Antimalarial activity of a new stilbene glycoside from *Parthenocissus tricuspidata* in mice

Won-Hwan Park¹, Sung Jae Lee², Hyung-In Moon³

Department of Diagnostics and Cardiovascular Medical Research Center, College of Korean Medicine, Dongguk University, Gyeong-Ju 780-714, South Korea¹; Department of Integrative Medicine, College of Medicine, Korea University, Anam-dong 5ga, Seongbuk-gu, Seoul 136-705, South Korea²; Department of Neurology and Inam Neuro Science Research Center, Wonkwang University Sanbon Medical Center, Kyunggi-Do 435-040, South Korea³

Correspondence: Dr. Hyung In Moon (Ph.D.) Inam Neuro Science Research Center, Department of Neurology, Wonkwang University Sanbon Medical Center, Sanbon-dong, Gunpo-City, Kyunggi-Do 435-040, South Korea

Fax: (+82)-313902414.
E-mail address: himoon@wonkwang.ac.kr
Abstract

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 4-d early infection, repository evaluation, and established infection, with a significant mean survival time comparable to that of the standard drug, chloroquine (5 mg·kg⁻¹·d⁻¹).

Keywords: Parthenocissus tricuspidata; Plasmodium berghei; piceid-(1→6)-β-D-glucopyranoside; antimalarial

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 power 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). Recently (9), methanol extracts of Parthenocissus tricuspidata (Vitaceae) were found to have potential antiplasmodial activity against P. falciparum in vitro, this activity being largely attributable to a piceid-(1→6)-β-D-glucopyranoside (PBG; Fig. 1). In the present study, PBG was also found to have potential antimalarial activity in vivo when tested against Plasmodium berghei in mice.
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 d 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 × $10^7$ infected erythrocytes ml$^{-1}$. Thirty ICR mice were then (at approximately 07.30 hours) each inoculated intraperitoneally with 0.2 ml of the inoculum before being randomly divided into six equal treatment groups of five mice each. On each morning (at 08:00–09:00 hours) from the day of infection (day 0) to 3 d later (day 3), mice in the respective groups were orally administered PBG in 0.2 ml distilled water (at 5, 10, or 25 mg PBG·kg$^{-1}$·d$^{-1}$), chloroquine in 0.2 ml distilled water (at 5 mg chloroquine·kg$^{-1}$·d$^{-1}$), 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 percentage parasitemia (by counting the number of parasitized erythrocytes per 200 erythrocytes in random fields). For each group of mice treated with PBG or chloroquine, the mean percentage chemosuppression was then calculated as 100[(A–B)/A], where A is the mean percentage 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 using the method described by Peters (7). For this, a further six
groups of mice (again with five mice/group) were respectively treated pre-infection with
0.2 ml oral doses of PBG in water (at 5, 10, or 25 mg·kg⁻¹·d⁻¹), pyrimethamine in water (at
1.2 mg·kg⁻¹·d⁻¹), or pure distilled water, for 4 consecutive days (days 0–3). On day 4, the
mice were inoculated with *P. berghei* (as in the 4-d test) and on day 7 (72 h post-infection)
their parasitemias were assessed. In order to evaluate the schizontocidal activity in
established infection (8), the 4-d 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)-d and parasitemias were evaluated on each day of the treatment. In addition,
mortality and weight changes in the mice were followed up to 30 d post-infection (day 29),
and the day-29 parasitemias of the survivors were evaluated. The median lethal dose (LD₅₀)
of PBG, when administered intraperitoneally, was also determined, using uninfected ICR
mice and the method of Lorke (6). Data were compared using Student’s *t*-tests.

