Antimalarial Activity of a New Stilbene Glycoside from *Parthenocissus tricuspidata* in Mice

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A novel stilbene glycoside [piceid-(1→6)-β-D-glucopyranoside; PBG] from *Parthenocissus tricuspidata* was tested in vivo against *Plasmodium berghei*. PBG exhibited significant blood schizontocidal activity in a 4-day early infection, a repository evaluation, and an established infection, with a significant mean survival time comparable to that obtained with the standard drug, chloroquine (5 mg · kg⁻¹ · day⁻¹). Antimalarial activity of PBG was assessed as described by Peters (7). For this, a further six groups of mice (again with five per group) were, respectively, treated preinfection with 0.2-ml oral doses of PBG in water (at 5, 10, or 25 mg · kg⁻¹ · day⁻¹), pyrimethamine in water (at 1.2 mg · kg⁻¹ · day⁻¹), or pure distilled water for 4 consecutive days (days 0 to 3). On day 4, the mice were inoculated with *P. berghei* (as in the 4-day test) and on day 7 (72 h postinfection) their parasitemias were assessed.

Malaria is the major tropical disease due to parasites and worldwide causes significant morbidity and mortality (10). Currently, there is a dramatic resurgence of the disease as a result of the increasing resistance of the vectors to insecticides and the progressive resistance of the causative parasites, particularly *Plasmodium falciparum*, to antimalarial drugs. Although the drug artemisinin is currently the most powerful weapon in the global war against chloroquine-resistant malaria, sooner or later resistance to artemisinin may develop (2). There is therefore an urgent need to discover and develop new effective and safe drugs for the treatment of this disease (1).

Isolation of PBG from plant material has been reported previously (9). As experimental hosts, we used 8-week-old outbred male ICR mice (body weight, 20 ± 2 g) purchased from Japan SLC (Hamamatsu, Japan). The animals were housed in standard cages, provided with standard feed and water ad libitum, and acclimatized for 10 days prior to the experiments. The malarial parasites used were of a chloroquine-sensitive strain of *P. berghei* (ATCC 50175; American Type Culture Collection, Manassas, VA), which had been maintained by serial blood passage in mice. The schizontocidal activity of PBG on early *P. berghei* infection was evaluated in a 4-day test (5). In this test, blood from an infected donor mouse was diluted with isotonic saline to yield an inoculum containing 5 × 10⁷ infected erythrocytes · ml⁻¹. Thirty ICR mice were then (at approximately 07.30 h) each inoculated intraperitoneally with 0.2 ml of the inoculum before being randomly divided into six treatment groups of five mice each. On each morning (08:00 to 09:00 h) from the day of infection (day 0) to 3 days later (day 3), mice in the respective groups were orally administered PBG (at 5, 10, or 25 mg · kg⁻¹ · day⁻¹) in 0.2 ml distilled water, chloroquine (at 5 mg · kg⁻¹ · day⁻¹) in 0.2 ml distilled water, or 0.2 ml distilled water. On day 4, 24 h after the last treatment, a thin smear was made from the tail blood of each mouse and stained with Giemsa’s stain in order to determine the percent parasitemia (by determining the number of parasitized erythrocytes per 200 erythrocytes in random fields). For each group of mice treated with PBG or chloroquine, the mean percent chemosuppression was then calculated as 100[ (A – B)/A], where A is the mean percent parasitemia of the mice treated only with distilled water (the negative controls) and B is the mean parasitemia in the test group. The possible “repository” activity of PBG was assessed as described by Peters (7). For this, a further six groups of mice (again with five per group) were, respectively, treated preinfection with 0.2-ml oral doses of PBG in water (at 5, 10, or 25 mg · kg⁻¹ · day⁻¹), pyrimethamine in water (at 1.2 mg · kg⁻¹ · day⁻¹), or pure distilled water for 4 consecutive days (days 0 to 3). On day 4, the mice were inoculated with *P. berghei* (as in the 4-day test) and on day 7 (72 h postinfection) their parasitemias were assessed. In order to evaluate schizontocidal activity in an established infection (8), the 4-day test was repeated but modified so that the first treatment did not take place until 72 h after the mice had been infected; the mice were treated daily for 5 (not 4) days, and parasitemia was evaluated on each day of treatment. In addition, mortality and weight changes in the mice were followed up to 30 days postinfection (day 29) and the day 29 parasitemias of the survivors were evaluated. The median 50% lethal dose (LD₅₀) of PBG, when administered intraperitoneally, was also determined by using uninfected ICR mice and the method of Lorke (6). Data were compared by using Student’s t tests.

