Activities of the Tetrahydrofuran Derivative, BA-41,799, Against Plasmodium cynomolgi Infections in Rhesus Monkeys†

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BA-41,799, a tetrahydrofuran derivative that at one time attracted considerable interest as an antimalarial agent because of a combination of structural novelty with activities against infections with Plasmodium berghei and Plasmodium berghei yoelii in mice, has been evaluated for its capacities to effect prophylaxis and radical cure in rhesus monkeys challenged or already infected with sporozoites of the drug-susceptible Ro strain or the pyrimethamine-resistant Ro/PM strain of Plasmodium cynomolgi, and for its capacity to effect suppressive cure of infections with trophozoites of these strains. At doses up to the maximum tolerated, BA-41,799 had only marginal activity against infections with the Ro strain and none against the Ro/PM strain. On the basis of past experiences, the above results suggest that BA-41,799 would have little to offer for prophylaxis, radical cure, or suppression of human infections with Plasmodium vivax.

In 1970 it was reported (5) that BA-41,799 [2-(p-chlorophenyl)-2-(4-piperidyl)-tetrahydrofuran], the most promising representative of a new chemical family of antimalarial agents (3), had substantial activity against infections with trophozoites of Plasmodium berghei in mice. Measured by the dose required to suppress parasitemia by 90%, BA-41,799 was approximately 1/4 as active as chloroquine, 1/2 as active as cycloguanil, and 1/80 as active as pyrimethamine against infections with a drug-susceptible strain of this plasmodium. By the same yardstick, BA-41,799 was essentially as active against infections with a variant strain that was 60-fold resistant to chloroquine as it was against infections with the parent susceptible strain. Against a variant strain that was 800-fold resistant to cycloguanil, it was only one-fifth as active as it was against the parent strain, an indication, albeit quite limited, of cross-resistance to an antimalar. BA-41,799 also had the capacity to prevent infections in mice challenged with sporozoites of a drug-susceptible strain (5) of Plasmodium berghei yoelii. These observations attracted the attention of those responsible for the Malaria Research Program of the United States Department of the Army at the Walter Reed Army Institute of Research. This led to the synthesis of substantial numbers of compounds structurally related to BA-41,799 and to evaluations of their activities against infections with trophozoites of P. berghei in mice and sporozoites of Plasmodium gallinaceum in chickens (1). None of these compounds displayed activities superior to those of BA-41,799 in these test systems (1, 4).

In late September 1966, stimulated by a review of data on the activities of BA-41,799 against infections with diverse strains of P. berghei (made available on 23 August 1966 by Wallace Peters, Ciba Ltd., Basel, Switzerland, and identical with those referred to above [5]), my laboratory undertook evaluations of the prophylactic, radical curative, and blood schizonticidal activities of this tetrahydrofuran against infections with Plasmodium cynomolgi in rhesus monkeys. Special attention was given to the impact of pyrimethamine resistance on these activities. Publication of the results of these studies, less promising in import than those on infections with P. berghei, was deferred, first because of concern of others with patent protection, later because of personal preoccupation with high-priority studies on infections with human plasmodia in owl monkeys (6). They are now being recorded in this brief report so as to complete the picture on what is currently known of the activity and potential of BA-41,799 as an antimalarial agent.

MATERIALS AND METHODS

All of the procedures pertinent to conduct of the experiments included in this report have been detailed previously (11–13, 15). The methodologies described cover: (i) the acquisition of feral monkeys, their conditioning for experimental use, and their maintenance and handling during therapeutic and other experiments; (ii) the routine maintenance of diverse stock strains of P. cynomolgi by serial monkey-to-mosquito-to-monkey passages; (iii) the acquisition of mosquitoes (Anopheles freeborni) heavily infected with sporozoites; (iv) the initiation of infections with sporozoites or trophozoites; (v) the monitoring of parasitic events in both untreated and treated infections, including ascertaining when infections are self-limited or cured; and (vi) the origins of the drug-susceptible Ro and the pyrimethamine-resistant Ro/PM strains used in the current investigation, the courses of untreated sporozoite- and trophozoite-induced infections with these strains, and the responses of such infections to treatment with standard drugs, including chloroquine, pyrimethamine, chloroquine, quinine, and primaquine.

