Anticancer effect of mountain ginseng Pharmacopuncture to the nude mouse of lung carcinoma induced by NCI-H460 human non-small cell lung cancer cells

Kwon Ki Rok*

*Dept. of Acupuncture & Moxibustion, Oriental Medical College, Sangji University

ABSTRACT

Objectives: This study was performed to examine the anticancer effect of mountain ginseng Pharmacopuncture (MGP) to the nude mouse of lung carcinoma induced by NCI-H460 human non-small lung cancer cells.

Methods: Human lung cancer (NCI-H460) cells were cultured and applied to evaluate anti-tumor activity in nude mice. After confirmed tumor growth in mice, MGP was treated per 0.1 ml/kg dose to intraperitoneal and intravenous injection everyday for four weeks. And checked the changes in body weights, tumor volume, mean survival time and percent, increase in life span, histo-pathological findings, organ weights, and blood chemistry levels.

Results: The results of in vivo study showed that MGP may have potential as growth inhibitor of solid tumor induced NCI-H460 without marked side effects. MGP inhibited dosage-dependently the growth of NCI-H460 cell-transplanted solid tumor compared with the control group. And mean survival time of MGP treated group was prolonged comparing with control group. Generally the group of intravenous injection is more effective than intraperitoneal injection.

Conclusion: These results were suggested that MGP may be a useful anticancer agent for therapy of human lung cancer. And follow study need for the certain evidence.

Key Words: mountain ginseng Pharmacopuncture (MGP), lung carcinoma, NCI-H460 human non-small lung cancer cells.

Received: 10.01.28
Accepted: 10.02.25

I. Introduction

Ginseng, *Panax ginseng* C.A. Meyer, is a commonly used herbal medicine in oriental countries including China, Japan and Korea for thousands of years [1]. The genus name ‘*Panax*’ was derived from Greek. *Pannax* means ‘cure all’. The herbal root is named ginseng, because it is shaped like a man. Ginseng is a deciduous perennial plant that belongs to the *Araliaceae* family [2].

The regulation of apoptosis, which is a programmed cell death, has become an area of extensive study in cancer research and has been considered an ideal way of eliminating precancerous and/or cancerous cells [3, 4]. Most cancer cells can block apoptosis, which allows them to survive despite the genetic and morphologic transformations. Therefore, the induction of apoptotic cell death is an important mechanism in many anti-cancer drugs [5].

In previous study, I confirmed the apoptotic...
effects of mountain ginseng Pharmacopuncture (MGP) on lung cancer cells[6].

Ginseng is widely accepted in both Korea and China that mountain ginseng is more active than cultivated ginseng in chemo-prevention[7]. And I reported the safety and anti-cancer effects of MGP in pre-studies[8-14].

But a lot of past studies related with MGP were performed by intravenous injection. And the problem is that intravenous injection is not traditional treatment in korean medicine. So, I want to compare the effect of intravenous and intraperitoneal injection, and confirming what is more effective method.

Now I attempts to elucidate the effect of MGP in human lung carcinoma cells and the underlying intra-cellular signal transform pathways involved in regulating apoptosis. And also evaluated the anti-tumor activity of MGP for NCI-H460 human lung carcinoma cells in vivo nude mouse xenograft model.

II. Materials and methods.

2-1 Manufacturing process of MGP

Cultivated mountain Ginsengs for MGP were estimated about 8-10 years old and manufactured under the following process: Cultivated mountain ginseng is rinsed in a running water(Fig. 1) and then decocted for 2 hours in distilled water. Remnants are then removed and decoction went through distillation before yielding the desired herbal acupuncture. Then the Pharmacopuncture was filtered using 0.45 μm, 0.2 μm and 0.1 μm filtering paper and then kept in the container. Finally, Pharmacopuncture is sterilized before being used(Fig. 2).

2-2 Cell line and culture conditions

Human lung cancer (NCI-H460) cells were from the Korean Cell Line Bank (Seoul, Korea). The cells were grown in the RPMI-1640 medium supplemented with 10% fetal bovine serum, 100U/ml penicillin and 100mg/ml streptomycin.

