Effects of Root Extracts from Panax ginseng C. A. Meyer (Araliaceae) of Different Ages on K562 Cells

Xunan Lyu1, Yizhen Zhang2, Qing Mu3, Zhou Cheng3, Jiakuan Chen1, Wenju Zhang1*

1Ministry of Education, Key Laboratory for Biodiversity Science and Ecological Engineering, Institute of Biodiversity Science, School of Life Sciences, Fudan University, Shanghai, China; 2School of Pharmacy, Fudan University, Shanghai, China; 3School of Life Science and Technology, Tongji University, Shanghai, China.

Email: *wjzhang@fudan.edu.cn

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ABSTRACT

It is well accepted in China that elder ginsengs have more bioactivity and value than younger ones. However, there is little research about the comparison of beneficial effects of ginsengs with different ages. In this study, ginseng root extracts (GRE) were extracted from ginsengs of 5, 8, 12, 14, and 16 years old, respectively, using 55% ethanol and their effects on human leukemic K562 cells within 48 hours were tested by using Cell Counting Kit-8. The results show that there are significant increases in the cell viability of all the GRE groups compared with Control group within 32 hours. Furthermore, the growth curves of GRE groups were obviously distinct from each other. The cell viability of 5-year-old and 8-year-old GRE groups kept a rapid increase while that of 16-year-old GRE group showed a strong fluctuation within 28 hours. Our results demonstrate that root extracts from ginsengs of different ages contain different bioactivity constituents and have different effects on cell.

Keywords: Panax ginseng; Root Extracts; Ages; K562 Cell Line

1. Introduction

Panax ginseng C. A. Meyer (ginseng) is a famous herbal medicine with a wide range of therapeutic benefits, such as inhibiting tumor growth, regulating immune system, inducing the cell differentiation, etc. [1,2]. It is well accepted that elder ginsengs have more bioactivity and value than younger ones based on Chinese conventional concept; however, there is little research about the comparison of beneficial effects of ginsengs with different ages.

K562 cell line was established from a patient with chronic myelogenous leukemia and has been widely used both in vitro experiments at present [3]. Ginsenosides and polysaccharides are regarded as the primary active ingredients of ginseng [1,2]. Total ginsenosides of ginseng could not only inhibit the proliferation of K562 cells but also induce the differentiation by inhibiting the expression of erythropoietin receptor protein [4,5]. Moreover, studies have provided clear evidence that polysaccharides from Ginseng also exert antiproliferative activity against K562 cells at a certain concentration range in vitro experiment [6,7]. Except for ginsenosides and polysaccharides, ginseng roots also contain some other constituents such as peptides, polyacetylenic alcohols, flavones, and fatty acids which also contribute to a wide range of beneficial effects of ginseng [1,2]. However, the general effects of ginseng root extracts (GRE) on K562 cells have not been previously investigated.

The purpose of this study was to compare the effects of GRE of different ages on K562 cell line.

2. Materials and Methods

2.1. Sample Collection

Roots of ginseng were collected from Huaien and Ji’an, Jilin province, China respectively (shown in Table 1). Ginseng samples of different ages were dried at 50˚C and crushed (40 mesh sieve), respectively. Each above sam-
ple was accurately weighed 6.000 g and 18.2 mL/g of 55% ethanol was added. After soaking 12 hours at room
temperature, the sample suspension was extracted under
ultrasonic for 42 min, then centrifuged and the super-
natant was collected. The extraction was repeated four
times. The supernatants were merged and evaporated to
dryness under vacuum at temperature 45°C. The dried
ginseng extracts were accurately weighed to calculate the
contents of total GRE and used as pharmacological ex-
periments. A stock solution was prepared by dissolving
GRE in ddH2O and stored at –20°C. To analysis amount
in different polarity, the extract resin was resuspended in
30 mL distilled water and then partitioned with chloro-
form (30 mL × 3). The chloroform extracts were merged
together and dried in a vacuum at 45°C and weighed ac-
curately to calculate the contents, then stored at 4°C. The
above remains of the ginseng extracts aqueous were then
extracted with butanol (30 mL × 3). The final remains of
the ginseng extracts aqueous were removed to dry and
weighed accurately to calculate the contents.

