Suppression of the Formation of Sister Chromatid Exchanges by Low Concentrations of Ginsenoside Rh2 in Human Blood Lymphocytes

Jin Hua Zhu, Tatsuya Takeshita, Isao Kitagawa, and Kanchisa Morimoto

Department of Hygiene and Preventive Medicine, Osaka University School of Medicine, 2-2 Yamada-oka, Suita, Osaka 565 [J. H. Z., T. T., K. M.], and Faculty of Pharmaceutical Science, Osaka University, 1-6 Yamada-oka, Suita, Osaka 565, Japan [I. K.]

Abstract

To assess the antimutagenic potentials of ginsenoside Rh2 (Rh2), its effects on the baseline and mitomycin C-induced sister chromatid exchange (SCE) were examined in human peripheral blood lymphocytes (PBLs). The SCE frequency in PBLs treated with various concentrations of Rh2 for 72 h decreased in a dose-dependent manner and was significantly lower than the baseline levels at 1.0 × 10^-10 M and 1.0 × 10^-7 M. The SCE frequency in PBLs treated with both Rh2 and mitomycin C was significantly (P < 0.001) less than that in PBLs treated with only mitomycin C. Cell cycle kinetics, as indicated by the proliferation and mitotic indices, was not significantly affected by Rh2 of various concentrations in the PBLs throughout the present experiments. This is the first report which showed convincingly a reduction of SCE in normal human cells. The mechanism remains to be elucidated in future studies.

Introduction

Ginseng has been widely used as a tonic remedy in China, Korea, and Japan for more than 4000 years, and is increasingly attracting attention. Ginseng radix rubra (red ginseng), extracted from the root of Panax ginseng C. A. Meyer, is especially highly valued because of its numerous beneficial effects such as correcting hyperlipidemia (1). Rh2, isolated from the methanol and butanol extract of red ginseng and was found specifically in red ginseng (2). Rh2 inhibited the growth of mouse melanoma cells and induced the differentiation of those cells in vitro (3, 4). Rh2 induced G1 phase arrest and/or S-phase prolongation in the tumor cell lines in vitro (5). These findings indicated the usefulness of Rh2 and/or red ginseng as an anticanccer agent. SCE has been regarded as a sensitive indicator of DNA damage (6) and is strongly correlated with the mutagenic activities of various chemicals (7). To investigate the antimutagenic potency of Rh2, we examined the baseline and mutagen-induced SCE frequencies in human lymphocytes. The findings revealed strong SCE-modifying effects of Rh2 at very low concentrations.

Materials and Methods

Subjects and Lymphocyte Cultures. Peripheral blood samples were drawn from healthy volunteers between 23 and 51 years old. The culture medium consisted of 5 ml RPMI 1640 (GIBCO) containing 15% fetal bovine serum (Bocknek, Rexdale, Canada), 3% phytohemagglutinin M (Difco), 20 μM 5-bromo-2'-deoxyuridine (Sigma), and penicillin and streptomycin (GIBCO). Whole blood (0.3 ml) was added to the culture medium and the cultures were continued for 72 h with 5% CO2 and 95% air at 37°C in complete darkness.

Subjects and Lymphocyte Cultures. Peripheral blood samples were drawn from healthy volunteers between 23 and 51 years old. The culture medium consisted of 5 ml RPMI 1640 (GIBCO) containing 15% fetal bovine serum (Bocknek, Rexdale, Canada), 3% phytohemagglutinin M (Difco), 20 μM 5-bromo-2'-deoxyuridine (Sigma), and penicillin and streptomycin (GIBCO). Whole blood (0.3 ml) was added to the culture medium and the cultures were continued for 72 h with 5% CO2 and 95% air at 37°C in complete darkness.

Assays for SCE and Cell Proliferation. A modified fluorescence-plus-Giemsa method (6) was used for the differential staining of sister chromatids. SCEs were scored blindly in 30 consecutive second division metaphases for each point. The mitotic index was calculated as the number of metaphases appearing in 1000 nuclei by scoring 3000 consecutive nuclei. Cell cycle kinetics was evaluated by the proportion of first (X1), second (X2), and third or more (X3) division cells in 200 consecutive metaphases. The proliferation index was calculated according to the following formula: Proliferation index = (1 × X1 + 2 × X2 + 3 × X3) / 100.

Statistical Analysis. We used Student's t test for the SCE frequency and mitotic index, and Wilcoxon's test for the proliferation index.

Results

The effects of Rh2 on the baseline SCE frequency were examined in human PBLs. The cells were treated with various doses of Rh2 ranging from 1.0 × 10^-22 to 1.0 × 10^-7 M for the whole culture period. As Table 1 shows, the SCE frequency decreased in a dose-dependent manner. The doses of 1.0 × 10^-10 and 1.0 × 10^-7 M produced significant reductions in the SCE frequency.

