Immunomodulatory Effects of Gynostemma pentaphyllum Makino on Human Immune Cells

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Keywords: immunomodulator, jiaogulan, lymphocyte proliferation, NK cell activity

Abstract

Use of herbs as health and/or dietary supplements has increased worldwide. The most common usage of botanicals is to improve the immune system. Water extract of Gynostemma pentaphyllum Makino was studied for its effects on lymphocyte proliferation and natural killer (NK) cell activity. Lymphocyte proliferation of normal peripheral blood mononuclear cells (PBMC) in response to G. pentaphyllum extract was increased at concentrations of (in ng/mL) 1, 10, 100, and (in µg/mL) 1, 5, 10 and 100. Aqueous extract of G. pentaphyllum reduced lymphocyte proliferation at 1 µg/mL, suggesting immunomodulating activities on human immunocompetent PBMC.

INTRODUCTION

Jiaogulan (Gynostemma pentaphyllum Makino; Cucurbitaceae) is a perennial climber, widely growing in China, Japan, Korea and Southeast Asia. It is used for treatment of inflammation, cough, hyperviscosity of sputums and chronic bronchitis (Jiang-Xu, 1979; Lin et al., 1993). Gypenosides, the total saponins with a dammarane-type basic structure (Piacente et al., 1995; Hu et al., 1996) isolated from jiaogulan, have been reported to decrease blood cholesterol, inhibit growth of tumor cells, heal peptic ulcer, relieve inflammation and pain and prevent platelet aggregation (Kimura et al, 1983; Li and Jin, 1989). Jiaogulan is also taken as tonic to improve the immune system. The effects and mechanisms of the activities on the immune system have not been well-defined. The objectives of this study were to investigate its capabilities on cell-mediated immune response (CMIR) by studying their effects on lymphocyte proliferation and natural killer (NK) cell activity of human peripheral blood mononuclear cells (PBMC).

MATERIALS AND METHODS

Preparation of Jiaogulan Aqueous Extract

Aerial parts of jiaogulan were collected from Chiang Mai, northern Thailand. Voucher specimen of jiaogulan (Bandsiddhi 43-21) was deposited at the Botanical section, Medicinal plant research institute, Department of medical sciences. Botanical identification was confirmed (Backer et al., 1963; Wu et al., 1983) and compared with authentic specimens at two herbaria, the Bangkok Herbarium (BK), Department of agriculture and the forest herbarium (BKF), Royal forest department, Ministry of agriculture and cooperative, Thailand. The plant was washed thoroughly, cut into segments, oven-dried at 40°C and ground. Fifteen grams of dried and ground aerial parts of jiaogulan were extracted with distilled water for 2 h using a reflux method. Filtrate was collected and residues further extracted with distilled water for 2 h. Filtrates collected from both extractions were pooled and dried under vacuum in a rotary evaporator. The amount of dried extract obtained was 3.79 g. The dried extract was dissolved in distilled water to make a stock concentration of 10 mg/mL, filtered and refrigerated until use.
Subjects
A total of 52 healthy Thai donors of the National blood bank, The Thai Red Cross society were recruited in this study. They were 20-50 y old, and none had a history of hepatitis B infection, nor had a risk for HIV-1 exposure.

Preparation of Mononuclear Cells
Mononuclear cells were separated from heparinized blood using Ficoll-Hypaque density gradient (Boyum, 1968). The mononuclear cells were counted and adjusted to an appropriate concentration in complete RPMI 1640 (RPMI 1640 medium supplemented with 2 mM glutamine, 10 mM HEPES, 100 U/mL penicillin G, and 100 µg/mL streptomycin) containing 10% fetal bovine serum (FBS; Grand Island Biological Company, Grand Island, NY, USA) for further assays.