2.3. Cell Culture and Cell Growth Curve
Detection
K562 human leukemia cell line was obtained from
Shanghai Institute of Cell, Chinese Academy of Sciences.
Cells were cultured in RPMI-1640 medium (Gibco-BRL,
Gaithersburg, MD) supplemented with 10% fetal bovine
serum (Gibco-BRL), 100 µg/ml streptomycin (Sigma
Chemical Co., St. Louis, MD) and 100 IU/ml penicillin
(Sigma) at 37°C. Cells were seeded at 1 × 10^5 cells/well
in 96-well plate for 48 hours with or without a final con-
centration of 1 mg/ml GRE named Control group, H5
(5-year-old) group, H8 (8-year-old) group, H12 (12-year-
old) group, H14 (14-year-old) group and H16 (16-year-
old) group respectively. Each group had 6 repeats. Cell
viability was analyzed by Cell Counting Kit-8 (CCK-8,
Dojindo, Kumamoto, Japan) every 4 hours within 48
hours.

2.4. Statistical Analysis
Results were expressed as the mean ± standard devia-
tions (SD). The effect on each parameter was examined
by one-way analysis of variance (ANOVA) and indepen-
dent-sample t test. P values less than 0.05 were consid-
ered statistically significant.

3. Results
3.1. Content of Total GRE and Their
Constituents
Table 1 shows contents of total GRE in ginseng of dif
ferent age groups. H5 group (5-year-old) has the lowest
content (30.87%) and H16 group (16-year-old) has the
highest (46.43%). Contents of ginsenosides and poly-
saccharides in GRE were measured and results were
showed in table. H16 group has the highest content of
ginsenosides in GRE (16.11%) while H5 has the highest
content of polysaccharides (56.37%).

3.2. Cell Viability of K562 Cells within 48 Hours
Compared with the cell viability of Control group, there
were significant increases in all groups treated with 1
mg/ml GRE between the 32-hour point and the 48-hour
point (data shown in supplementary Table 1). However,
growth curves of groups treated with GRE of different
ages were obviously distinct from each other within 48
hours.

3.3. Cell growth Curves of H5 and H8 GRE
Groups
Compared with Control group, H5 group and H8 group,
which possessed similar growth curves, had significant
proliferation in cell viability within 48 hours except at
the 4-hour point when H5 group had no significant dif
ference with Control group. Furthermore, the cell viabil
ity of H8 group was significantly increased compared
with that of H5 group within 24 hours and had no sig
nificant difference from the 28-hour point to the 48-hour
point (Figure 1).

3.4. Cell Growth Curve of H12 GRE Group
There was a cyclical fluctuation with a period of 8 hours
in the growth curve of H12 group within 24 hours. Com-
pared with Control group, there was no significant dif
ference within 28 hours in cell viability except at the 12-
hour point when the cell viability of H12 group was sig
nificantly decreased. After 28 hours, the growth curve
grew slowly. Moreover, the cell viability of H12 group was
always significantly higher than that of Control group but
lower than that of other GRE groups after 32 hours.

Table 1. Location, age, individual number, voucher specimen, and collection time of materials used in this study.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Age (years)</th>
<th>Location</th>
<th>Individual</th>
<th>Voucher specimen.</th>
<th>Collection Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>H5</td>
<td>5</td>
<td>Ji’an, Jilin</td>
<td>5</td>
<td>JR1</td>
<td>2006</td>
</tr>
<tr>
<td>H8</td>
<td>8</td>
<td>Huaiiren, Jilin</td>
<td>5</td>
<td>HR5</td>
<td>2004</td>
</tr>
<tr>
<td>H12</td>
<td>12</td>
<td>Huaiiren, Jilin</td>
<td>5</td>
<td>HR6</td>
<td>2004</td>
</tr>
<tr>
<td>H14</td>
<td>14</td>
<td>Huaiiren, Jilin</td>
<td>5</td>
<td>HR11</td>
<td>2009</td>
</tr>
<tr>
<td>H16</td>
<td>16</td>
<td>Huaiiren, Jilin</td>
<td>4</td>
<td>HR8</td>
<td>2004</td>
</tr>
</tbody>
</table>

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3.5. Cell Growth Curve of H14 GRE Group

The growth curve of H14 group was similar to that of HR12 within 12 hours but significantly higher than the latter from the 16-hour point to the 40-hour point. At the 12-hour point, the cell viability of H14 group was significantly decreased compared with that of Control group.

