In Vitro and In Vivo Antimalarial Activities of T-2307, a Novel Arylamidine

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T-2307, a novel arylamidine, has been shown to exhibit broad-spectrum antifungal activities against clinically significant pathogens. Here, we evaluated the in vitro and in vivo antimalarial activity of T-2307. The 50% inhibitory concentrations (IC50s) of T-2307 against Plasmodium falciparum FCR-3 and K-1 strains were 0.47 and 0.17 μM, respectively. T-2307 at 2.5 to 10 mg/kg of body weight/day exhibited activity against blood stage and liver stage parasites in rodent malaria models. In conclusion, T-2307 exhibited in vitro and in vivo antimalarial activity.

Malaria, caused by protozoan parasites of the genus Plasmodium, is one of the world’s leading killer infectious diseases. There are an estimated 200 to 300 million new cases of the disease each year worldwide, and 0.8 to 1 million deaths (5, 15). Many of these deaths occur in children and are the result of severe and cerebral malaria caused by Plasmodium falciparum, the most pathogenic of the four species that infect humans (5).

In the absence of a vaccine for malaria and in view of widespread resistance of the parasites to antimalarial drugs in current use, new agents are urgently needed to combat malaria (4).

We had previously reported that T-2307, a novel arylamidine, exhibits broad-spectrum in vitro and in vivo antifungal activity against clinically significant pathogens (7, 8, 16). The analogous arylamidine derivatives, such as pentamidine and DB75, exhibit antiprotozoan activities against Plasmodium, Trypanosoma, and Leishmania (2, 3, 14).

Accordingly, T-2307, similar to pentamidine and DB75, is expected to exhibit antiprotozoan activity. In the present study, we investigated the in vitro and in vivo antimalarial activity of T-2307.

The in vitro antimalarial activity of T-2307 against P. falciparum was examined. Parasite cultures were maintained in human erythrocytes suspended at 5% hematocrit in RPMI 1640 containing 0.5% L-glutamine, and 50 mg of hypoxanthine per liter. After the parasites had been synchronized to the ring stage by sorbitol lysis (6), T-2307 and reference agents were added to the synchronized parasite culture (ring stage, >90%, and parasitemia, 0.5%) in a 96-well plate. The plate was incubated for one intraerythrocytic life cycle (FCR-3, 40 h, and K-1, 48 h) at 37°C under a gas mixture of 5% O2 and 5% CO2. Parasite growth was measured by SYBR green I-based fluorescence assay, and IC50s were calculated using WinNonlin software.

Next, morphological effect (%) was calculated by assessing the morphology of 50 parasites at each stage. The definition of the morphological effect is given in detail in the Fig. 1b legend. These compounds had no effect on the morphology in ring stage parasites (Fig. 1a).

The stage-specific activities of T-2307 and pentamidine against P. falciparum K-1 strain were assessed by evaluating a morphological effect. The synchronized ring, trophozoite, and schizont stages were exposed to approximately the 5 times the IC50 of T-2307 and pentamidine against the K-1 strain (800 nM and 400 nM, respectively) for 12 h.

The morphology of the parasites treated with these agents was compared with that of untreated parasites by using microscopy. T-2307 and pentamidine caused altered morphologies, such as condensation in trophozoite stage parasites and abnormal cell division in schizont stage parasites (Fig. 1a). On the other hand, these compounds had no effect on the morphology in ring stage parasites (Fig. 1a).

The IC50s of T-2307 and reference agents against P. falciparum FCR-3 and K-1 in vitro are shown in Table 1. The IC50s of T-2307 against FCR-3 and K-1 strains were 0.47 and 0.17 μM, respectively, indicating that T-2307 exhibited no cross-resistance against chloroquine.

The stage-specific activities of T-2307 and pentamidine against P. falciparum K-1 strain were assessed by evaluating a morphological effect. The synchronized ring, trophozoite, and schizont stages were exposed to approximately the 5 times the IC50 of T-2307 and pentamidine against the K-1 strain (800 nM and 400 nM, respectively) for 12 h.

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Next, morphological effect (%) was calculated by assessing the morphology of 50 parasites at each stage. The definition of the morphological effect is given in detail in the Fig. 1b legend. These results indicated that both T-2307 and pentamidine exhibited potent activity against mature stages, especially the schizont stage (Fig. 1b).

TABLE 1 IC50s of T-2307 and reference agents against P. falciparum FCR-3 and K-1 in vitro

<table>
<thead>
<tr>
<th>Compound</th>
<th>FCR-3</th>
<th>K-1</th>
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<tbody>
<tr>
<td>T-2307</td>
<td>0.47 ± 0.01</td>
<td>0.17 ± 0.00</td>
</tr>
<tr>
<td>Pentamidine</td>
<td>0.18 ± 0.00</td>
<td>0.083 ± 0.003</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>0.051 ± 0.002</td>
<td>1.4 ± 0.0</td>
</tr>
</tbody>
</table>

*IC50s, chloroquine, and pentamidine were added to the 0.5% parasitemia culture (>90% ring stage). Then, the culture was incubated for one intraerythrocytic life cycle (FCR-3, 40 h; K-1, 48 h) at 37°C under a gas mixture of 5% O2 and 5% CO2. Parasite growth was assessed by SYBR green I-based fluorescence assay, and IC50s were calculated using WinNonlin software.

*The results are expressed as means ± standard errors of the means of duplicate experiments.