The studies presented here were served by 42 feral juvenile rhesus monkeys (Macaca mulatta), males ranging from 2.6 to 3.9 kg in weight at time of assignment to experiment. Of this total, 26 were inoculated with sporozoites: 13 with the Ro strain and 13 with the Ro/PM strain. The inocula were 7.5 × 10⁵ and 2.3 × 10⁵ sporozoites for challenges with the respective strains. The remaining 16 monkeys were inoculated with trophozoites: eight with the Ro strain and eight with the Ro/PM strain. In both cases, the inoculum was 5 × 10⁵ trophozoites.

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With respect to sources of compounds, BA-41,799 was supplied as the water-soluble hydrochloride salt by Ciba Ltd., Basel, Switzerland. Primaquine and chloroquine were supplied as the water-soluble diphosphates by Eli Lilly & Co., Indianapolis, Ind., and Sterling-Winthrop Research Institute, Rensselaer, N.Y., respectively. Freshly prepared solutions of the individual agents or combinations of primaquine with chloroquine were administered via stomach tube (12) between 8 and 9 a.m., approximately 15 h after access to the afternoon food ration of the previous day. Although the test agents were administered as salts, all doses were calculated as drug base and are referred to as such in text and tables.

To simplify evaluations of the several experiments, their special design features are being set forth along with the results of the studies that they served.

**RESULTS**

**Assessment of prophylactic activity.** All 26 monkeys inoculated with sporozoites were initially committed to evaluation of the prophylactic activity of BA-41,799 with appropriate untreated and treated controls. One subject in each group of 13 served as an untreated control. Three members of each group received primaquine in doses of 1.0 mg/kg 2 h before sporozoite challenge and daily thereafter for the next

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### TABLE 1. Prophylactic activity of BA-41,799

<table>
<thead>
<tr>
<th>P. cynomolgi strain</th>
<th>Regimen</th>
<th>Daily dose (mg/kg)</th>
<th>No. of monkeys fully protected/no. inoculated</th>
<th>Days from inoculation to patenty</th>
<th>Delay to patenty (days) ret controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ro</td>
<td>None (control)</td>
<td>0</td>
<td>0/1</td>
<td>8</td>
<td></td>
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<tr>
<td></td>
<td>BA-41,799</td>
<td>1.0</td>
<td>0/3</td>
<td>8, 8, 8</td>
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<tr>
<td></td>
<td></td>
<td>5.0</td>
<td>0/3</td>
<td>9, 9, 9</td>
<td>1, 1, 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.0</td>
<td>0/3</td>
<td>17, 17, 19</td>
<td>9, 9, 11</td>
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<tr>
<td>Primaquine</td>
<td>1.0</td>
<td>3/3</td>
<td>Not patent</td>
<td></td>
<td></td>
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<tr>
<td>Ro/PM</td>
<td>None (control)</td>
<td>0</td>
<td>0/1</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BA-41,799</td>
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<td>0/3</td>
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<tr>
<td></td>
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<td>0/3</td>
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<td></td>
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<td>25.0</td>
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<tr>
<td>Primaquine</td>
<td>1.0</td>
<td>3/3</td>
<td>Not patent</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The inocula were 7.5 x 10^7 and 2.3 x 10^7 sporozoites for the Ro and Ro/PM strains, respectively.
* These doses were administered 2 h before sporozoite inoculation and once daily thereafter for 7 consecutive days.
* Thick blood films were negative for 63 consecutive days after inoculation. Each subject was rechallenged with 5 x 10^7 sporozoites on day 63; parasitemias became patent 8 days later, indicating full susceptibility to infection.

