NEW, ANTIMALARIAL, TRICYCLIC 1,2,4-TRIOXANES: EVALUATIONS IN MICE AND MONKEYS*

GARY H. POSNER, CHANG H. OH, H. KYLE WEBSTER, ARBA L. AGER, JR., AND RICHARD N. ROSSAN

Department of Chemistry, The Johns Hopkins University, Baltimore, Maryland; Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, District of Columbia; Center for Tropical Parasitic Diseases, University of Miami School of Medicine. Miami, Florida; Gorgias Memorial Laboratory, Panama, Panama

Abstract. We have concluded initial preclinical studies with synthetic trioxanes numbered 3–9 and have compared them with artemisinin (numbered 1) using CD-1 mice infected with Plasmodium berghei. Based on their antimalarial effectiveness in mice, two of these synthetic trioxanes were selected for evaluation in Aotus monkeys infected with multidrug-resistant (MDR) P. falciparum. Trioxane numbered 8 (12 and 48 mg/kg), trioxane numbered 9 (12 and 48 mg/kg) and arteether (numbered 2, 48 mg/kg) were administered intramuscularly in three 12-hr doses to A. lemurinus lemurinus (Panamanian owl monkeys) infected with the Vietnam Smith/RE strain of P. falciparum and monitored for parasitemia. Trioxane numbered 8 at 12 mg/kg cleared parasitemia in two monkeys, but recrudescence occurred in one animal. Treatment of the recrudescent infection with 48 mg/kg was curative. Infections in two monkeys treated initially with 48 mg/kg were cured (six-month follow-up). Trioxane numbered 9 produced a similar outcome: 12 mg/kg suppressed parasitemia in two monkeys but was not curative; however, 48 mg/kg cured infections in all four monkeys treated. These preliminary observations show synthetic trioxanes numbered 8 and 9 to be as effective as arteether (numbered 2) against MDR in P. falciparum in the Aotus monkey.

Among infectious diseases, malaria is most widespread. Worldwide, approximately 300 million people are infected, and 1–2 million die each year. In many places, the incidence of malaria is increasing dramatically, in large part because of the growing multidrug resistance of malaria parasites to the standard alkaloidal drugs such as quinine and its synthetic analogs. Therefore, there is an urgent need for new ways to prevent malaria (e.g., with antimalarial vaccines) and to treat malaria with effective drugs.

Important new progress in malaria chemotherapy has been made using the endoperoxide sesquiterpene artemisinin (qinghaosu, numbered 1) (Figure 1) and its derivatives, such as arteether (numbered 2); several of these 1,2,4-trioxanes are being clinically used as antimalarial drugs with impressive activity against multidrug-resistant (MDR) forms of Plasmodium falciparum.

Total synthesis of tetracyclic artemisinin, however, is a complex and expensive process. Recently, we designed and synthesized a series of simpler, tricyclic 1,2,4-trioxanes related structurally to artemisinin. Several of these compounds proved to be extraordinarily potent against P. falciparum in vitro. Based on these promising in vitro results, in vivo tests have been performed.

In this report, we describe preclinical in vivo evaluation of these structurally simplified trioxanes in two standard test systems: CD-1 mice infected with P. berghei and Aotus monkeys infected with MDR P. falciparum.

MATERIALS AND METHODS

Trioxanes numbered 3–9

These tricyclic trioxanes were prepared in six or seven chemical steps from inexpensive and readily available cyclohexanone, as described previously by Posner and others. The trioxane primary alcohol numbered 3 served as a common precursor to alcohol derivatives numbered 4–9.

Plasmodium berghei-mouse test systems

The P. berghei-mouse test system provides a measure of the antimalarial efficacy of candidate

* Dedicated to the memory of the late Dan Klayman, a scholar and friend.
drugs administered subcutaneously in graded doses by the response of *P. berghei* strain KBG 173 malaria parasites in young CD-1 Swiss mice. The activity of a compound is measured by its ability to increase survival time or cure infected mice. A comparison of single versus multiple dosing regimens was performed in CD-1 mice five weeks of age.

**Single-dose test**

Mice in groups of five, each weighing 18 grams, were inoculated intraperitoneally (ip) with 0.02 ml of heparinized donor blood containing $6 \times 10^6$ erythrocytes parasitized with *P. berghei* (KBG-173 strain), which was designated the MM-line. The mice were housed at 75°F for the duration of the experiment. The compounds were administered only once subcutaneously (sc), three days after infection, to mice with a parasitemia of approximately 15%. Each compound was mixed in peanut oil in doses of 640, 160, and 40 mg/kg. Antimalarial activity was determined by following survival times of treated groups compared with infected, nontreated controls (these mice die routinely on day 6 or 7 postinfection). A compound was considered active if it extended the survival time at least twice as long that of the infected, untreated controls (12–14 days postinfection). Mice surviving 60 days postinfection were considered cured.

**Multiple-dose test**

Mice in groups of five, each weighing 18 grams, were inoculated ip with $5 \times 10^6$ erythrocytes parasitized with *P. berghei* (KBG-173 strain), which was designated the P-line. The mice were housed in a room maintained at 75°F for the entire experiment. The compounds were mixed in peanut oil and administered sc once a day for three days beginning on day 3 postinfection. Antimalarial activity was assessed by following survival times of treated mice compared with infected, nontreated controls (these mice die between days 7 and 10 postinfection). A compound was considered active if it extended the survival time twice as long as that of the controls. Mice surviving 60 days postinfection were judged cured.