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The in vivo antimalarial activities of T-2307 and pentamidine were evaluated in mice infected with *Plasmodium vinckei* PV strain. It has been reported that *P. vinckei* infection was a good murine model of *falciparum* malaria for testing the in vivo activity of diamidine derivatives (1). BALB/c mice (male, 5 to 6 weeks of age) were intravenously injected with 0.2 ml of the parasitized erythrocytes (1 × 10⁸ cells/mouse), and thereafter, T-2307 or a reference agent was subcutaneously administered once a day for 8 days beginning 2 h postinfection. Animal experiments in this study were carried out in compliance with the Guide for Animal Experimentation at Obihiro University of Agriculture and Veterinary Medicine.

As shown in Fig. 2a, the parasitemia in the mice administered T-2307 at 2.5 mg/kg of body weight/day decreased significantly compared to that in the control group. At the same dose, T-2307 exhibited antimalarial activity superior to that of pentamidine,
TABLE 2 | in vivo antimalarial activity of T-2307 against liver stage parasites of P. berghei ANKA

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. infected/no. injected</th>
<th>Prepatent period (days)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>5/5</td>
<td>6.0 ± 0.3</td>
</tr>
<tr>
<td>T-2307</td>
<td>4/4</td>
<td>7.0 ± 0.0*</td>
</tr>
<tr>
<td>Primaquine</td>
<td>0/4</td>
<td>&gt;7*</td>
</tr>
</tbody>
</table>

*a* Mice were intravenously injected with a sporozoite suspension (1 × 10⁵ cells/mouse). T-2307 at 2.5 mg/kg was subcutaneously administered 4 times a day for 4 days and primaquine at 50 mg/kg was intraperitoneally administered once a day for 4 days beginning 2 days before infection. Parasitemia was determined between 5 and 7 days after infection.

*b* No. infected, number of mice that developed >0.1% parasitemia at the blood stage until 7 days after infection; no. injected, number of mice injected with sporozoites.

*c* Prepatent period, number of days between sporozoite infection and detection of 0.1% parasitemia at the blood stage. The results are expressed as the means ± standard errors of the means of 4 of 5 experiments. The prepatent period of the T-2307-treated group and that of the control group were compared by paired t test using SAS analytical software, release 8.2 (SAS Institute Japan Ltd., Tokyo, Japan).

*d* P < 0.05.

*e* The prepatent period could not be calculated because parasite was not detected. Therefore, statistical analysis could not be performed.

while chloroquine administration at 5 mg/kg/day resulted in a decrease in parasitemia to an undetectable level.

Similar results were obtained when the *in vivo* activity of T-2307 was evaluated in the mice infected with *Plasmodium berghei* ANKA strain or *Plasmodium chabaudi* AI strain. In both models, parasitemia in the mice administered T-2307 at 0.25 and 2.5 mg/kg/day decreased significantly compared to that in the control group, with a greater decrease at 2.5 mg/kg/day (Fig. 2b and c), while chloroquine at 5 mg/kg/day decreased parasitemia to a nearly undetectable level.

It has been observed that pentamidine is almost inactive against infection with *P. berghei* but is effective against *P. vinckei* infection (1). In contrast, T-2307 showed antimalarial activity *in vivo* against not only *P. vinckei* but also *P. berghei* and *P. chabaudi*. This suggests that there is a difference in the spectrum of activity between T-2307 and pentamidine in these rodent malaria models.

The *in vivo* antimalarial activity of T-2307 against the liver stage was assessed by evaluating a prepatent period for the blood stage parasites in the mice after the sporozoite inoculation (11). Sporozoites were isolated from the salivary glands of *P. berghei*-infected *Anopheles stephensi* mosquitoes. BALB/c mice (male, 6 weeks of age) had been intravenously injected with 0.2 ml of the sporozoite suspension (1 × 10⁵ cells/mouse). T-2307 at 2.5 mg/kg was subcutaneously administered 4 times a day for 4 days, and primaquine at 50 mg/kg was intra-peritoneally administered once a day for 4 days, both beginning 2 days before infection. Parasitemia was observed between 5 and 7 days after infection. The prepatent period was defined as the number of days between sporozoite infection and detection of 0.1% parasitemia of the blood stage parasites. The prepatent period of mice administered T-2307 was found to be approximately 1 day longer than that of the control group, indicating that T-2307 decreased the liver stage parasite burden by approximately 10-fold compared with that of the control group (17), while no parasites were detected in mice treated with 50 mg/kg of primaquine (Table 2).

It has been shown that pentamidine targets the hemoglobin degradation pathway and that DB75 targets the nucleus (9, 13). Although several hypotheses have been proposed, the precise mechanism of diamidine compounds against the malaria parasites remains unclear. We had previously reported that T-2307 disrupts mitochondrial function in yeast (10). However, the mechanism of action of T-2307 against the malaria parasites also remains unclear at present. On the basis of the difference in the spectrum of activity of T-2307 and pentamidine against the rodent malaria models, the mechanism of action of T-2307 against malaria parasites may be different from that of pentamidine. Further studies will be required to elucidate the mechanism of action of T-2307.

In the present study, we showed that T-2307 exhibited *in vitro* and *in vivo* antimalarial activities against *P. falciparum* and the rodent malaria parasites, respectively.

Further research is now required to confirm the characteristics of efficacy and safety of T-2307 and its derivative compounds as new antimalarial agents.

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REFERENCES