Experimental and epidemiological evidence on non-organ specific cancer preventive effect of Korean ginseng and identification of active compounds

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Abstract
Panax ginseng C.A. Meyer has been the most highly recognized medicinal herb in the Orient. The prolonged administration of red ginseng extract significantly inhibits the incidence of hepatoma and also proliferation of pulmonary tumors induced by aflatoxin B1 and urethane. Statistically significant anticarcinogenic effects were in aged or heat treated extracts of ginseng and red ginseng made by steaming in a 9 weeks medium-term anticarcinogenicity test using benzo[a]pyrene. In case–control studies, odds ratios (OR) of the cancer of lip, oral cavity and pharynx, larynx, lung, esophagus, stomach, liver, pancreas, ovary, and colorectum were significantly reduced. As to the type of ginseng, the ORs for cancer were reduced in user of fresh ginseng extract intakers, white ginseng extract, white ginseng powder, and red ginseng. In a cohort study with 5 years follow-up conducted in a ginseng cultivation area, ginseng users had a decreased relative risk (RR) compared with non-users. The relative risks (RRs) of ginseng users were decreased in gastric cancer and lung cancer. These findings strongly suggest that Panax ginseng C.A. Meyer cultivated in Korea has non-organ specific cancer preventive effects against various cancers. To investigate the active components for cancer prevention, several fractions of fresh and red ginseng and four semi-synthetic ginsenoside Rh1, Rh2, Rg3 and Rg5, the major saponin components in red ginseng, were prepared among the ginsenosides. By using Yun’s model, Rg3 and Rg5 showed statistically significant reduction of lung tumor incidence and Rh2 had a tendency to decrease the incidence. In conclusion, these results strongly suggested that Panax ginseng C.A. Meyer cultivated in Korea is a non-organ specific cancer preventive against human cancers and also indicated that the anticarcinogenicity or human cancer preventive effect of Panax ginseng is due to ginsenoside Rg3, Rg5 and Rh2.

Keywords: Panax ginseng C.A. Meyer; Anticarcinogenesis; Cancer chemoprevention; Case–control studies; Cohort study; Active compounds

1. Introduction
Cancer research in Korea started later than other countries. In 1949, Dr. Il Sun Yun analyzed 632 cases of histologically diagnosed cancer patients who were admitted between 1925 and 1939 to the Severance Medical College (the forerunner of Yonsei Medical School), and reported the finding in Cancer Research [1]. This paper showed findings similar to those in Western countries with no remarkable exceptions; namely, carcinomas of penile skin and liver, which were prevalent among Koreans. Subsequently, he studied the influence of splenic extract on mouse skin...
cancer induced by methylcholanthrene, and presented a series of papers. Seventeen years later, in 1966, the Korean cancer Research Association was established. In 1977, growing interests of molecular biologists, immunologists, biochemists, and medical doctors in mutagenesis and carcinogenesis research led to the birth of the Korean Society of Environmental Mutagens and Carcinogens.

In spite of incessant new discoveries and clinical application of cancer chemotherapeutics [2–4], 5 years survival rates of cancer patients were less than one in five in the 1930s, and increased to one in four in the 1940s and one in three in the 1960s. By the late 1970s, the goal of 5 years cancer survival rate was to achieve one out of two patients, but it was not successful [5]. At present, it is still only two out of five patients, corresponding to 40% of “observed survival rate”.

At that time, a few Korean scientists who realized such limitations in cancer treatment came to the conclusion that primary prevention was a means of non-toxic natural products. Since then, we have been trying to find non-toxic cancer chemopreventives or immunopreventives in natural products which have been used historically.

Ginseng has been used as one of the most valuable natural tonics in the Orient for over 2000 years (Fig. 1) [6]. In Asia, it has been widely believed that ginseng is a miraculous medicine or mysterious tonic, improves one’s physical condition, and prolongs life with long-term administration. Considering Shenong’s report in Liang Dynasty, China, sixth century that the long-term use of ginseng led to prolongation of life span [7]. We hypothesized that the life-prolongation effect of ginseng described by Shenong might be due to potential prevention of uncontrollable diseases such as cancer that could not effectively be treated even by modern medicine. Therefore, long- and medium-term experiments, two case–control studies, cohort study based on population and identification of active compounds in ginseng were carried out to evaluate the effect of ginseng on inhibition or prevention of carcinogenesis or cancer and results obtained are summarized below.
2. Pre-clinical studies on anticarcinogenicity of *Panax ginseng* C.A. Meyer

2.1. Long-term anticarcinogenicity study

In 1978, a long-term anticarcinogenicity experiments, lasting 67 weeks involving 2000 mice was carried out to evaluate the effect of ginseng on inhibition or prevention of carcinogenesis, induced by various chemical carcinogens such as 9,10-dimethyl-1,2-benzanthracene (DMBA), urethane, *N*-2-fluorenylacetamide (FAA), aflatoxin B1 and tobacco smoke condensates. In the group sacrificed at 28 weeks after the treatment (urethan combined with red ginseng extract), there was 22% decrease (*P* < 0.05) in the incidence of lung adenoma. In the group sacrificed at 56 weeks after birth (aflatoxin B1 combined with red ginseng extract), there was 75% (*P* < 0.05) decrease in the incidence of liver cancer, thus demonstrating that these natural products could offer hope for human cancer prevention (Table 1) [8,9].