To assess the effects of Rh2 on the cell cycle kinetics of the cells, we scored the proliferation index and mitotic index on the same slides. In the dose range examined, we could find no significant effects of Rh2 on the proliferation index or the mitotic index (Table 1).

Next we evaluated the effects of Rh2 on the SCE frequency and cell cycle kinetics when administered simultaneously with MMC, a DNA-alkylating agent. As shown in Table 2, at 1.0 × 10^-7 M, Rh2 significantly reduced the SCE frequency of the cells treated with either 3.0 × 10^-8 or 6.0 × 10^-8 M of MMC. Even at a much lower concentration (1.0 × 10^-19 M) Rh2 significantly decreased the SCE frequency when administered with MMC at 6.0 × 10^-8 M.

In these experiments, the addition of Rh2 did not cause any significant changes in the proliferation index or mitotic index compared to the cultures with only 0.25% ethanol.

Discussion

In order to assess the possibility of antimutagenic potentials of Rh2, we examined the potency of Rh2 to modify the baseline and MMC-induced SCE frequencies in human PBLs, since SCE sensitively reflects mutagenic damage (6) and correlates well with the mutation frequencies (7). Rh2 significantly suppressed both the baseline and MMC-induced SCEs without any apparent changes in cell cycle kinetics as indicated by the proliferation and mitotic indices.

Protein synthesis inhibitors such as cycloheximide significantly suppressed the frequency of UV light-induced SCE, but not of baseline SCE (9). Rh2 inhibited protein synthesis in an ovarian cancer cell line (10), only at much higher concentrations (≥60 μM) than those

Received 12/16/94; accepted 2/2/95.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported in part by Grants-in-Aid for scientific research from the Ministry of Education, Science, and Culture of Japan and by a Grant-in-Aid for cancer research from the Ministry of Health and Welfare of Japan.

2 To whom requests for reprints should be addressed.

3 The abbreviations used are: Rh2, ginsenoside Rh2; SCE, sister chromatid exchange; MMC, mitomycin C; PBLs, peripheral blood lymphocytes.
used in the present study. Since we did not observe any inhibition of cell proliferation with the dosages examined, Rh2 is not considered to have reduced the SCE frequency through the inhibition of protein synthesis.

Ginseng extracts partly prevented pulmonary damage induced by cigarette smoke in rats (11). Rh2 might also have acted as an antioxidant in the present study resulting in the reduction of a fraction of SCE related to the reactive oxygen species. However, we could find no antioxidative agent that reduced the baseline SCE level in mammalian cells. A fraction of MMC-induced SCEs could have been canceled by Rh2 at 1.0 × 10⁻⁷ M through its direct attachment to MMC. However, Rh2 at a much lower concentration could also significantly reduce the MMC-induced SCEs. Hence, Rh2 would have more than the effect of a simple antioxidant or a desmutagen.

Rh2 is a plant glycoside with a dammarane skeleton resembling the steroid (2, 3). Rh2 was found to be taken up in the lipid fraction of cell membranes (4), which might implicate some important role of steroids (2, 3). Rh2 was found to be taken up in the lipid fraction of cigarette smoke in rats (11). Rh2 might also have acted as an antioxidant in the present study resulting in the reduction of a fraction of SCE and cell proliferation with the dosages examined, Rh2 is not considered to have reduced the SCE frequency through the inhibition of protein synthesis.

The effects of several steroid hormones on the SCE frequency have been examined (12). Estriol at 5.0 × 10⁻⁵ M significantly increased the SCE frequency in the PBLs of pregnant women (12), although not showing convincingly a reduction in baseline SCEs in normal human cells. Further studies to elucidate the scientific bases for the biological effects of Rh2 would be of great help for both shedding light on the mechanism underlying SCE formation and applying Rh2 in cancer therapy and health promotion.

Acknowledgments

We thank H. Ogura for technical assistance.