2-3. Experimental animal

Balb/c nude mice (male, 9-11 weeks, n=25) weighing 21-25g were purchased from Japan SLC, Inc (SLC Inc., Shizuoka, Japan) and were housed under specific pathogen-free conditions according to the guidelines of Chungbuk National University Animal Care and Use Committee.

The animal room was controlled for temperature (22 ± 2°C), light (12 hr light/dark cycle) and humidity (50 ± 10%). All laboratory feed pellets and beddings were autoclaved.

2-4. Experimental design

The tumor regression model on nude mouse has been successfully applied to evaluate anti-tumor activity on common. So the model was used to evaluate suppression of solid tumor on MGP. When the tumor volume reached 100mm³, the nude mice, xenografted tumor fragment were randomly distributed into three groups (positive control group, MGP 0.1ml with intraperitoneally treated group, and MGP 0.1ml with intravenous injection treated group; injected dose(0.1ml) was selected by clinical use), and each group was consist of seven mice. MGP was intraperitoneally, intravenous injected everyday for four weeks.

2-5. Cell preparation

NCI-H460 cell were cultured in 260ml tissue culture flasks in Eagle's minimum essential medium (EMEM) containing 100U/ml penicillin and 10%
heat inactivated fetal calf serum in an incubator with 95% air and 5% CO₂ at 37 ºC. When the cells became confluent, they were washed twice with Hank's balanced salt solution (HBSS), trypsinized with 0.25% trypsin in HBSS, washed twice with fresh culture medium[15].

2-6. Xenografts

NCI-H460 cells were washed twice with Hank's balanced salt solution (HBSS), trypsinized with 0.25% trypsin, and washed twice with fresh culture medium. NCI-H460 cells, 1x10⁶ cells/mouse in 0.1 ml HBSS, were injected subcutaneously into the flank of mice using a 26 gauge needle. After 14-16 day observation, apparent solid tumor mass was removed from 3 mice out of 5 mice inoculated with NCI-H460 cells. Tumor fragments (3x3x3 mm³) were made by trimming with a knife, and xenografted into the flank of new mice using a troca. The suppressive effect of anticancer agents on solid tumor was evaluated in a tumor-regression model. In brief, from the day tumor volume reached 100 mm³, the mice xenografted with a tumor fragment were administrated intra-peritoneal and intravenous injection with MGP every day for 28 days.

2-7. Changes in tumor volume

The changes in the size of tumor mass were recorded twice a week by measuring with a digital calipers. That is, the largest and smallest diameters were measured in each mouse, and the tumor volume was estimated according to the formula[16, 17].

\[ V(\text{mean tumor volume}) = \frac{(A*B^2)}{2} \] (A=the largest diameter, B=smallest diameter)

where V is the tumor volume in mm³, and A and B are the largest and smallest tumor diameters in mm, respectively. Based on the regression of tumor volume, the antitumor activities of treatment were expressed by inhibition rate.

\[ IR(\text{inhibition rate})(\%) = \frac{[(CV-TV)/TV]}{TV} \times 100 \]

(CV=Control group tumor volume, TV=Treatment group tumor volume) where CV and TV are tumor volumes in control (water) and treatment groups, respectively. Also, the tumor weights were measured on the final day after sacrifice of animals and removal of the tumor mass.

2-8. Mean survival time and percent increase in life span

To compare the life span of mice xenografted with NCI-H460 tumor fragments, survival time was estimated from the day when the tumor volume reached 1,500 mm³ as described previously, and % increase in life span (%ILS) were calculated according to the equation:

\[ \%\text{ILS}(\text{increase in life span}) = \frac{[(T-C)/C]}{T} \times 100, \]

where C and T are mean survival days of mice in control and treatment groups, respectively[16, 17].