2.2. Medium-term anticarcinogenicity test (Yun’s model) on ginseng

Soon thereafter, we realized that it was necessary to develop a medium-term model for further experimentation. N-GP (S) newborn mice less than 24 h old were injected once in the scapular region subcutaneously with 0.02 ml of benzo(a)pyrene (0.5 mg suspension of BP in aqueous gelatin). After weaning, red ginseng extract made of *Panax ginseng* C.A. Meyer cultivated in Korea were administered for 6 weeks through drinking water or diets. All mice were sacrificed at the 9th week after birth and the index of lung tumor incidence was scored by the method described previously [10–13].

To verify the utility of this model, ascorbic acid, carrot, β-carotene, soybean lecithin, spinach, *Sesamum indicum*, *Ganoderma lucidum*, caffeine, biochanin A, red ginseng extract (6 years old), fresh ginseng (4 years old) and 13-cis retinoic acid, some of which are known to have anticarcinogenic activity in various animals, were also tested. Ascorbic acid, soybean lecithin, *Ganoderma lucidum*, caffeine and red ginseng extract showed inhibition of lung tumor incidence, while fresh ginseng, carrot, β-carotene, spinach and 13-cis retinoic acid did not (Table 2) [10–15]. The observation on β-carotene was consistent with the negative results of ATBC trial [16], CARET trial [17,18] and Physicians’ Health Study [19,20], thereby suggesting that this model for lung tumor induced by 0.5 mg of BP was useful for the screening of cancer preventive agents.

Recently, loci responsible for mouse lung tumor susceptibility have been mapped to chromosomes 6, 9, 17, and 19, while those linked to lung tumor resistance have been mapped to chromosome 4, 11, 12, and 18. Known candidate susceptibility or resistance genes include the K-ras proto-oncogene on chromosome 6 and the p16 tumor suppressor gene on chromosome 4. With evidence of considerable overlap between the genetic alterations that underlie human and mouse tumorigenesis, the mouse lung tumor model has been expanded to include pre-clinical screening of chemopreventive agents.

### Table 1

<table>
<thead>
<tr>
<th>Sacrifice (weeks)</th>
<th>Weight of lung</th>
<th>Incidence of lung adenoma</th>
<th>Diffuse infiltration</th>
<th>Incidence of hepatoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMBA 48</td>
<td>21% decrease</td>
<td>–</td>
<td>63% decrease</td>
<td>–</td>
</tr>
<tr>
<td>Urethane 28</td>
<td>–</td>
<td>22% decrease*</td>
<td>–</td>
<td>75% decrease</td>
</tr>
<tr>
<td>Aflatoxin B1 56</td>
<td>29% decrease</td>
<td>–</td>
<td>–</td>
<td>75% decrease</td>
</tr>
</tbody>
</table>

DMBA: 9,10-dimethyl-1,2-benzanthracene.

* P < 0.05.

### Table 2

<table>
<thead>
<tr>
<th>Negative</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrot</td>
<td>Ascorbic acid</td>
</tr>
<tr>
<td>Fresh ginseng (4 years old)</td>
<td>Soybean lecithin</td>
</tr>
<tr>
<td>Spinach</td>
<td><em>Ganoderma lucidum</em></td>
</tr>
<tr>
<td>β-Carotene</td>
<td>Red ginseng extract (6 years)</td>
</tr>
<tr>
<td>Sesameum indicum</td>
<td>Caffeine</td>
</tr>
<tr>
<td>13-cis retinoic acid</td>
<td>Capsaicin</td>
</tr>
<tr>
<td>French wine</td>
<td>Biochanin A</td>
</tr>
<tr>
<td>Refined rice wine</td>
<td>2-Allylthiopyrazine</td>
</tr>
<tr>
<td>Authentic honey</td>
<td></td>
</tr>
</tbody>
</table>
agents against human lung cancers [21]. This mouse lung tumor model has been adopted by researchers, including Chemoprevention Branch of the NCI. Furthermore, this model system also confirmed negative anticarcinogenicity effect of 9-cis retinoic acid, 4-HPR and oltipraz that were known to be promising cancer preventive agents in NCI recommended models [22].