3.6. Cell Growth Curve of H16 GRE Group

The growth curve of H16 group appeared a strong cyclical fluctuation with a period of 8 hours within 32 hours. After that, it grew similarly with the growth curve of H14 group. The cell viability of H16 group was always significantly higher than that of Control group except at the 28-hour point. In particular, the cell viability of H16 group was significantly higher than that of Control group and the other GRE groups at the 4-hour point.

4. Discussion

In previous studies, ginsenosides and polysaccharides, mainly contributing to the beneficial biological effects of ginseng [1,2], inhibited the proliferation of K562 cells in vitro experiments separately [4-7]. Furthermore, it was reported that ginsenosides of 100 - 400 μg/ml (the range of this study was 127 - 161 μg/ml) [4] and polysaccharides of 20 - 800 μg/ml (the range of this study was 237 - 563 μg/ml) [6] could inhibit the proliferation of K562 cells. However, our experimental data showed that the cell viability of all GRE groups significantly higher than that of control group from the 32-hour point to the 48-hour point, meaning that GRE increased rather than inhibited the proliferation of K562 cells. One of the possible reasons is that except for ginsenosides and polysaccharides, there are some nonpolar chemical constituents in the GRE, such as polyacetylenes [8], flavonones and fatty acids, which might contribute to the effects of ginseng [1,2]. In this study, these other constituents account for a high percentage in the GRE (from 30.06% to 63.48%, Table 2) and possibly lead to the strong proliferation on K562 cells or change the combined effects via antagonism and/or synergy [9]. In addition, some certain ginsenosides have been proved to have the ability to stimulate cell proliferation. For example, panaxatriol could significantly stimulate the proliferation of K562 cells at the low concentration but inhibit the proliferation at the middle concentration [10] and ginsenoside Rg1, have the ability to promote the proliferation of neural stem cells [11,12]. These special ginsenosides might play an important role in the GRE groups of this study.

Table 2. Content of total Ginseng root extract (GRE) and content of GRE components.

<table>
<thead>
<tr>
<th></th>
<th>H5</th>
<th>H8</th>
<th>H12</th>
<th>H14</th>
<th>H16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Content of GRE in ginseng (%)a</td>
<td>30.87</td>
<td>33.35</td>
<td>39.98</td>
<td>34.36</td>
<td>46.43</td>
</tr>
<tr>
<td>Content of nonpolar constituents in GRE (%)b</td>
<td>30.06</td>
<td>42.31</td>
<td>63.48</td>
<td>36.15</td>
<td>51.63</td>
</tr>
<tr>
<td>Content of ginsenosides in GRE (%)c</td>
<td>13.57</td>
<td>15.62</td>
<td>12.76</td>
<td>15.83</td>
<td>16.11</td>
</tr>
<tr>
<td>Content of polysaccharides in GRE (%)d</td>
<td>56.37</td>
<td>42.07</td>
<td>23.76</td>
<td>48.02</td>
<td>32.26</td>
</tr>
</tbody>
</table>

aExtract amount and content from ginseng material of the different age; bNonpolar constituents extracted by chloroform from the total ginseng extract; Content of nonpolar constituents in the total extract; cGinsenosides constituents extracted by butanol from the ginseng extract that after being extracted by chloroform; Content of ginsenosides in the total extract; dPolyasaccharides constituents, the remains that being removed chloroform and butanol soluble parts from the ginseng total extract; Content of polysaccharides in the total extract.
Supplementary Table showed that the proportions of ginsenosides, polysaccharides and nonpolar constituents in GRE have remarkable difference in different age groups. These differences may be one reason why K562 cells have different growth curves when adding the GRE with same content but from different age ginsengs. The H5 and H8 groups, possessing the similar growth curves, both had a strong proliferation effect on K562 cells compared with Control group within 48 hours. However, there was a strong fluctuation in the growth curve of H16 group within 32 hours. In addition, the cell viability of H12, H14 and H16 groups all decreased sharply at the 12-hour point while that of H5 and H8 groups increased significantly at the same time, demonstrating an obvious difference in biological effects between elder GRE groups and younger groups. The elder ginseng has been shown to possess some kinds of ginsenosides, such as Rh2 and Compound K, which do not exist in the younger ones [2], so we guess that in addition to the difference in the proportions of total ginsenosides, polysaccharides and nonpolar constituents, the elder ginseng containing some special ginsenosides may be one reason for obvious differences in biological effects between elder GRE groups and younger groups. Some evidence from previous studies also clearly indicated that Rh2 and compound K have strong effects on some cultured cancer cells in vitro experiments [13-18]. Moreover, it has been reported that ginsenosides in ginseng roots will increase slowly in the first several years and then increase sharply in the fifth and sixth years [19].