Lymphoproliferation Assay
Lymphocyte proliferative response was done as described earlier (Sriwanthana and Chavalittumrong, 2001). Purified mononuclear cells (2x10^6 cells/mL) were cultured in triplicates in 96-well microtiter plates (Costar, Cambridge, MA, USA) with the extract at final concentrations of (in ng/mL) 1, 10, 100, and (in µg/mL) 1, 5, 10 and 100 in complete RPMI 1640 containing 10% FBS. The cultures were incubated at 37°C with 5% CO₂ for 72 h. Lymphocyte proliferation was determined by uptaking of ^3H-thymidine at 18 h before harvesting. The radioactivity was measured by a liquid scintillation counter (Topcount Microplate Scintillation and Luminescence Counter, Packard Instrumental Co., CT, USA). The degree of activation was expressed as a stimulation index [S.I., the ratio of the ^3H-thymidine uptake in count per minute (CPM) of samples with extract to those without extract]. Phytohemagglutinin HA16/17 (Murex Diagnostics Limited, Dartford, England) at 2 µg/mL was added to the culture system to check for cell survival.

NK Cell Activity Assay
PBMC (2x10^6 cells/mL) were incubated in the presence or the absence of jiaogulan extract at the final concentrations of 10 ng/mL, 100 ng/mL, 1 µg/mL, 5 µg/mL, and 10 µg/mL at 37°C for 18 h. After incubation, the cultures were washed and then used as effector cells for the assay of NK cell activity. K 562 cells were used as target cells and were grown in complete RPMI 1640 containing 10% FBS. The target cells (2x10^6 cells) were labeled with 100 µCi of Na_2^{51}CrO_4 (specific activity 37.0 MBq/µg; Amersham, Buckinghamshire, UK) at 37°C, 5% CO₂ for 60 min, washed 3 times with RPMI 1640 containing 10% FBS.

Cytotoxicity assay was performed as described earlier (Sriwanthana and Chavalittumrong, 2001). In brief, 2x10^5 target cells/well and a PBMC effector-to-target cell ratios (E:T) of 90:1, 30:1, 10:1, and 3:1 were set up in triplicate in 96-well round-bottom microtiter plates (Corning Incorporated, Corning, NY, USA). The plates were incubated for 4 h at 37°C with 5% CO₂. After incubation, supernatants from each well (100 µL) were transferred into tubes and counted in a Gamma counter (Cobra Series Gamma Counter Systems, Packard Instrumental Co., CT, USA). The percentage of cytolyis was calculated as: %cytolysis = (experimental release - spontaneous release) / (maximal release - spontaneous release). Spontaneous release was measured by incubation of target cells with medium alone, while maximal release was measured by lysis of target cells with 5% Triton X-100. NK cell activity was expressed as lytic units (LU)/10⁷ PBMCs as determined by least squares analysis derived from the percentage of specific lysis of all E:T ratios. One LU was defined as the number of effector cells required for 20% specific lysis of 1x10⁴ target cells.

Statistical Analysis
Data were expressed as mean ± SE and compared using Student’s paired t-test.
RESULTS

Lymphocyte Proliferation
The extract-induced proliferative responses were examined from 52 PBMC in cultures containing jiaogulan. Similar patterns of blastogenesis of lymphocytes were found in responses to jiaogulan. The responses were elevated with the water extract of jiaogulan ranging from concentrations of 1 ng/mL to 100 µg/mL (P < 0.05) (Table 1).

NK Cell Activity
Reduction in NK activity was observed in PBMC treated with 1 µg/mL of jiaogulan extract compared with untreated ones (P = 0.02) (Table 2).

DISCUSSION
Humoral and cellular immunity are defense mechanisms against foreign bodies. Botanicals are suggested to act primarily on cellular rather than humoral immune responses. Several medicinal plants are traditionally believed to promote health by their immunomodulatory activities. Our studies were designed to investigate the in vitro effects of jiaogulan on lymphocyte proliferation and NK cell activity of human PBMC.