In both the 4-d 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·d⁻¹)
producing significant reductions in parasitemia (*P* < 0.05). The highest dose of PBG tested
(25 mg·kg⁻¹·d⁻¹) was not as effective as the lowest dose tested (5, or 10 mg·kg⁻¹·d⁻¹) and
did not achieve the level of chemosuppression seen with the drugs used as positive controls
- chloroquine at 5 mg·kg⁻¹·d⁻¹ or pyrimethamine at 1.2 mg·kg⁻¹·d⁻¹ (Table 1). The *in vivo*
antimalarial activity of PBG is presented in Fig. 2. The procedure followed was that of the
classical 4-d suppressive test of Peters (7) in which the test compound is administered to
the malaria-infected mice for 4 d. This procedure is proposed by the WHO as the first-line
primary screen for in vivo testing of potential antiplasmodial compounds. All 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$^{-1}$·d$^{-1}$ died before day 29 (on day 20), as did two of the five mice treated with 5 mg·kg$^{-1}$·d$^{-1}$ (on days 17 and 23), and all five of the negative control mice (on days 11–18). None of the mice administered the highest dose of PBG and none of those administered chloroquine died during the follow-up period. The mean survival times of the mice administered 5, 10, or 25 mg PBG·kg$^{-1}$·d$^{-1}$, chloroquine, and water were 25.0, 27.0, 30.0, 30.0, and 16 d, respectively. The mice still alive on day 29 (all of which had been treated with PBG or chloroquine) were aparasitemic. In the tests of activity against established infection, the highest tested doses of PBG appeared as effective as chloroquine in terms of the day-7 parasitemias (Fig. 2) and the day-29 survival. At 5, 10, or 25 mg·kg$^{-1}$·d$^{-1}$, parasitemia was reduced on D3 by 6.25, 18.75 or 34.37% and on D7 by 73.6, 86.3 or 89.4 % (with a $P = 0.0004$ vs. negative control) and survival was increased by 96 hours.

In the toxicity tests, all the mice administered PBG at 5–500·mg·kg$^{-1}$ exhibited insignificant signs of toxicity, ranging from writhing and gasping (LD$_{50}$ of >500 mg·kg$^{-1}$) to 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 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 either by causing elevation of erythrocytic oxidation (3) or inhibiting protein synthesis (4). PBG clearly merits further investigation.

ACKNOWLEDGMENTS
This work was supported by the MRC program of MOST/KOSEF (grant #: R13-2005-013-01000-0), Korea.

REFERENCES


Fig. 1. Structures of piceid-(1→6)-β-D-glucopyranoside (PBG) isolated from *P. tricuspidata*.
Table 1. Blood schizontocidal activity of PBG, as measured against *P. berghei* in mice

<table>
<thead>
<tr>
<th>Compound/Drug</th>
<th>Dose (mg·kg(^{-1})·day(^{-1}))</th>
<th>Four-day test</th>
<th>Repository activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average % parasitemia</td>
<td>Average % parasitemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Average % suppression</td>
<td>Average % suppression</td>
</tr>
<tr>
<td>PBG</td>
<td>5</td>
<td>31.3 ± 0.23(^*)</td>
<td>23.2 ± 0.42(^*)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>22.4 ± 0.21(^*)</td>
<td>16.5 ± 0.30(^*)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>15.2 ± 0.12(^*)</td>
<td>11.3 ± 0.11(^*)</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>5</td>
<td>6.2 ± 0.15(^*)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>6.2 ± 0.15(^*)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>6.2 ± 0.15(^*)</td>
<td>ND</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>1.2</td>
<td>ND(^***)</td>
<td>4.6 ± 0.21(^*)</td>
</tr>
<tr>
<td>Control (D.W.,(^**))</td>
<td>0.2ml</td>
<td>56.3 ± 0.16(^*)</td>
<td>4.6 ± 0.21(^*)</td>
</tr>
</tbody>
</table>

\(^*\) Data are expressed as mean ± S.D for five animals per group. *P < 0.05 when compared with the control.\(^*\)

\(^**\) D.W.: Distilled water

\(^***\) ND, Not determined.
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·d⁻¹ or chloroquine at 5 mg·kg⁻¹·d⁻¹, on days 3–7. Data are shown as the mean ± S.D for five mice per condition. The test was performed as described by Ryley (8). Mice were inoculated with trophozoites and were treated 2 h later with drug or vehicle at the indicated dose by the intraperitoneal route (ip). The injection was repeated daily for a total of 4 d.
Control

PBG. 5 mg·kg\(^{-1}\)·day\(^{-1}\)

PBG. 10 mg·kg\(^{-1}\)·day\(^{-1}\)

PBG 25 mg·kg\(^{-1}\)·day\(^{-1}\)

Chloroquine

***p = 0.0004 vs. negative control
**p = 0.0006 vs. negative control
*p = 0.03 vs. chloroquine
(Student’s t-test)

% Parasitaemia

Days of observation

D3  D4  D5  D6  D7

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