	Y	Cure	312
6	20/53	1.00	55	14	5+/Pos	4+/Pos	Imp	Y	Cure	249
7	12/24	1.00	26	14	5+/Pos	4+/Pos	Imp	Y	Cure	273
8	12/34	1.00	35	14	3+/Pos	2+/Pos	Imp	Y	Cure	395
9	27/54	0.75	40	28	4+/Pos	2+/Neg	Imp	Y	Cure	121
10	30/53	1.00	55	28	5+/Pos	0/Neg	Cure	N	NA	396
11	12/25	1.00	25	28	5+/Pos	0/Neg	Cure	N	NA	375
12	10/23	1.00	25	28	4+/Pos	0/Neg	Cure	N	NA	368
13	19/45	1.00	45	28	4+/Pos	3+/Pos	Imp	Y	Cure	368
14	17/47	1.00	45	28	5+/Pos	0/Pos	Cure‡	N	NA	180
15	24/53	1.00	55	28	5+/Pos	3+/Pos	Imp	Y	Cure	76
16	28/49	1.00	50	28	5+/Pos	4+/Pos	Imp	Y	Cure	284

NOTE. Pos = positive; Cont = contaminated; N = no; NA = not applicable; Imp = improvement; Y = yes; Neg = negative.
* See *Patients and Methods* section for explanation of grading system.
† This patient withdrew from the study after 5 days of therapy with WR6026.
‡ The spleen was not palpable at follow-up.

Clinical improvement occurred in all of the eight patients treated for 14 days (table 1). Histologic examination showed that one patient was cleared of amastigotes and that the number of parasites in the other seven decreased by 1 logarithm (90%). Cultures showed that treatment eliminated parasites in one of the eight patients, and for seven it increased the number of days until promastigotes were apparent. In three patients, a reduction in spleen size was noted. Patient 1, who was initially cleared of amastigotes (as evidenced by staining and culture results), had no parasites when staining and cultures were performed at 2-month, 6-month, and 12-month follow-ups; he was clinically well and was considered cured. This patient became afebrile 10 days after the initiation of treatment. The seven who improved clinically but for whom there was no histologic or cultural evidence of improvement were treated with stibogluconate. At a 12-month follow-up they were clinically well, and none of those whose spleens were palpable and aspirated had parasites revealed by stain or culture. One additional patient withdrew from the study for family reasons after 5 days of therapy with the 8-aminoquinoline. He was subsequently cured with stibogluconate.

After 14 days of treatment, significant improvements in the following mean (±SD) values were noted for the eight patients: weight (initially 39 ± 10 kg) at 4 weeks was 41 ± 11 kg (P = .0195); liver size (initially 4 ± 2 cm) at 4 weeks was 3 ± 2 cm (P = .0203); spleen size (initially 13 ± 3 cm) at 4 weeks was 12 ± 2 cm (P = .1945); hemoglobin concentration (initially 7 ± 2 g/dL) at 4 weeks was 9 ± 1 g/dL (P = .0109); leukocyte count (initially 3.1 ± 1.2 × 10^3/mm^3) at 4 weeks was 3.6 ± 0.8 × 10^3/mm^3 (P = .346); eosinophil percentage (initially 0 ± 0%) at 4 weeks was 1 ± 1% (P = .3457); platelet count (initially 160 ± 40 × 10^3/mm^3) at 4 weeks was 244 ± 127 × 10^3/mm^3 (P = .4537); and the maximum total protein concentration (initially 93 ± 11 g/L) was 109 ± 8 g/L (P = .0007) (paired, two-tailed t-test).

The conditions of all eight patients treated for 28 days clinically improved (table 1). Histologic findings showed that four were cleared of leishmanial amastigotes and that in the other four the number of parasites was reduced by 1 or 2 logarithms (90% to 99%). Cultures revealed that the 8-aminoquinoline eliminated parasites in three of the eight patients by the time the first posttreatment aspirate was obtained; another patient's second aspirate was culture-negative, which brings the number of cures to four. The 8-aminoquinoline increased the number of days until promastigotes were apparent in seven of the eight patients. Seven patients' spleens were reduced in size, as determined by palpation. The four patients whose conditions improved clinically and who were cleared of parasites did not receive antimony during follow-up. Their spleens were not palpable and their splenic aspirates were not positive at follow-up (at 12 months for three patients and at 6 months for one). Patient 9 became afebrile on day 23; a reduction in the number of parasites was histopathologically evident. Patients 10, 11,

1[illegible]4 became afebrile at a mean of 11 days (range, 5–12 days); these patients had no histopathologically evident parasites post-treatment. Patient 15 did not become afebrile and was not cleared of parasites.