In this study, if the time interval of detection is 4 hours, the fluctuation of K562 cell growth curves of H12, H14 and H16 groups, implying the imbalance between proliferation and apoptosis of cells, will be detected. However, if the time interval is 8 hours, the all the growth curves will have no fluctuation. In previous studies, the time interval of detection was 12 hours or even longer [3-7, 20-22]. It is a possible reason that the cyclical fluctuation of cell growth curves was not detected in previous studies. Our results demonstrate that the time interval should be shortened to observe the imbalance between proliferation and apoptosis.

In conclusion, elder ginsengs not only often accumulate more metabolic products than younger individuals, but also the GRE of the former has quite different bioactivity from that of the latter. Although ginsenosides and polysaccharides are regarded as the primary active ingredients of ginseng and can inhibit the proliferation of K562 cells, total GRE can significantly stimulate the proliferation of these cells.

5. Acknowledgements

We thank Dr. Jianhui Xie and Dr. Haiyan Zhu for their help in experiments. We also thank Mr. Fugui Lyu and Mr. Shuiwen Wang for their help in sample collection. This study was supported by Science and Technology Community of Shanghai (Traditional Chinese Medicine Research Program, Grant No.08DZ1972500).

REFERENCES


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Supplementary Table. OD values of control, H5, H8, H12, H14 and H16 groups within 48 hours.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Control</th>
<th>H5</th>
<th>H8</th>
<th>H12</th>
<th>H14</th>
<th>H16</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.888 ± 0.0549&lt;sup&gt;cef&lt;/sup&gt;</td>
<td>0.881 ± 0.049&lt;sup&gt;cdef&lt;/sup&gt;</td>
<td>0.960 ± 0.0281&lt;sup&gt;abf&lt;/sup&gt;</td>
<td>0.954 ± 0.0300&lt;sup&gt;abf&lt;/sup&gt;</td>
<td>0.984 ± 0.0407&lt;sup&gt;abf&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>0.968 ± 0.0564&lt;sup&gt;bcef&lt;/sup&gt;</td>
<td>0.986 ± 0.0430&lt;sup&gt;cdef&lt;/sup&gt;</td>
<td>1.204 ± 0.0454&lt;sup&gt;abcdef&lt;/sup&gt;</td>
<td>1.022 ± 0.0512&lt;sup&gt;cdef&lt;/sup&gt;</td>
<td>1.059 ± 0.0492&lt;sup&gt;bief&lt;/sup&gt;</td>
<td>1.184 ± 0.0377&lt;sup&gt;bde&lt;/sup&gt;</td>
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<td>8</td>
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<td>1.326 ± 0.0320&lt;sup&gt;abcdef&lt;/sup&gt;</td>
<td>0.877 ± 0.0418&lt;sup&gt;abcdef&lt;/sup&gt;</td>
<td>0.892 ± 0.0643&lt;sup&gt;abcdef&lt;/sup&gt;</td>
<td>1.074 ± 0.0320&lt;sup&gt;bde&lt;/sup&gt;</td>
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</tr>
</tbody>
</table>

Values are expressed as mean ±SD. *means P < 0.05 vs. Control group. †means P < 0.05 vs. H5 group. ‡means P < 0.05 vs. H8 group. §means P < 0.05 vs. H12 group. ¶means P < 0.05 vs. H14 group. ‖means P < 0.05 vs. H16 group.