![Figure 1. Trioxane derivatives used in this study.](image)

**Plasmodium falciparum-Aotus monkey test system**

Laboratory-adapted *A. lemurinus lemurinus* (Panamanian owl monkeys) served as hosts for the preclinical drug evaluations. Animal procedures and husbandry have been described previously. The parasite used for experimental infection was the Vietnam Smith/RE strain of *P. falciparum*, a recrudescent isolate of the Vietnam Smith strain, obtained from an Aotus monkey after administration of chloroquine. The Vietnam Smith strain is resistant to maximum tolerated doses of chloroquine, pyrimethamine, and quinine.

Intravenous inoculation of $5 \times 10^6$ trophozoites from an untreated strain-passage monkey produced parasitemias ranging from $1 \times 10^7$ to $88 \times 10^7$ mm$^3$ on the fifth day postinoculation when treatment was initiated. Drug stock solutions were prepared at appropriate concentrations in sesame oil (a commonly used inert carrier similar to peanut oil) and kept at room temperature. All drugs were administered intramuscularly at selected doses at 8:00 AM, 8:00 PM, and 8:00 AM.

The course of parasitemia was followed daily, beginning on the day after inoculation, by examination of Giemsa-stained blood smears. Infections were considered to be cured if no par-
asites were detected for a minimum of 100 days after the end of treatment. In conducting this research, the investigators adhered to the Guide for the Care and Use of Laboratory Animals (1985), as promulgated by the National Research Council.

RESULTS

Plasmodium berghei-mouse test systems

Single doses of the drug were not effective (Table 1), with only trioxane numbered 8 showing three of five cures at 640 mg/kg; no antimalarial activity was found with a single dosage of 160 mg/kg. Artemisinin (numbered 1) was included as a positive drug control in the single-dose test, with one of five cures at a dosage of 640 mg/kg. One of five cures was also achieved also with trioxane sulfonate ester numbered 7.

Multiple doses of the synthetic trioxanes numbered 3-9 (640 mg/kg) were more effective (Table 2). The most effective antimalarials were trioxane diphenyl phosphate ester numbered 8 and trioxane benzyl ether numbered 9, with five of five and four of five cures, respectively.

Plasmodium falciparum-Aotus monkey test system

Treatments against Vietnam Smith/RF infections are summarized in Table 3. Trioxane numbered 8 at a dose of 12.0 mg/kg cleared parasitemia in each of two monkeys, but a recrudescence occurred in one subject. Retreatment with 48.0 mg/kg and initial treatment with this dose cured infections.

A dose of 12.0 mg/kg of trioxane numbered 9 only suppressed parasitemia in each of two monkeys. Initial treatment with a dose of 48.0 mg/kg as well as retreatment with this dose cured the infection in four of four animals.

Arteether (numbered 2) was included as a
positive drug control. Infections in two Aotus monkeys were cured with a dose of 48.0 mg/kg.\textsuperscript{6} These preliminary results show that synthetic trioxanes numbered 8 and 9 are as effective as arteether (numbered 2) against MDR P. falciparum in the Aotus monkey.

DISCUSSION

Chemotherapy of falciparum malaria is becoming increasingly complex with the spread of MDR forms of the parasite. In Thailand, for example, the decrease in the effectiveness of both mefloquine and halofantrine has resulted in the operational use of artemisinin derivatives, usually in combination with a second drug such as mefloquine (World Health Organization, unpublished data). In addition to the artemisinin derivatives arteether and artesunate, another derivative, arteether,\textsuperscript{17} is currently being developed by the World Health Organization. Although resistance to artemisinin compounds has not been reported from the field, their short circulating half-lives complicate the use of single-dose formulations. A further possible complication in the use of these drugs is their potential toxicity, even though few serious side effects have been documented for arteether and artesunate, which are widely used in China for treatment of acute falciparum malaria. However, potential toxicity with long-term use has not been clinically evaluated. Nevertheless, these derivatives of artemisinin, alone or in combination, represent a significant new chemical class of antimalarials for use against MDR falciparum malaria. New synthetic trioxanes, therefore, represent an important approach for development of artemisinin-like antimalarials.

We have shown here specifically that synthetic trioxanes numbered 8 and 9 are likely to be effective antimalarial agents in humans because 1) the Aotus monkey model is considered to be the best primate model for experimental treatment of blood-induced infections of MDR P. falciparum and 2) trioxanes numbered 8 and 9 are comparable in potency to both arteether and arteether,\textsuperscript{17} and arteether has already been shown to be highly effective in human patients with malaria.\textsuperscript{13,14}

Synthetic trioxanes numbered 8 and 9 therefore offer important, relatively inexpensive, and easily prepared alternatives to artemisinin and its derivatives. Work is continuing on establishing the pharmacokinetics of these trioxanes and on designing and preparing even more desirable analogs (e.g., those having good oral bioavailability).

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Authors’ addresses: Gary H. Posner, Department of Chemistry, The Johns Hopkins University, Baltimore, MD 21218-2685; Chang H. Oh, Department of Chemistry, Inje University, Kimhae 621-749, Republic of Korea; H. Kyle Webster, Walter Reed Army Institute of Research, Washington, DC 20307-5100; Arba L. Ager, Jr., Center for Tropical Parasitic Diseases, Department of Microbiology and Immunology, University of Miami, School of Medicine, Miami, FL 33179; Richard N. Rossman, PSC #02, Box 2209, APO AA 34002.

Reprint requests: Gary H. Posner, Department of Chemistry, The Johns Hopkins University, Baltimore, MD 21218-2685.

REFERENCES

11. Posner GH, Oh CH. Gerena I., Milhous WK.