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### TABLE 2. Radical curative activity of BA-41,799

<table>
<thead>
<tr>
<th>Primary curative regimen</th>
<th>Compound</th>
<th>Daily dose (mg/kg)</th>
<th>No. of infections treated</th>
<th>Response</th>
<th>Days to relapse after chloroquine treatment</th>
<th>No. of infections cured</th>
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<tbody>
<tr>
<td>P. cynomolgi strain</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ro</td>
<td>BA-41,799</td>
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<td>3</td>
<td>0</td>
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<td>5, 7, 7</td>
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<tr>
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<td>9</td>
<td>9</td>
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<tr>
<td>Ro/PM</td>
<td>BA-41,799</td>
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<td>3</td>
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</tr>
<tr>
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<td>3</td>
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<tr>
<td>Primaquine*</td>
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<td>9</td>
<td>9</td>
<td>9</td>
<td>NA</td>
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</tr>
</tbody>
</table>

* These doses were administered once daily for 7 consecutive days.
* The relapse interval was measured from the day of the last dose of test compound.
* Chloroquine was administered in daily doses of 5.0 mg/kg for 7 consecutive days, beginning the day after the last dose of BA-41,799 for infections with persisting parasitemias and when a parasitemia of approximately 10 parasites per 10^6 erythrocytes was attained in a relapse.
* NA, Not applicable.
* Doses of 5.0 mg of chloroquine per kg were administered concomitantly with primaquine in this regimen.
* None of the infections in this group relapsed within a 90-day post-dosage observation period.
FIG. 1. Responses of infections with trophozoites of the Ro and RolPM strains to treatment with daily doses of 25.0, 50.0, or 100.0 mg of BA-41,799 per kg. Monkeys were infected with the Ro strain (○ and △) or the RolPM strain (□ and ▽). T, seven-day treatment interval; R, retreated with a different agent; C, cured; †, died.

7 days. The capacity of this primaquine regimen to prevent infections with the Ro and RolPM strains is well established (14, 16). The remaining nine members of each major group, in three equally sized subgroups, received BA-41,799 in doses of 1.0, 5.0, or 25.0 mg/kg once daily for 8 consecutive days starting 2 h before sporozoite challenge. With dosage calculated on comparable surface area-body weight ratios (2), these doses, delivered to a 3.0-kg monkey, covered the range of doses required to suppress infections with trophozoites of P. berghei and prevent infections with sporozoites of P. berghei yoelii in 20-g mice. The 25.0-mg/kg dose also approached what was thought to be the maximum dose tolerated by the rhesus monkey (personal communication, W. Peters).

Routine monitoring of parasitic events in the prophylactic study was initiated on day 7 after sporozoite challenge and repeated daily throughout the evaluation period. As the results summarized in Table 1 show, BA-41,799 provided no protection against infections with sporozoites of the Ro strain at doses of 1.0 or 5.0 mg/kg and effected only a 9- to 11-day delay in onset of parasitemia at a dose of 25.0 mg/kg. This summary also shows that BA-41,799 was completely without effect on development of infections with the RolPM strain. Thus, at all dose levels, from 1.0 to 25.0 mg/kg, parasitemias became patent on day 8 after sporozoite challenge, the same time as in the untreated control. In keeping with previous experience (14, 16), primaquine at a dose of 1.0 mg/kg provided full protection against infections with sporozoites of both the Ro and RolPM strains.

Assessment of radical curative activity. The capacity of BA-41,799 to effect radical cure was evaluated against the infections that developed in the 18 monkeys who had received this tetrahydrofuran in the prophylactic regimen with little or no benefit. Doses of 1.0, 5.0, or 25.0 mg/kg were
administered once daily for 7 days to subgroups of three monkeys formed by inclusion of one subject from each of the subgroups in the prophylactic experiment. Treatment was initiated on day 3 or 4 after the onset of parasitemy, when parasitemias of 10 to 50 parasites per 10⁴ erythrocytes prevailed. Administration of BA-41,799 was followed by dosage with chloroquine, 5.0 mg/kg daily for 7 days, whenever parasitemia persisted or upon relapse after apparent clearance of parasitemia. This was done to ensure that the effects of BA-41,799 on the exoerythrocytic stages were not being masked by persisting parasitemia. All infections that relapsed subsequent to this course of chloroquine were retreated with the potentially curative (10, 14, 16) combination of primaquine and chloroquine at doses of 0.75 and 3.0 mg/kg, respectively, once daily for 7 days.

