Antimalarial Activity of Herbal Extracts Used in Traditional Medicine in Korea

Hyun Park, a Myung Soo Kim, b Byung Hun Jeon, c Tae Kyun Kim, c Yong Man Kim, a Joohong Ahn, d Dong-Yeul Kwon, e Yoshiaki Takan a, f Yusuke Wataya, f and Hye-Sook Kim a, f

a Department of Parasitology, College of Medicine, Wonkwang University; b Department of Pathology, College of Oriental Medicine, Wonkwang University; Iksan 570–749, Korea; c Department of Life Science, Kwangju Institute of Science and Technology; Kwangju 500–712, Korea; d Life Sciences Division, Korea Institute of Science and Technology; Seoul 130–650, Korea; e Faculty of Pharmacy, Meijo University; Toyoyama, Tempaku-ku, Nagoya 468–8503, Japan; and f Faculty of Pharmaceutical Sciences, Okayama University; Tsushima, Okayama, Okayama 700–8530, Japan.

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Aqueous extracts of 6 traditional Korean medicines used to treat malaria were tested in vitro for their antimalarial activity against Plasmodium falciparum. The EC50 values for the herbal extracts were in the range 1.4—8.1 μg/ml. Significant antimalarial activity was observed with Coptis japonica (EC50 = 1.4 μg/ml), but it demonstrated no selective toxicity (selectivity = 1). In contrast, Kalopanax pictus showed antimalarial activity (EC50 = 4.6 μg/ml) and higher selective toxicity (>4). This indicated that K. pictus may be potent for a new antimalarial agent.

Key words Plasmodium falciparum; traditional Korean medicine; antimalarial drug; drug-resistant malaria; Coptis japonica; Kalopanax pictus

Plasmodium falciparum, the most widespread etiological agent of human malaria, is becoming increasingly resistant to conventional antimalarial drugs, which necessitates a continuous effort to search for new antimalarial drugs.1,2) In the endemic area where malaria prevails, traditional herbal medicines are often used for antipyretic therapy. However, very little scientific information is available to assess the efficacy of these herbal remedies. Therefore, it is important to investigate the efficacy of the antimalarial activities of these medicinal plants in order to determine their potential as sources in the development of new antimalarial drugs.

Korea was declared to be free of vivax malaria by the WHO in 1979. However, it recurred in military soldiers near the De-Militarized Zone (DMZ) in the northern part of the country in 1993. This single outbreak has resulted in an exponential spread in the northwestern part of South Korea, causing more than 1700 cases in 1997 and approximately 4000 cases in 1999.3) Malaria has now become the most important parasitic disease in Korea.

In the course of our study, we have collected 6 medicinal plants which have been traditionally used to treat malaria and fever in Korea. The present paper reports on in vitro antimalarial activity of the herbal extracts against P. falciparum strain FCR-3 and their cytotoxicity against mouse mammary FM3A cells, which serve as a host model.

MATERIALS AND METHODS

Preparation of Traditional Medicine Samples Information on antimalarial and antipyretic therapies was collected through traditional healers and research on “Dong-yi-bo-gam” written by J. Hur in the Chosun dynasty.4) Solanum nigrum, Artemisia capillaris, Kalopanax pictus, Scutellaria baicalensis, Coptis japonica, and Phellodendron amurense were purchased from the oriental drug store, Bohwa Dang (Chunju, South Korea). The traditional medicines (100 g) were extracted with distilled water (500 ml) at 70°C for 5 h. The extract was filtered through a 0.45-mm filter, lyophilized, and kept at 4°C. The yield of dried extract (black color) from starting crude materials was about 8.9% (w/w). The dried extract was dissolved in water before use. Quinine hydrochloride, a clinically used antimalarial drug, was from Sigma (St. Louis, MO, U.S.A.).

Malaria Parasites P. falciparum (ATCC 30932, FCR-3 strain) was used in our study. P. falciparum was cultivated by a modification of the method of Trager and Jensen5) using a 5% hematocrit of type A human red blood cells suspended in RPMI 1640 medium (Gibco, NY, U.S.A.), and supplemented with heat-inactivated 10% type A human serum. The plates were placed in a CO2–O2–N2 incubator (5% CO2, 5% O2, and 90% N2 atmosphere) at 37°C, and the medium was changed daily until 5% parasitemia (which means the existence of 5 parasite-infected erythrocytes in every 100 erythrocytes).

Mammalian Cells Mouse mammary tumor FM3A cells (wild-type, subclone F28-7)6) were supplied by the Japanese Cancer Research Resources Bank (JCRB). FM3A cells were maintained in a suspension culture at 37°C in a 5% CO2 atmosphere in plastic bottles containing ES medium (Nissui Pharmaceuticals, Tokyo, Japan) supplemented with 2% heat-inactivated fetal bovine serum (Gibco, NY, U.S.A.).