2-9. Blood chemistry levels

Blood samples were centrifuged at 1,400 x g at 4 ºC for 10min. The supernatants (serum) were used for the determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT), with an automatic analyzer(7170, Hitachi, Tokyo, Japan).

2-10. Statistical analysis

The results are presented as mean ± S.D., and the significance of difference between the mean of control and treatment groups was analyzed using one-way analysis of variance (ANOVA) followed by a
Dunnett's t-test correction, paired t-test, and linear regression analysis. Statistical significance was determined at the level of p<0.05 or p<0.01 (SPSS Version 10.0).

III. Results

3-1. Changes in body weights

Changes in body weights of each group were shown in Fig. 3. The mean body weights of MGP treated group were mild increased than those NCI-H460 cell only group (25.33-26.64g), with value of 25.70-27.50g in intraperitoneally treated and 27.30-31.18g in intravenous injection treated group. But no significant differences were observed (MGP treated groups and positive control group) (Fig. 3).

3-2. Changes in tumor volume

Treatment with MGP (intraperitoneally, intravenous injection) inhibited the growth of NCI-H460 cell-transplanted solid tumor compared with value of positive control group (Fig. 4). In 28nd day, the mean tumor volume of MGP with intraperitoneally and intravenous injection treatment group were lower significantly than that of the positive control group throughout the study period.

3-3. Inhibition rate (I.R) on tumor volume

IR (%) of each group are shown in Table 1. From eighth day to final day (28 day), each of IR (%) were tendency to dose-dependence (intraperitoneal injection G. I.R 69.96% < intravenous injection G. I.R 41.95% at 14 day) (Table 1.).

3-4. Tumor weights and volume (plethysmography)

Final tumor weights and volume of each group are shown Table 2. Tumor weight and volume of positive control group (NCI-H460 only) was 2.52 ±0.75g and 4.67±1.18cm³ on the final day. Tumor weight and volume of MGP intraperitoneal group was 2.20±0.93g and 4.65±1.97cm³. Tumor weight and volume of MGP intravenous injection group was 2.39±1.25g and 2.42±1.19cm³. Compared to the NCI-H460 cell alone group, tumor weight and volume of MGP intravenous injection group was significantly decreased.

3-5. Mean survival time and Percent increase in life span (% ILS)

Mean survival time and Percent increase in life span are shown in Table 3. and Fig. 5. The positive control group (NCI-H460 cell alone) survived 18.29±3.59 days. Mean survival time of low MGP with intraperitoneal treated group was extended to 18.71±5.31 days and 2.30 %ILS. Also mean survival time of MGP with intravenous injection treated group was 19.13±7.10 days and 4.59 %ILS. Each of %ILS were increased in dose dependent manner. MGP with intravenous injection treated group was slightly difference compared to the positive control (NCI-H460 cell alone) group.

3-6. Histopathological findings (light microscopy) and organ weights

Absolute organ weights of kidney, liver, spleen, heart and lung are shown Table 4. Especially MGP (intraperitoneal and intravenous injection) treated group were a marked decreased in liver weights (p<0.01). But light microscopic histopathological examination showed liver tissue of mice treated with MGP intraperitoneal and intravenous injected
group did not show any specific lesions compared to the liver tissue of NCI-H460 cells-bearing mice with positive control (Fig. 6).

**3-7. Blood chemistry levels, on the final day.**

The test substances in the blood level CA, CRE, ALT, UN results showed reduction in the significance MGP intravenous injected group ($p<0.05$, $p<0.01$), and ALP results showed significantly increased by NCI-H460 cell alone group ($p<0.01$) (Table 5).

**IV. Discussion and Conclusion**

The major aim of this study was to examine whether MGP is effective and what is more useful method between intraperitoneal and intravenous injection for the MGP in human lung carcinoma NCI-H460 cells in vivo.

Human tumor xenografts in immunodeficient animal models provide a means to evaluate potential anti-tumor drugs in preclinical studies and are applicable for studying many different types of human malignancies[19].