In the eight patients who received treatment for 28 days, there were significant improvements in the following mean (± SD) values: weight (initially 44 ± 13 kg) at 6 weeks was 49 ± 14 kg (P = .001); liver size (initially 4 ± 2 cm) at 6 weeks was 3 ± 2 cm (P = .007); spleen size (initially 16 ± 4 cm) at 6 weeks was 12 ± 4 cm (P = .016); hemoglobin concentration (initially 7 ± 1 g/dL) at 6 weeks was 9 ± 2 g/dL (P = .009); leukocyte count (initially 2.2 ± 1.0 × 10^3/mm^3) at 6 weeks was 3.6 ± 1.4 × 10^3/mm^3 (P = .009); eosinophil percentage (initially 0 ± 2%) at 6 weeks was 2 ± 2% (P = .142); platelet count (initially 193 ± 113 × 10^3/mm^3) at 6 weeks was 135 ± 39 × 10^3/mm^3 (P = .223); and the maximum total protein concentration (initially 86 ± 20 g/L) was 117 ± 24 g/L (P = .009) (paired, two-tailed t-test).

The adverse effects of the 8-aminoquinoline were mild to moderate and transient. Four patients had headaches, one had abdominal cramps, and one had epigastric distress.

[illegible] were no severe adverse effects associated with the 1[illegible] treatment regimen, during which the following means (± SD) of the maximum values were noted: methemoglobin (initially 1.3 ± 0.4% of total hemoglobin), 2.3 ± 0.8% of [illegible] = .0067); alkaline phosphatase (initially 0.6 ± 0.3 time[illegible] per limit of normal), 1.1 ± 0.7 times upper limit of normal (P = .0294); ALT (initially 0.7 ± 0.6 times upper limit of normal), 1.9 ± 0.8 times upper limit of normal (P = .0294); AST (initially 1.0 ± 0.9 times upper limit of normal), 2.0 ± 1.0 times upper limit of normal (P = .0178); bilirubin (initially 13.3 ± 4.7 μmol/L), 19.8 ± 3.7 μmol/L (P = .0046); LDH (initially 0.9 ± 0.3 times upper limit of normal), 1.0 ± 0.2 times upper limit of normal (P = .2943); and triglycerides (initially 1.8 ± 0.4 mmol/L), 2.0 ± 0.3 mmol/L (P = .9001) (paired, two-tailed t-test).

There also were no severe adverse effects associated with the 28-day regimen, during which the following means (± SD) of the maximum values were noted: methemoglobin (initially 1.2 ± 0.5% of total hemoglobin), 2.6 ± 1.7% of total (P = .060); alkaline phosphatase (initially 0.8 ± 0.2 times upper limit of normal), 1.2 ± 0.4 times upper limit of normal (P = 0.151); ALT (initially 0.4 ± 0.3 times upper limit of normal), 1.2 ± 1.1 times upper limit of normal (P = .133); AST (initially 0.5 ± 0.3 times upper limit of normal), 1.4 ± 1.4 times upper limit of normal (P = .120); bilirubin (initially 11.9 ± 6.3 μmol/L), 17.3 ± 6.6 μmol/L (P = .112); LD[illegible] tially 0.9 ± 0.3 times upper limit of normal), 1.1 ± 0.2 [illegible] upper limit of normal (P = .090); and triglycerides (initially 1.5 ± 0.4 mmol/L), 2.2 ± 0.5 mmol/L (P = .001) (pai[illegible]vo-tailed t-test). Transient elevation of ALT and AS[illegible]entrations in one patient (number 12) may have been due to concomitant hepatitis B. In all patients, methemoglobin levels, which increased to a maximum of 5.5% of total hemoglobin levels, were not suggestive of toxicity; they peaked at 7–14 days and returned to normal at 14–28 days. The concentration of fasting triglycerides increased mildly to a maximum of 3.0 mmol/L and returned to normal after treatment ceased.