In both the 4-day test and the test of repository activity, oral PBG produced dose-dependent chemosuppression (Table 1), with even the lowest dose tested (5 mg · kg⁻¹ · day⁻¹) producing significant reductions in parasitemia (*P* < 0.05). The high-

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The highest dose of PBG tested (25 mg·kg\(^{-1}\)·day\(^{-1}\)) was not as effective as the lowest dose tested (5 or 10 mg·kg\(^{-1}\)·day\(^{-1}\)) and did not achieve the level of chemosuppression seen with the drugs used as positive controls—chloroquine at 5 mg·kg\(^{-1}\)·day\(^{-1}\) and pyrimethamine at 1.2 mg·kg\(^{-1}\)·day\(^{-1}\) (Table 1). The in vivo antimalarial activity of PBG is presented in Fig. 2. The procedure followed was that of the classical 4-day suppressive test of Peters (7), in which the test compound is administered to malaria-infected mice for 4 days. This procedure is proposed by the WHO as the first-line primary screen for in vivo testing of potential antiplasmodial compounds. All of the mice administered PBG exhibited a gradual decrease in body weight from day 7; however, this weight loss persisted for only a few days, after which the mice exhibited daily gains in weight. In contrast, mice in the negative control group lost weight each day throughout the follow-up period. One of the five mice administered 10 mg PBG·kg\(^{-1}\)·day\(^{-1}\) died before day 29 (on day 20), as did two of the five mice treated with 5 mg·kg\(^{-1}\)·day\(^{-1}\) (on days 17 and 23) and all five of the negative control mice (on days 11 to 18). None of the mice administered PBG in the tests of antimalarial activity exhibited any signs of acute toxicity. In the toxicity tests, all of the mice administered PBG at 5 to 500 mg·kg\(^{-1}\)·day\(^{-1}\) died before day 29 (all of which had been treated with PBG or chloroquine) were aparasitic. In the tests of activity against an established infection, the highest tested doses of PBG appeared as effective as chloroquine in terms of day 7 parasitemia (Fig. 2) and day 29 survival. At 5, 10, or 25 mg·kg\(^{-1}\)·day\(^{-1}\), parasitemia was reduced on day 3 by 6.25, 18.75, or 34.37% and on day 7 by 73.6, 86.3, or 89.4% (\(P = 0.0004\) versus the negative control) and survival was increased by 96 h.

In the toxicity tests, all of the mice administered PBG at 5 to 500 mg·kg\(^{-1}\)·day\(^{-1}\) exhibited insignificant signs of toxicity, ranging from writhing and gasping (LD\(_{50}\) of >500 mg·kg\(^{-1}\)) to a decreased respiratory rate, decreased limb tone, and death. The LD\(_{50}\) was calculated to be >500 mg·kg\(^{-1}\), however, and none of the mice administered PBG in the tests of antimalarial activity exhibited any signs of acute toxicity.

The present results indicate that PBG possesses useful blood

**TABLE 1.** Blood schizontocidal activity of PBG against *P. berghei* in mice

<table>
<thead>
<tr>
<th>Compound or drug</th>
<th>Dose(^a)</th>
<th>Four-day test(^b)</th>
<th>Repository activity(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% Parasitemia</td>
<td>% Suppression</td>
</tr>
<tr>
<td>PBG</td>
<td>5</td>
<td>31.3 ± 0.23(^c)</td>
<td>44.3</td>
</tr>
<tr>
<td>PBG</td>
<td>10</td>
<td>22.4 ± 0.21(^b)</td>
<td>60.2</td>
</tr>
<tr>
<td>PBG</td>
<td>25</td>
<td>15.2 ± 0.12(^b)</td>
<td>73.2</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>5</td>
<td>6.2 ± 0.15(^b)</td>
<td>89.3</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>1.2</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Control (DW(^e))</td>
<td>0.2</td>
<td>56.3 ± 0.16</td>
<td>ND</td>
</tr>
</tbody>
</table>

\(^a\) Drug doses are in milligrams per kilogram per day, and the control DW dose is in milliliters.

\(^b\) Data are expressed as means ± standard deviations for five animals per group.

\(^c\) \(P < 0.05\) when compared with the control.

\(^d\) DW, distilled water.

\(^e\) ND, not determined.

**FIG. 1.** Structure of PBG isolated from *P. tricuspidata.*

**FIG. 2.** Effect of PBG on established *P. berghei* infections in mice. The experimental hosts were infected on day 0 and treated orally with distilled water; PBG at 5, 10, or 25 mg·kg\(^{-1}\)·day\(^{-1}\); or chloroquine at 5 mg·kg\(^{-1}\)·day\(^{-1}\) on days 3 to 7. Data shown are the mean ± the standard deviation for five mice per condition. The test was performed as described by Ryley and Peters (8). Mice were inoculated with trophozoites and treated 2 h later with the drug or the vehicle at the indicated doses by the intraperitoneal route. The injection was repeated daily for a total of 4 days.

30.0, 30.0, and 16 days, respectively. The mice still alive on day 29 (all of which had been treated with PBG or chloroquine) were aparasitic. In the tests of activity against an established infection, the highest tested doses of PBG appeared as effective as chloroquine in terms of day 7 parasitemia (Fig. 2) and day 29 survival. At 5, 10, or 25 mg·kg\(^{-1}\)·day\(^{-1}\), parasitemia was reduced on day 3 by 6.25, 18.75, or 34.37% and on day 7 by 73.6, 86.3, or 89.4% (\(P = 0.0004\) versus the negative control) and survival was increased by 96 h.

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The present results indicate that PBG possesses useful blood
schizontocidal activity when used at doses that cause no marked toxicity in mice. Although the mechanism of action of this compound has not been elucidated, some plants and/or plant compounds are known to exert antimalarial activity by either causing an elevation of erythrocytic oxidation (3) or inhibiting protein synthesis (4). PBG clearly merits further investigation.

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REFERENCES