The increase in lymphocyte proliferation, quantified as stimulation index (S.I.), was demonstrated in a dose-dependent response at concentrations of 1 ng/mL to 1 µg/mL. The response decreased slightly at concentrations over 1 µg/mL, which may be due to amounts of active constituents in the extract or other factors induced during lymphocyte blastogenesis.

NK cells are known to play an important role as one of the first lines of host defense mechanisms against a variety of infections and cancers. We measured the effect of the aqueous extracts of jiaogulan on NK cell activity of normal PBMC, in 13 donors. Our result showed a reduction in the function of NK cells at 1 µg/mL. The reduction in NK activity was not due to the toxicity of jiaogulan, because the viability of PBMC was greater than 95% in the presence or the absence of the extract (data not shown). In contrast, the NK activity at other concentrations revealed no difference compared to the control. Jiaogulan extract at 1 µg/mL may induce some soluble factors that could decrease the activity.

It has been reported that gypenosides, saponins isolated from jiaogulan, enhanced T and B lymphocyte proliferation and interleukin-2 (IL-2) production of splenocytes in both normal and immunosuppressed mice (Li and Xing, 1992). Gypenosides also demonstrated their effect on humoral and cellular immunocompetence in γ-irradiated mice (Chen et al., 1996). Moreover, it was found to possess antioxidant effect in human and murine phagocytes (Li et al., 1993). Our findings showed similar results of aqueous extract on human immune cells. Enhancement of lymphocyte proliferation, may be due to the effect of gypenosides.

Our study suggested that aqueous extracts of jiaogulan possess immunomodulatory activities on human PBMC. Chronic toxicity of the extract in Wistar rats performed indicated no toxicity at concentrations as high as 750 mg/kg/d jiaogulan (P. Chavalittumrong, pers. commun., 2002). Further studies to evaluate safety and efficacy of the extract in humans are needed to use for benefits of both immunocompetence and immunocompromised, as those with HIV-infection or cancers.

ACKNOWLEDGEMENTS
We acknowledge staffs of the National blood bank, The Thai Red Cross society for providing blood from their regular donors. We also thank staffs in phytochemistry and toxicology units at Medicinal plant research institute for their help.

Literature Cited
### Tables

Table 1. Effect of jiaogulan (*Gynostemma pentaphyllum*) on lymphocyte proliferation of normal PBMC (*n*=52).

<table>
<thead>
<tr>
<th>Extract concentrations</th>
<th>Stimulation Index (S.I.) (Mean ± SE)</th>
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<tbody>
<tr>
<td>Control</td>
<td>1.00 ± 0.00&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>1 ng/mL</td>
<td>1.41 ± 0.08&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>10 ng/mL</td>
<td>1.61 ± 0.12&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>100 ng/mL</td>
<td>2.01 ± 0.15&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>1 µg/mL</td>
<td>2.86 ± 0.20&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>5 µg/mL</td>
<td>2.36 ± 0.15&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>10 µg/mL</td>
<td>2.14 ± 0.13&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>100 µg/mL</td>
<td>2.45 ± 0.23&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>PHA 2 µg/mL</td>
<td>120.17 ± 14.1&lt;sup&gt;*&lt;/sup&gt;</td>
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<sup>NS</sup> Not significant,  <sup>*</sup> Significant at *p* < 0.05

Table 2. NK cell activity by normal PBMC in the presence of jiaogulan (*Gynostemma pentaphyllum*) extract (*n*=13).

<table>
<thead>
<tr>
<th>Extract concentrations</th>
<th>Lytic units (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>85.16 ± 18.04&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>10 ng/mL</td>
<td>84.67 ± 17.33&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>100 ng/mL</td>
<td>76.81 ± 14.71&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>1 µg/mL</td>
<td>52.54 ± 9.89&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>5 µg/mL</td>
<td>66.13 ± 14.51&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>10 µg/mL</td>
<td>71.63 ± 10.92&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>NS</sup> Not significant,  <sup>*</sup> Significant at *p* < 0.05