So, we also conducted in vivo experiments of nude mice with NCI-H460 cell-transplanted tumor during 28 days, which were treated with MGP 0.1 ml with intraperitoneal and intravenous injection. For example, as time-dependence changes of tumor volume were measured by a digital calipers. And results of removed tumors volume were measured by a plethysmometer at final day. All tumor volume results were suppressed with dose-dependence tendency, but each one of groups was different in significantly tendency.

The results showed that MGP inhibited dosage-dependently the growth of NCI-H460 cell-transplanted solid tumor compared with the control group. At 21 day, numerical value of solid tumor showed 1793.48mm$^3$ (MGP with intraperitoneal injected group) and 2806.47mm$^3$ (Control group) respectively (Fig. 4). We calculated the results of 21 day because the death rate overwhelmed 50% on the 21 day. Compared with the control group, experimental group showed tumor growth inhibition from 11th day (MGP intraperitoneal injected group I.R. 79.81% < MGP with intravenous injected group I.R 51.52%) in dose dependent manner (Table 1). At the final day, Tumor weights showed 2.20 ± 0.93 MGP intraperitoneal injected group, 2.39 ± 1.25g(MGP with intravenous injected group) and 2.52 ± 0.75g (Control group) (Table 2.).

Mean survival time and the rate of increasing life span of dosage MGP with intraperitoneal injected group was 18.71 ± 5.31 days and 2.30% ILS. Dosage MGP with intravenous injected group showed 19.13 ± 7.10 days and 4.59% ILS. The control group (NCI-H460 cell alone) survived only 18.29 ± 3.59 days (Fig. 5, Table 3.).

In relative organ weights, especially MGP treated groups were a marked decreased in liver weights ($p<0.01$). But light microscopic histopathological examination showed liver tissue of mice treated with MGP did not show any specific lesions compared with liver tissue of NCI-H460 cells-bearing mice with positive control (Table 4., Fig. 6).

Biochemical blood analysis AST, PHOS levels did not significance result in test. The test substances in the blood level CA, CRE, ALT, UN results showed reduction in the significance MGP with intravenous injected group ($p<0.05$, $p<0.01$). And ALP levels MGP with intravenous injected group($7.97 ± 2.76$IU/L) compared to NCI-H460 cell alone group($67.62 ± 7.27$ IU/L) significance increased. ($p<0.01$). Also UN levels MGP with intraperitoneal injected group($22.02 ± 2.26$mg/dl) showed significance decreased($p<0.01$) compared to NCI-H460 cell alone group($29.38 ± 3.68$ mg/dl) (Table 5.).

The results showed that MGP may have potential as growth inhibitor of solid tumor induced NCI-
H460 without marked side effects. These results were suggested that MGP may be a useful anti-cancer agent for therapy of human lung cancer and intravenous injection is more effective than intraperitoneal injection to treating MGP method.

V. References

Anticancer effect of mountain ginseng Pharmacopuncture to the nude mouse of lung carcinoma induced by NCI-H460 human non-small cell lung cancer cells


After nude mice with NCI-H460 cell-transplanted, tumor were treated with 28 daily dose of 0.1 ml (intraperitoneally, intravenous injection) MGP. The body weights of nude mice in the positive control, NCI-H460 cell alone (■, n=7), MGP with intraperitoneal group(◇, n=7), MGP 0.1 ml with intravenous injection(▲ n=7) was measured two times a week.

Fig. 1 Shape of cultivated mountain ginseng

Fig. 2 Manufacturing process of MGP

Fig. 3 Changes in body weights in nude mice bearing NCI-H460 cell solid tumor
After nude mice with NCI-H460 cell-transplanted, tumor were treated with 28 daily dose of 0.1 ml (intraperitoneally, intravenous injection) MGP. The body weights of nude mice in the positive control, NCI-H460 cell alone (■, n=7), MGP with intraperitoneal group(◇, n=7), MGP 0.1 ml with intravenous injection(▲ n=7) was measured two times a week.
Fig. 4 Time-course of increase in tumor volumes in NCI-H460 cells-bearing mice treated with MGP
The mice xenografted with tumor fragments were treated with the anticancer agents from the day when tumor mass reached 100 mm$^3$. The length and width of solid tumor in the positive control, NCI-H460 cell alone (■, n=7), MGP with intraperitoneal group (◆, n=7), and MGP with intravenous group (▲ n=7) were measured two times a week and tumor volume was evaluated.