2.3. Dependency of anticarcinogenicity on the types and ages of ginseng

Using Yun’s model, we further investigated whether fresh or white ginseng had similar anticarcinogenic effects and also whether these effects were dependent on the types and ages of the ginseng. Among fresh ginseng of 1.5, 3, 4, 5, and 6 years of age, significant anticarcinogenic effects were observed with powders and extracts of 6 years dried fresh ginseng, 5 and 6 years white ginsengs and 4, 5 and 6 years red ginsengs. It was concluded from the above results that the anticarcinogenicity of ginseng varied depending on the type and age (Table 3) [23–26].

3. Epidemiological studies

3.1. Case–control study on 905 pairs

The effect of ginseng consumption on the risk of cancer was evaluated by interviewing 905 pairs of cases and controls matched by age, sex, and date of admission to the Korea Cancer Center Hospital, Seoul. Of the 905 cases, 562 (62%) had history of ginseng intake compared to 674 of the control (75%), a statistically significant difference ($P < 0.01$). The odds ratio (OR) of cancer in relation to ginseng intake was 0.56 (95% confidence interval (CI), 0.45–0.69). Ginseng extract and powder were shown to be more effective than fresh sliced ginseng, juice, or tea in reducing the OR. ORs for decreasing levels of ginseng intake were 1.00, 0.58, 0.43, and 0.25 for males and 1.00, 0.81, 0.56, and 0.52 for females. A trend test showed significant decrease in the number of cancer cases among those who reported increasing frequency of ginseng intake for males ($P < 10^{-5}$) as well as for females (Table 4) ($P < 0.05$). The reliability of recall for ginseng use was assessed twice by interviewing one-tenth of the randomly selected subjects using the same questionnaire. The overall agreement in reported ginseng use between the two interviews was 0.85, and the Kappa value was 0.71 ($P < 0.01$). These results strongly support the hypothesis that ginseng has cancer preventive effects, as suggested by previous animal experiments [27]. The Lancet stated in an editorial in 1992 that ginseng consumption reduces the risks for all cancer types. The article included an example of the “non-organ specific approach” to cancer chemoprevention [28].

3.2. Case–control study on 1987 pairs

In order to further explore (a) the types of ginseng products that have the most prominent cancer

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Fresh ginseng Powder</th>
<th>Extract</th>
<th>White ginseng Powder</th>
<th>Extract</th>
<th>Red ginseng Powder</th>
<th>Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP</td>
<td>41.1</td>
<td>63.9</td>
<td>45.0</td>
<td>41.3</td>
<td>48.6</td>
<td>47.5</td>
</tr>
<tr>
<td>BP + 1.5 years</td>
<td>31.2</td>
<td>48.3</td>
<td>–</td>
<td>–</td>
<td>37.9</td>
<td>40.7</td>
</tr>
<tr>
<td>BP + 3</td>
<td>30.0</td>
<td>52.5</td>
<td>41.3</td>
<td>32.0</td>
<td>41.7</td>
<td>35.0</td>
</tr>
<tr>
<td>BP + 4</td>
<td>31.3</td>
<td>51.8</td>
<td>38.9</td>
<td>46.0</td>
<td>31.7*</td>
<td>30.1*</td>
</tr>
<tr>
<td>BP + 5</td>
<td>30.3</td>
<td>47.5</td>
<td>31.6*</td>
<td>44.0</td>
<td>28.3**</td>
<td>30.0*</td>
</tr>
<tr>
<td>BP + 6</td>
<td>27.8*</td>
<td>44.1*</td>
<td>25.3***</td>
<td>26.5*</td>
<td>25.4***</td>
<td>26.3*</td>
</tr>
</tbody>
</table>

BP: benzo(a)pyrene. Years: age of ginseng at harvest.

* $P < 0.05$.
** $P < 0.02$.
*** $P < 0.01$. 

Table 3
Anticarcinogenic effects of Panax ginseng C.A. Meyer according to type and age; using Yun’s 9 weeks medium-term anticarcinogenicity model
Table 4

<table>
<thead>
<tr>
<th>Frequency of ginseng intake</th>
<th>Male</th>
<th>Female</th>
<th>Odds ratio (95% CI)</th>
<th>Male</th>
<th>Female</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No intake</td>
<td>117</td>
<td>56</td>
<td>1.00</td>
<td>226</td>
<td>175</td>
<td>1.00</td>
</tr>
<tr>
<td>1–3 times/year</td>
<td>132</td>
<td>108</td>
<td>0.58 (0.38–0.90)</td>
<td>111</td>
<td>106</td>
<td>0.81 (0.57–1.15)</td>
</tr>
<tr>
<td>4–11 times/year</td>
<td>104</td>
<td>115</td>
<td>0.43 (0.28–0.67)</td>
<td>75</td>
<td>103</td>
<td>0.56 (0.39–0.82)</td>
</tr>
<tr>
<td>Once/month or more</td>
<td>83</td>
<td>157</td>
<td>0.25 (0.16–0.39)</td>
<td>57</td>
<td>85</td>
<td>0.52 (0.35–0.78)</td>
</tr>
<tr>
<td>Total</td>
<td>436</td>
<td>436</td>
<td>469</td>
<td>469</td>
<td>469</td>
<td></td>
</tr>
</tbody>
</table>