In Vitro Antimalarial Activity of Traditional Medicines The following procedures were used to assay antimalarial activity.7,8) Asynchronously cultivated P. falciparum strain FCR-3 and their cytotoxicity against mouse mammary FM3A cells, which serve as a host model.

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* To whom correspondence should be addressed. e-mail: hs kim@cc.okayama-u.ac.jp © 2003 Pharmaceutical Society of Japan
cultures were run simultaneously. All data points represent the mean of three experiments. Parasitemia in the control reached between 4 and 5% at 72 h. The EC_{50} value refers to the concentration of the compound necessary to inhibit the increase in parasite density at 72 h by 50% of the control.

**Toxicity against Mammalian Cell Line** FM3A cells grew with a doubling time of about 12 h. Prior to exposure to drugs, the cell density was adjusted to 5\times 10^4 cells/ml. A cell suspension of 990 \mu l was dispensed to the test plate, and the compound at various concentrations suspended in distilled water (10 \mu l) was added to individual wells in the 24-well multi-dish. The plates were incubated at 37°C in a 5% CO₂ atmosphere for 48 h. All of the test compounds were assayed in duplicate at each concentration. Cell numbers were measured using a cell counter CC-130 (Toa Medical Electric Co., Japan). All data points represent the mean of three experiments. The EC_{50} value refers to the concentration of the compound necessary to inhibit by 50% the increase in cell density of the control at 48 h. Selectivity refers to the mean of the EC_{50} value for FM3A cells per the mean of the EC_{50} value for *P. falciparum*.

**RESULTS AND DISCUSSION**

Six aqueous extracts of herbal medicine used for malarial and antipyretic therapies in Korea were assessed for their antimalarial activities. The results of the *in vitro* antimalarial activity and cytotoxicity of the extracts against *P. falciparum* and mammalian cells are summarized in Table 1. The extracts of *C. japonica* showed high antimalarial activity (EC_{50} = 1.4 \mu g/ml), but it had no selective toxicity (selectivity = 1). Five other extracts showed evident antimalarial activity with EC_{50} values ranging from 3.5 to 8.1 \mu g/ml, and exhibiting greater than 2 times the selective activities. In addition, *K. pictus* had moderate antimalarial activity (EC_{50} = 4.6 \mu g/ml) with no cytotoxicity for mammalian cells treated at 20.0 \mu g/ml, and weak antimalarial activity relative to quinine (EC_{50} = 0.05 \mu g/ml against *P. falciparum*). The stage specific effect of *K. pictus* was determined on the parasite-infected erythrocytes. *K. pictus* showed morphological changes of the parasites at the transition from trophozoites to the schizont stages (data not shown).

This is the first report on antimalarial activities of herbal medicine traditionally used for malaria and antipyretic therapy in Korea, and all extracts in this study showed antimalarial activities. In particular, the extract of *K. pictus* revealed antimalarial activity with no cytotoxicity *in vitro*. Our results indicate that Korean traditional medicine would be promising for further investigation for new antimalarial agents.

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<table>
<thead>
<tr>
<th>Plant name</th>
<th><em>P. falciparum</em> EC_{50} (\mu g/ml)</th>
<th>FM3A EC_{50} (\mu g/ml)</th>
<th>Selectivity^a</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Solanum nigrum</em> L.</td>
<td>5.8</td>
<td>&gt;15.0 (100)^b</td>
<td>&gt;2</td>
</tr>
<tr>
<td><em>Artemisia capillaris</em> THUNB</td>
<td>8.1</td>
<td>&gt;15.0 (100)^b</td>
<td>&gt;2</td>
</tr>
<tr>
<td><em>Kalopanax pictus</em> NAKAI</td>
<td>4.6</td>
<td>&gt;20.0 (100)^b</td>
<td>&gt;4</td>
</tr>
<tr>
<td><em>Scutellaria baicalensis</em> GEORG</td>
<td>4.2</td>
<td>12.0</td>
<td>3</td>
</tr>
<tr>
<td><em>Coptis japonica</em> MAKINO</td>
<td>1.4</td>
<td>1.4</td>
<td>1</td>
</tr>
<tr>
<td><em>Phellodendron amurense</em> RUPR.</td>
<td>3.5</td>
<td>14.4</td>
<td>4</td>
</tr>
<tr>
<td>Quinine</td>
<td>0.05</td>
<td>36.1</td>
<td>722</td>
</tr>
</tbody>
</table>

^a Selectivity refers to the ratio of the EC_{50} value for the FM3A cells and the EC_{50} value for *P. falciparum*. ^b The value in parenthesis shows the growth (%) of each dose.

**REFERENCES**