The results of the above study (Table 2) show that, over the range of doses tested, BA-41,799 did not effect cure of infections with either the Ro or Ro/PM strain. Doses of 25.0 mg/kg led to temporary clearance of parasites of the Ro strain but not those of the Ro/PM strain. In only one subject, a monkey infected with the Ro/PM strain, was there a significant lengthening of the interval between the end of treatment with chloroquine and relapse, a phenomenon for which no explanation can be offered. As would have been expected (10, 14, 16), all 18 infections not cured by BA-41,799 were cured by the primaquine-chloroquine regimen.

Assessment of blood schizonticidal activity. Of the 16 monkeys inoculated with trophozoites (8 with the Ro strain and 8 with the Ro/PM strain), two infected with each strain served as untreated controls (Fig. 1D). Treatment of the remaining 12 monkeys was initiated on day 7 after inoculation, when parasitemias of 9 to 36 parasites per 10⁴ erythrocytes prevailed. Pairs of monkeys infected with each strain were scheduled to receive doses of 25.0, 50.0, or 100.0 mg of BA-41,799 per kg once daily for 7 days. The limited control of parasitemia attained with doses of 25.0 mg/kg in the radical cure study led to the inclusion of 50.0- and 100.0-mg/kg doses in the current study, with full recognition that they might evoke untoward reactions of considerable severity.

Doses of 25.0 and 50.0 mg of BA-41,799 per kg effected slow reductions in the parasitemias of monkeys infected with the Ro strain, leading to clearances on the day after the last dose, followed by recrudescences 4 days later (Fig. 1A and B). These doses were without effect on the parasitemias of monkeys infected with the Ro/PM strain. Doses of 100.0 mg/kg were lethal for three of four recipients, one of the two infected with the Ro strain and both of those infected with the Ro/PM strain (Fig. 1C). These subjects died approximately 28 h after the second, third, or sixth dose, exhibiting severe depression, somnolence, and collapse of peripheral circulation 16 to 30 h antemortem. The infection of the fourth recipient of the 100.0-mg/kg dose, a subject infected with the Ro strain, was cured.

DISCUSSION

There is little that can be added to the above descriptions of the failure of BA-41,799 to effect prophylaxis, radical cure, or suppressive cure of infections with either the drug-susceptible Ro strain or the pyrimethamine-resistant Ro/PM strain of P. cynomolgi. These negative results dim prospects for successful use of this tetrahydrofuran in the treatment of human malaria, its interesting and substantial accomplishments in mice infected with P. berghei or P. berghei yoelli notwithstanding. The past predictive records of these rodent infections, especially as they pertain to prophylaxis and cure of human infections with Plasmodium vivax, are poor (9), whereas those of infections with P. cynomolgi in rhesus monkeys are excellent (9). It can be argued that infections with trophozoites of P. berghei predict reasonably well for infections with Plasmodium falciparum (8). Even so, indirect evidence in that area is not promising. WR-179,305 (4-[1-(p-chlorophenyl)-1-ethoxyethyl]-piperidine), a product of the Department of the Army chemical synthesis program and a close chemical relative of BA-41,799 that equalled the latter agent in activity against infections with sporozoites of P. gallinaceum in chickens and trophozoites of P. berghei in mice (4), effected no more than temporary clearance of parasitemia when administered at maximum tolerated doses to owl monkeys infected with trophozoites of the pyrimethamine-susceptible, chloroquine-resistant Oak Knoll strain of P. falciparum (7; unpublished data).

In view of the negative results of this study, one could argue that investigation of the tetrahydrofurans as antimalarial agents could well be terminated at this point. Such a position seems premature. It would seem wiser to recognize that infections with P. berghei have little to offer to evaluations of the potentials of representatives of this class of compounds and turn to a more predictive system for monitoring the products of systematic chemical syntheses. As pointed out previously (9), when manipulated judiciously, the P. cynomolgi-rhesus monkey model is neither so costly nor unwieldy that it cannot be used for drug screening, even though it is more expensive and demanding of personnel attention than the P. berghei-mouse model.

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LITERATURE CITED