Linear trend test (1 d.f.) 45.59 (P < 0.0001) 3.98 (P < 0.05)

\( \chi^2 \) homogeneity test (3 d.f.) 47.28 (P < 0.0001) 16.53 (P < 0.001)

preventive effect, (b) the reproducibility of the dose–response relationship, (c) the duration of ginseng consumption to have significant preventive effect, (d) the types of cancer that can be prevented by ginseng, and (e) the effect of ginseng on cancers associated with smoking, we increased the number of subjects for a case–control study to 1987 pairs. In this study, as with the other study ginseng users had a lower risk (OR; 0.50) for cancers compared with non-users. As for the type of ginseng, fresh ginseng extract users had 0.37 ORs for cancer, 0.57 for white ginseng extract users, 0.30 for white ginseng powder users, and 0.20 for red ginseng users. Those who took fresh ginseng slices, fresh ginseng juice, and white ginseng tea, however, showed no decrease of risk. Overall, the risk decreased as the frequency and duration of ginseng intake increased, thus showing a dose–response relationship. As for the sites of cancers, the ORs were 0.47 for cancer of lip, oral cavity, and pharynx; 0.36 for stomach cancer; 0.42 for colorectal cancer; 0.48 for liver cancer; 0.22 for pancreatic cancer; 0.18 for laryngeal cancer; 0.55 for lung cancer; 0.15 for ovarian cancer; and 0.48 for other cancers. In cancers of female breast, uterine cervix, urinary bladder, and thyroid gland, however, there was no association with ginseng intake (Table 5). In cancers of lung, lip, oral cavity and pharynx, and liver, smokers who took ginseng showed decreased OR compared with smokers with no ginseng intake. These findings support the view that ginseng use decreases the risk for most cancers compared to non-use [29,30].

Table 5

<table>
<thead>
<tr>
<th>Site of cancer</th>
<th>Cases (never taken/ever taken)</th>
<th>Controls (never taken/ever taken)</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lip, oral cavity, and pharynx</td>
<td>67/92</td>
<td>40/119</td>
<td>0.47 (0.29 ± 0.76)</td>
</tr>
<tr>
<td>Esophagus</td>
<td>40/47</td>
<td>14/73</td>
<td>0.20 (0.09 ± 0.38)</td>
</tr>
<tr>
<td>Stomach</td>
<td>142/138</td>
<td>76/224</td>
<td>0.16 (0.09 ± 0.32)</td>
</tr>
<tr>
<td>Colon and rectum</td>
<td>55/63</td>
<td>32/68</td>
<td>0.42 (0.24 ± 0.74)</td>
</tr>
<tr>
<td>Liver</td>
<td>108/156</td>
<td>67/197</td>
<td>0.48 (0.33 ± 0.70)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>12/11</td>
<td>5/18</td>
<td>0.22 (0.05 ± 0.95)</td>
</tr>
<tr>
<td>Larynx</td>
<td>21/19</td>
<td>8/32</td>
<td>0.18 (0.06 ± 0.54)</td>
</tr>
<tr>
<td>Lung</td>
<td>120/156</td>
<td>81/195</td>
<td>0.55 (0.38 ± 0.79)</td>
</tr>
<tr>
<td>Female breast</td>
<td>82/92</td>
<td>70/109</td>
<td>0.63 (0.40 ± 1.05)</td>
</tr>
<tr>
<td>Cervix uteri</td>
<td>156/146</td>
<td>31/270</td>
<td>0.72 (0.52 ± 1.01)</td>
</tr>
<tr>
<td>Ovary</td>
<td>17/5</td>
<td>8/14</td>
<td>0.15 (0.04 ± 0.60)</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>23/40</td>
<td>16/47</td>
<td>0.64 (0.28 ± 1.47)</td>
</tr>
<tr>
<td>Thyroid gland</td>
<td>16/24</td>
<td>14/26</td>
<td>0.96 (0.38 ± 2.44)</td>
</tr>
<tr>
<td>Other</td>
<td>53/81</td>
<td>35/79</td>
<td>0.48 (0.27 ± 0.85)</td>
</tr>
</tbody>
</table>

Adjusted for age, sex, marital status, education, smoking, and alcohol consumption.
4. Prospective study for population