References


Table 1 Effects of various doses of Rh2 on baseline SCE frequency in human lymphocytes

<table>
<thead>
<tr>
<th>Dose of Rh2 (m)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (distilled water)</td>
<td>8.4 ± 2.0</td>
<td>9.8 ± 3.1</td>
<td>9.9 ± 3.3</td>
<td>9.3 ± 2.5</td>
<td>9.3 ± 6.6</td>
</tr>
<tr>
<td>Control (0.25% ethanol)</td>
<td>8.4 ± 2.2</td>
<td>9.4 ± 3.0</td>
<td>9.4 ± 2.4</td>
<td>9.5 ± 2.5</td>
<td>9.2 ± 5.5</td>
</tr>
<tr>
<td>1.0 × 10⁻⁸</td>
<td>8.0 ± 2.1</td>
<td>9.9 ± 3.5</td>
<td>9.8 ± 3.5</td>
<td>10.5 ± 3.2</td>
<td>9.5 ± 5.1</td>
</tr>
<tr>
<td>1.0 × 10⁻⁷</td>
<td>8.0 ± 2.8</td>
<td>9.4 ± 3.5</td>
<td>8.8 ± 2.2</td>
<td>8.7 ± 0.6</td>
<td>8.7 ± 0.6</td>
</tr>
<tr>
<td>1.0 × 10⁻⁶</td>
<td>7.9 ± 2.1</td>
<td>8.8 ± 2.8</td>
<td>9.1 ± 3.4</td>
<td>8.4 ± 3.9</td>
<td>8.6 ± 0.6</td>
</tr>
<tr>
<td>1.0 × 10⁻⁵</td>
<td>7.4 ± 2.2</td>
<td>8.7 ± 2.6</td>
<td>8.3 ± 2.8</td>
<td>8.6 ± 2.6</td>
<td>8.3 ± 0.7</td>
</tr>
<tr>
<td>1.0 × 10⁻⁴</td>
<td>7.3 ± 2.2</td>
<td>8.0 ± 2.4</td>
<td>7.4 ± 3.4</td>
<td>8.0 ± 3.7</td>
<td>7.7 ± 0.4</td>
</tr>
</tbody>
</table>

* Mean ± SD for donors.
** Mean ± SD for cells.
* Marginally significant (P = 0.06) against control (0.25% ethanol).
** Significant (P < 0.05) against control (0.25% ethanol).

Table 2 Effects of Rh2 on MMC-induced SCE frequency, proliferation index, and mitotic index in human lymphocytes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SCE frequency/cell</th>
<th>Proliferation index</th>
<th>Mitotic index/ thousand cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>9.3 ± 0.4</td>
<td>2.3 ± 0.2</td>
<td>57.0 ± 14.0</td>
</tr>
<tr>
<td>Ethanol (0.25%)</td>
<td>9.2 ± 0.3</td>
<td>2.3 ± 0.2</td>
<td>57.2 ± 14.6</td>
</tr>
<tr>
<td>Rh2 (1.0 × 10⁻⁷ M)</td>
<td>7.7 ± 0.1</td>
<td>2.4 ± 0.3</td>
<td>77.0 ± 29.5</td>
</tr>
<tr>
<td>MMC (3.0 × 10⁻⁴ M)</td>
<td>25.9 ± 1.4</td>
<td>2.5 ± 0.2</td>
<td>86.0 ± 31.5</td>
</tr>
<tr>
<td>Rh2 (1.0 × 10⁻⁷ M) + MMC (3.0 × 10⁻⁴ M)</td>
<td>19.1 ± 0.9</td>
<td>2.3 ± 0.1</td>
<td>67.7 ± 14.0</td>
</tr>
<tr>
<td>MMC (6.0 × 10⁻⁴ M)</td>
<td>41.6 ± 2.0</td>
<td>2.3 ± 0.2</td>
<td>70.0 ± 12.8</td>
</tr>
<tr>
<td>Rh2 (1.0 × 10⁻⁷ M) + MMC (6.0 × 10⁻⁴ M)</td>
<td>28.5 ± 2.4*</td>
<td>2.4 ± 0.03</td>
<td>62.7 ± 21.8</td>
</tr>
<tr>
<td>Rh2 (1.0 × 10⁻⁷ M) + MMC (6.0 × 10⁻⁴ M)</td>
<td>32.4 ± 1.5</td>
<td>2.2 ± 0.2</td>
<td>59.0 ± 17.8</td>
</tr>
<tr>
<td>Rh2 (1.0 × 10⁻⁷ M) + MMC (6.0 × 10⁻⁴ M)</td>
<td>39.0 ± 1.8</td>
<td>2.3 ± 0.2</td>
<td>65.5 ± 15.0</td>
</tr>
</tbody>
</table>

* The effects of Rh2 on the MMC-induced SCE frequency were assessed by comparing the SCE frequency after simultaneous treatment with Rh2 and MMC with those after treatment with only the same dose of MMC. Mean (± SD) values of four subjects are shown.
** Significant (P < 0.01) against control (0.25% ethanol).


Suppression of the Formation of Sister Chromatid Exchanges by Low Concentrations of Ginsenoside Rh₂ in Human Blood Lymphocytes

Jin Hua Zhu, Tatsuya Takeshita, Isao Kitagawa, et al.


Updated version
Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/55/6/1221

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.