Urinalysis revealed no significant abnormalities in the 16 patients, and their electrocardiograms and chest roentgenograms were normal.

Drug levels in serum were available for only 13 patients for logistical reasons. The mean peak level was 273 ng/mL (range, 90–520 ng/mL); the mean trough level was 41 ng/mL (range, 10–90 ng/mL). Seven of these 13 patients had been treated for 28 days, and for the 4 who were cured the mean peak drug level was 252 ng/mL (range, 90–252 ng/ml); for the other 3, whose conditions only improved, the mean peak level was 196 ng/mL (range, 170–240 ng/ml). There was no significant difference between the mean peak drug levels for the 4 patients who were cured and the 3 patients whose conditions only improved (P = .6402; Fischer's ratio [F] = .247, degrees of freedom [DF] = 6, n = 7; analysis of variance). For the 4 patients who were cured the mean trough drug level was 31 ng/mL (range, 15–40 ng/mL) and for the 3 patients whose conditions only improved the mean trough drug level was 50 ng/mL (range, 30–70 ng/mL). There was no significant difference between the mean trough levels for the 4 patients who were cured and the 3 whose conditions only improved (P = .1767, F = 2.473, DF = 6, n = 7). These data suggest that a high peak level may correlate with cure; however, the small sample size may preclude any demonstration of statistical significance.

Discussion

The WR6026 compound was safe and effective against visceral leishmaniasis in a subpopulation (four of the eight patients treated for 28 days) in this limited clinical-efficacy trial. In addition, it cured one patient who was treated for 14 days. Improvement was seen in all patients' conditions. There appeared to be a correlation between elimination of fever and absence of parasites in the spleen. Efficacy may change in accordance with peak plasma level, area under the concentration curve, and half-life, all of which varied in a previous study of healthy volunteers [31]. The coincidence of a positive stain and a negative culture is possible, because parasites may be visible but not viable.

Blood or tissue drug levels of active metabolites of WR6026 may be important. The monitoring of drug levels in serum during therapy could enable adjustment of dosages and thus possibly increase the proportion of patients for whom such therapy is efficacious.

The highest level of methemoglobin was 5.5% of total hemoglobin, a finding compatible with previously reported data regarding a 30-mg/d, 14-day regimen (2.0%–3.1% methemoglobin) [9]. Four patients had headaches and two had

abdominal symptoms, the only findings attributable to methemoglobinemia. Methemoglobin levels associated with symptoms such as headache and abdominal cramps usually are 10%–20%. The fact that the methemoglobinemia is mild and reversible supports the use of the 8-aminoquinoline at higher doses and for longer periods.

Further studies of this compound, administered at higher doses and in longer regimens, are indicated; drug levels should be determined during treatment for dosage adjustments. Patients who cannot tolerate stibogluconate, pentamidine, or amphotericin (or for whom therapy with these drugs fails) could be treated with 8-aminoquinoline alone or in combination with other drugs.

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- Future Projects

It is our understanding that WR 6026 is currently undergoing further clinical investigation for the treatment of New World Leishmaniasis under a collaborative arrangement between the U. S. Army Medical Research and Materiel Command (USAMRMC) and the Brazilian Ministry of Health. These studies are designed to gain additional information with regards to dose and treatment duration. Once this additional information is obtained, we would propose expanding the clinical experience with WR 6026 to include patients with Old World Leishmaniasis, a well recognized, distinct clinical entity. Such future collaborative efforts between the U. S. Army Medical Research and Materiel Command and the Kenya Medical Research Institute, Nairobi would be greatly welcomed. We would propose this work be conducted as a joint, collaborative project involving co-investigators from USAMRMC permanently assigned to the U. S. Army Medical Research Institute - Kenya with formal visiting scientist status at our institution. Such arrangements would provide for enhanced opportunities for scientists from both institutes to work together and expand our understanding of treatment of this mutually relevant tropical infectious disease.