Since the above promising findings were obtained at the beginning of our case–control study and also there were no human studies on the preventive effects of ginseng on cancer, we performed a more reliable cohort study in ginseng cultivation area, Kangwha-eup from August 1987 to December 1992. We studied 4634 (2362 men, 2272 women) adults over 40 years old who completed a questionnaire on ginseng intake. Among 355 (7.7%) total deaths, cancers accounted for 79 (22.8%). Subjects with cancers totaled 137 (3.0%), with 58 (1.3%) alive at the end of the study period and 79 (1.7%) deaths. Of 4634 persons eligible for analysis, 70.5% (3267) were ginseng users. Ginseng intakers had a decreased risk (RR = 0.40, 95% CI: 0.28–0.56), compared with non-intakers. On the type of ginseng, the RRs was 0.31 (95% CI: 0.13–0.74) for fresh ginseng extract users and 0.34 (95% CI: 0.20–0.53) for users of multiple combinations. There was no cancer death among 24 red ginseng intakers. There was a decreased risk with increasing frequency of ginseng intake, showing a dose–response relationship. Newly diagnosed cancer cases were identified: 42 stomach, 24 lung, 14 liver and 57 at other sites (Table 6). The RR of ginseng intakers were 0.33 (95% CI: 0.18–0.57) in gastric cancer and 0.30 (95% CI: 0.14–0.65) in lung cancer. Among ginseng preparations, fresh ginseng extract users were significantly associated with a decreased risk of gastric cancer (RR = 0.33, 95% CI: 0.12–0.88). These results strongly suggest that Panax ginseng C.A. Meyer has non-toxic and non-organ specific preventive effects against cancers [31,32].

5. Identification of active components in ginseng

To identify its active components, various extracts of red and fresh ginseng were tested for anticarcinogenicity using Yun’s 9 weeks medium model. For fractionation of red ginseng, powdered red ginseng of 6 years old Panax ginseng cultivated in Korea was used for water extract, panaxadiol, panaxatrol type saponin and hexane fraction [33,34]. For the fractionation of fresh ginseng, air-dried and powdered fresh ginseng was used for 70% ethanol extract, water extract, total saponin and polysaccharide [33,35]. For the preparation of ginsenoside Rg3 and Rgs mixture, the ginsenoside Rh1 obtained from Korean ginseng was used [33,36]. The ginseng fractions were administered to newborn mice after weaning for 6 weeks:

Table 6

<table>
<thead>
<tr>
<th>Ginseng intake</th>
<th>No. of subjects</th>
<th>Stomach (42)</th>
<th>Lung (24)</th>
<th>Liver (14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No intake</td>
<td>1283</td>
<td>23 1.00</td>
<td>14 1.00</td>
<td>4 1.00</td>
</tr>
<tr>
<td>Ginseng intake</td>
<td>3167</td>
<td>19 0.33*</td>
<td>10 0.30*</td>
<td>10 0.86</td>
</tr>
<tr>
<td>Slices and juice</td>
<td>236</td>
<td>2 0.57</td>
<td>1 0.67</td>
<td>10 1.97</td>
</tr>
<tr>
<td>Extract</td>
<td>296</td>
<td>1 0.53*</td>
<td>0.12–0.88</td>
<td>1 0.28</td>
</tr>
<tr>
<td>White ginseng</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Powder</td>
<td>147</td>
<td>1 0.24</td>
<td>0.03–1.84</td>
<td>2 0.35</td>
</tr>
<tr>
<td>Extract</td>
<td>68</td>
<td>2 1.34</td>
<td>0.50–5.97</td>
<td>1 0.67</td>
</tr>
<tr>
<td>Tea</td>
<td>442</td>
<td>6 0.64</td>
<td>0.26–2.63</td>
<td>4 0.80</td>
</tr>
<tr>
<td>Red ginseng</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boiled chicken with</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>young ginseng root</td>
<td>381</td>
<td>5 0.43</td>
<td>0.12–1.43</td>
<td>1 0.35</td>
</tr>
</tbody>
</table>

RR: relative risks, adjusted for age, sex, education, smoking and alcohol consumption. CI: confidence interval. Value in parentheses indicate number of cancer cases.

* P < 0.05.
lung adenoma incidence was 46.8% with 0.5 mg of benzo(a)pyrene. However, when treated together with red ginseng extract (2 ml/m, drinking water) the incidence was significantly reduced to 27.5% (inhibition rate of 36.8%). Panaxadiol type saponin (56.6 μg/ml), hexane fraction (21.9 μg/ml) and water fraction (811.4 μg/ml) showed 42.3, 41.3, 40.0 and 41.3% incidence, respectively, with no significant reduction observed [33].

The next step was to compare anticarcinogenicity of 6 years fresh ginseng fractions of 70% ethanol extract (4.72 mg/ml), water extract (6.4 mg/ml), total saponin (0.44 mg/ml) and polysaccharide (1.32 mg/ml). Lung adenoma incidence was 58.3% in 0.5 mg of BP alone treated mice. Treatment with ethanol extracts and total saponin together with BP reduced lung tumor incidence significantly to 44.1% (inhibition rate 25.7%) and 43.3% (inhibition rate 24.4%), respectively, but the incidence of polysaccharide treatment was 50.0%, thus showing no significant reduction.

A third experiment was to examine which components of red ginseng were responsible for anticarcinogenicity. For the experiment, Rg3 and Rg5 mixtures were selected, because they are present in large amounts in red ginseng and their semi-syntheses are possible. Lung adenoma incidence was 60.0% in 0.5 mg of BP treated mice, however, treatment with Rg3 + Rg5 mixture along with BP significantly reduced the incidence to 45.0% (inhibition rate 25.0%). The results showed that Rg3 + Rg5 had anticarcinogenic effect in Yun’s medium-term model [33].

6. Identification of active compounds in red ginseng

6.1. Effective components of red ginseng

For long-term experiment, we used red ginseng extract for oral administration because it was water soluble, and found that there was significant decrease in the incidence of lung adenoma and liver cancer by red ginseng administration [8,9]. However, fresh ginseng (4 years old) was ineffective as anticarcinogenic or cancer preventive agent both in experimental animal models [11] and in human case–control [27,28] and cohort study [31]. However, when treated with heat, the fresh or white ginseng and red ginseng were highly effective in cancer prevention.

Thirty-five kinds of ginsenosides have so far been isolated from fresh, white or red ginseng, among which 22 kinds of ginsenosides are protopanaxadiol type and 12 of them are protopanaxatriol type, and only one ginsenoside Ro is oleanane type [32]. Since ginsenosides are generally labile under acidic conditions, ordinary acidic hydrolysis is always accompanied by many side reactions such as cyclization of side chains, glycosyl elimination and epimerization of carbone-20 by SN1 reaction. Therefore, the chemical transformations of secondary metabolites occur during steaming preparative process for red ginseng. The unique components of red ginseng are known as 20(S)-ginsenoside Rg3, ginsenosides Rh2, Rs1, or Re, Re1, Rx1 and Rg5, plus notoginsenoside-Rh1 in protopanaxadiol group, and 20(R)-ginsenoside Rg2, 20(R)-ginsenoside-Rh1, ginsenoside Rh2 and F4 in protopanaxatriol group. Malonyl-ginsenoside Rh1, Rb1, Re, and Rd are found only in white ginseng [32].

However, most of the ginsenosides are present in red ginseng in minute quantities, there, it is extremely difficult to obtain enough amount for in vivo study.

6.2. Nine weeks medium-term anticarcinogenicity test on ginsenosides of red ginseng

Ginsenoside Rg3 was isolated as previously described [36], and Rg3 and Rh2 were by usual procedure from Korean red ginseng [37,38]. In brief, a mixture of 20(R)- and 20(S)-ginsenoside Rg3 was obtained under mild acidic hydrolysis from protopanaxadiol saponins, ginsenoside Rb1, Rb2, Rc and Rd. The product was acetylated to give peracetates, which were further converted into 20(S)-ginsenoside Rg5, 20(R)-ginsenoside Rg3, 20(S)-ginsenoside Rh2 and 20(R)-ginsenoside Rh2 by direct alkaline treatment, Rh1 was prepared from ginsenoside Re by similar procedure [38,39]. All of the ginsenosides obtained were identified by physiochemical and spectral analysis (IR, MASS, H-NMR, 13C-NMR). N-GP (S). Subsequently, employing Yun’s 9 weeks medium-term mouse lung tumor anticarcinogenicity test model, mice were subcutaneously injected once with 0.02 ml of BP suspension (0.5 mg, in 1% aqueous gelatin). Two control groups consisted of normal animals, (no ginseng was given) and red ginseng administered (but not BP-treated). Red ginseng
Fig. 2. Chemical structure of ginsenoside Rh$_1$, Rh$_2$, Rg$_3$, and Rg$_5$. Glc-: $\beta$-d-glucopyranosyl; Glc-Glc-: $(1\rightarrow2)\beta$-d-glucopyranosyl.

Table 7. Anticarcinogenicity of ginsenosides Rh$_1$, Rh$_2$, Rg$_3$, and Rg$_5$ using Yun’s 9 weeks medium-term anticarcinogenicity model.

<table>
<thead>
<tr>
<th>Experimental groups and treatment</th>
<th>Dose</th>
<th>Route</th>
<th>Sex</th>
<th>No. of mice</th>
<th>Incidence</th>
<th>Multiplicity (mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>M</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>M + F</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>BP: 0.5 mg/head SC</td>
<td>M</td>
<td>25</td>
<td>14 (56.0)</td>
<td>1.20 ± 1.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>25</td>
<td>16 (64.0)</td>
<td>1.40 ± 2.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>M + F</td>
<td>50</td>
<td>30 (60.0)</td>
<td>2.00 ± 1.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BP:</td>
<td>M</td>
<td>30</td>
<td>15 (50.0)</td>
<td>1.20 ± 1.54</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>30</td>
<td>16 (53.3)</td>
<td>1.49 ± 1.86</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>M + F</td>
<td>60</td>
<td>51 (51.7)</td>
<td>1.63 ± 1.27</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BP: 0.5 mg/head SC</td>
<td>M</td>
<td>30</td>
<td>13 (43.3)</td>
<td>0.77 ± 1.14</td>
<td></td>
</tr>
<tr>
<td>Rh$_1$: 80 $\mu$g/ml DW</td>
<td></td>
<td>F</td>
<td>30</td>
<td>16 (53.3)</td>
<td>1.53 ± 1.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>M + F</td>
<td>60</td>
<td>29 (48.3)</td>
<td>1.15 ± 1.61</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BP:</td>
<td>M</td>
<td>30</td>
<td>13 (43.3)</td>
<td>0.67 ± 0.96</td>
<td></td>
</tr>
<tr>
<td>Rh$_2$: 80 $\mu$g/ml DW</td>
<td></td>
<td>F</td>
<td>30</td>
<td>15 (50.0)</td>
<td>1.03 ± 1.27</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>M + F</td>
<td>60</td>
<td>28 (46.7)</td>
<td>0.85 ± 1.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BP:</td>
<td>M</td>
<td>30</td>
<td>13 (43.3)</td>
<td>0.85 ± 1.21</td>
<td></td>
</tr>
<tr>
<td>Rg$_3$: 80 $\mu$g/ml DW</td>
<td></td>
<td>F</td>
<td>30</td>
<td>14 (46.7)</td>
<td>1.33 ± 2.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>M + F</td>
<td>60</td>
<td>27 (45.0)</td>
<td>1.08 ± 2.21</td>
<td></td>
</tr>
</tbody>
</table>

SC: subcutaneous administration; DW: drinking water.

* $P < 0.05$. 
extract (2 mg/ml of drinking water) was given immedi-
ately after weaning. The following ginsenosides
were administered in drinking water (80 μg/ml) for 6
weeks; ginsenosides Rh1, Rh2, Rg3 and Rg5 (Fig. 2).
Drinking water was changed every other day and diet
was prepared every other week. All mice were sac-
crificed at the 9th week after birth, and the adenomas
were counted. No lung tumor was observed in both
normal control mice (no BP administered) and mice
treated singularly with ginsenoside Rh1, Rh2, Rg3 or
Rg5. However, a lung tumor incidence of 60% was
found with the group of mice which were given once
with 0.5 mg of BP. On the other hand, when treated
with 2 mg of red ginseng extract for 6 weeks after
BP pretreatment, a 43.3% of incidence was observed
(27.8% decrease), which was statistically significant.
Ginsenoside Rh1 (51.7% lung incidence) had no signif-
icant effect on the BP-induced lung tumor. Although
ginsenoside Rh2 together with BP caused 48.3%
(19.5% decrease) incidence of tumor, this was consid-
ered to be a trend toward inhibition but not statistically
significant.

When given with 80 μg/ml concentration for 6
weeks after BP administration, Rg3 showed statisti-
cally significant decrease (22.2%) of lung tumor
incidence (46.7%; P < 0.05), whereas Rg3 and BP
had biologically significant 45.0% (25.0% decrease)
incidence (P < 0.05) (Table 7).

The above results obtained by Yun’s model, there-
fore, demonstrated that, among the four ginsenosides
purified from red ginseng, Rg3 and Rg5 yield a statis-
tically significant reduction of lung tumor incidence,
while Rh2 had a tendency of decreasing the incidence.
These results suggest on overall efficacy of ginseng as
a cancer chemopreventive [38].

7. Discussion

Our strategy now to ameliorate this scourge of can-
cer is to switch from therapeutic approaches to chemoprevention by identifying effective natural products as chemopreventive agents. Anticarcinogenic effects of Korean red ginseng were earlier observed in 1980 by long-term [8,9] or Yun’s 9 weeks medium-term exper-
iments [11–13] with mouse lung tumor, and the anti-
carcinogenicity of ginseng was found to be dependent
on the type and age of ginseng [23–25].

Recently, there have been many reports from
various countries to support anticarcinogenicity of
ginseng. Ginsenosides from Panax notoginseng
(Sanchi ginseng) can inhibit early antigen activa-
tion of Epstein-Barr virus, and also show anticar-
cinogenic effects in a two-stage mouse skin model
with DMBA and in lung carcinogenesis induced by
4-nitroquinolin-1-oxide [40]. Anticarcinogenic effects
of majonoside from Vietnamese ginseng have also
been shown in two-stage tests of mouse skin [41]. In
a study of ginseng on the development of diethylni-
trosamine (DEN) induced liver cancer in rats, only
one of seven animals developed a tumor, when given
ginseng, compared with all of the six control rats with
tumor [42]. Tissue-culture biomass tincture obtained
from cultured cells of Panax ginseng had strong
inhibitory effect on rat mammary adenocarcinoma
induced by methyl-N-nitrosourea in rats [43] and also
on the development of experimental uterine cervix
and vaginal tumors induced by intravaginal appli-
cation of 7,12-dimethylbenz(a)anthracene (DMBA)
in mice [44]. Ginseng inhibited the development of
brain and spinal cord tumors induced by transplacen-
tal administration of N-ethyl-N-nitrosourea (ENU)
in rats [45]. Red ginseng extracts had significant in-
hibitory effect on skin cancer formation in a two-stage
carcinogenesis mouse model. At 50–400 mg/kg, red
ginseng extract inhibited development of skin papil-
lomas in mice induced by DMBA and croton oil, and
decreased the incidence, while prolonging the latent
period before tumor occurrence, and reduced tumor
number per mouse in a dose-dependent manner [46].

Dietary administration of red ginseng powder in
the initiation stage of carcinogenesis in the colon
of rats suppressed preneoplastic lesions induced by
1,2-dimethylhydrazine; this effect was associated
with suppression of cell proliferation [47]. Moreover,
when red ginseng powder was delivered during the
initiation phase of carcinogenesis, simultaneously
with exposure to aflatoxin B1 (AFB1) only a mod-
est inhibition of aberrant crypt foci (ACF) was noted
at only 0.5 mg/kg dose of ginseng. Thus, the effect of
ginseng during the initiation of ACFs from the nor-
mal mucosa is relatively weak. However, after ACFs
have been established in the colon during a 4 weeks
outgrowth period, an additional 4 weeks exposure to
red ginseng at a dose of 2 mg/kg significantly reduced
the incidence of ACFs. It was also noted that the
ginseng powder during the post-initiation time decreased the number of multiple crypt containing foci. These results suggest that some factor in red ginseng powder inhibits the growth of preneoplastic lesions in the rat colon induced by azoxymethane [48]. In MCF-7 breast-cancer cells, the ability of American ginseng to induce estrogen-regulated gene pS2 and affects cell-cycle were assessed by northern blot analysis and by flow cytometry, respectively. Both American ginseng and oestradiol equally induced pS2 RNA expression, but only the ginseng decreased cell proliferation \( (P < 0.005) \) in a dose-dependent manner [49]. Oral administration of red ginseng extracts significantly suppressed spontaneous liver tumor formation in C3H/He male mice [50].

We succeeded to purify and identify four ginsenosides, including ginsenoside Rh2, Rg2, Rg3 and Rf. Among the four ginsenosides, Rg3 and Rg5 showed statistically significant reduction of lung tumor incidence and Rh2 had a tendency of decreasing the incidence. These results strongly demonstrate that the anticarcinogenicity or human cancer preventive effect of ginseng is due to ginsenoside Rg3, Rg5 and Rh2 present in Korean red ginseng [38,51].

There are a few reports regarding possible mechanism of ginsenosides on cancer preventive effects. The methanol extract of heat-processed Panax ginseng C.A. Meyer attenuates the lipid peroxidation in rat brain homogenates and is also capable of scavenging superoxide generated by xanthine oxidase or by 12-O-tetradecanoylphorbol-13-acetate (TPA) in differentiated human promyelocytic leukemia (HL-60) cells. topical application of the same extract onto back of ICR mice also suppressed TPA-induced skin tumor promotion [52]. Likewise, topical application of ginsenoside Rg3 significantly inhibited TPA-induced mouse epidermal ornithine decarboxylase activity and skin tumor promotion. Expression of cyclooxygenase-2 (COX-2) in TPA-stimulated mouse skin was markedly suppressed by Rg3 pretreatment. In addition, Rg3 inhibited TPA-stimulated activation of NF-κB and extracellular-regulated protein kinase (ERK), one of the mitogen-activated protein (MAP) kinases in mouse skin as well as cultured human breast epithelial cells (MCA-10A) [53].

In case-control studies of 905 pairs and 1987 pairs, there was noticeable decrease in cancer risk for takers of ginseng extract compared to users of fresh ginseng, and the decrease was greatly dependent on frequency of ginseng intake [27,28]. The OR of ginseng consumers decreased in all kinds of cancers. These results strongly support the hypothesis that ginseng has cancer preventive effects, as suggested by the earlier animal experiments. Furthermore, the results of cohort study also suggest that Panax ginseng C.A. Meyer has non-organ specific preventive effect against cancer [54], in support of the previous case-control studies [31].

In conclusion, ginseng has been proven to be non-organ specific cancer preventive. Since minor ginsenosides in red ginseng are shown to be active components, it is hoped that synthetic ginsenosides would be available as soon as possible and their genuine merit as cancer preventive should be tested by clinical intervention.

References