Fig. 5 Mean survival time in NCI-H460 cells-bearing mice treated with MGP
Results are shown as mean ± S. D.
* Significant difference from positive control (NCI-H460 cell alone) group at p(0.05.

Fig. 6 Gross finding of liver of NCI-H460 cells-bearing mice with positive control
NCI-H460 cell alone (A), MGP with intraperitoneal group (B), MGP with intravenous group (C)(×100).
Table 1. Inhibition rate (I.R) on tumor volume of NCI-H460 tumor-bearing mice(%).

<table>
<thead>
<tr>
<th></th>
<th>0 day</th>
<th>4 day</th>
<th>7 day</th>
<th>11 day</th>
<th>14 day</th>
<th>18 day</th>
<th>21 day</th>
<th>25 day</th>
<th>28 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCI-H460 cell alone</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>MGP with intraperitoneal G.</td>
<td>100.00</td>
<td>84.13</td>
<td>74.88</td>
<td>79.81</td>
<td>69.96</td>
<td>72.82</td>
<td>63.90</td>
<td>68.28</td>
<td>68.87</td>
</tr>
<tr>
<td>MGP with intravenous G.</td>
<td>135.18</td>
<td>79.40</td>
<td>56.80</td>
<td>51.52</td>
<td>41.95*</td>
<td>32.24*</td>
<td>55.08</td>
<td>54.35</td>
<td>63.60</td>
</tr>
</tbody>
</table>

Table 2. Tumor weights in mice xenografted with NCI-H460 cells on the final day.

<table>
<thead>
<tr>
<th></th>
<th>NCI-H460 cell alone</th>
<th>MGP intraperitoneal injection G.</th>
<th>MGP intravenous injection G.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor weight (g)</td>
<td>2.52 ± 0.75</td>
<td>2.20 ± 0.93</td>
<td>2.39 ± 1.25</td>
</tr>
<tr>
<td>Tumor volume (g^3)</td>
<td>4.67 ± 1.18</td>
<td>4.65 ± 1.97</td>
<td>2.42 ± 1.19**</td>
</tr>
</tbody>
</table>

Table 3. Percent increase in life span (%ILS) of NCI-H460 tumor-bearing mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean survival time (day)</th>
<th>%ILS</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCI-H460 cell alone</td>
<td>18.29 ± 3.59</td>
<td>0</td>
</tr>
<tr>
<td>MGP intraperitoneal injection G.</td>
<td>18.71 ± 5.31</td>
<td>2.30</td>
</tr>
<tr>
<td>MGP intravenous injection G.</td>
<td>19.13 ± 7.10</td>
<td>4.59</td>
</tr>
</tbody>
</table>

Table 4. Organ weights, on the final day, of NCI-H460 tumor-bearing mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Absolute organ weights</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body weight (g)</td>
</tr>
<tr>
<td>NCI-H460 cell alone</td>
<td>26.64 ± 1.61</td>
</tr>
<tr>
<td>MGP intraperitoneal injection G.</td>
<td>27.50 ± 2.15</td>
</tr>
<tr>
<td>MGP intravenous injection G.</td>
<td>31.18 ± 2.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Absolute organ weights</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kidney (g)</td>
</tr>
<tr>
<td></td>
<td>L</td>
</tr>
<tr>
<td>NCI-H460 cell alone</td>
<td>0.22 ± 0.06</td>
</tr>
<tr>
<td>MGP intraperitoneal injection G.</td>
<td>0.23 ± 0.03</td>
</tr>
<tr>
<td>MGP intravenous injection G.</td>
<td>0.25 ± 0.02</td>
</tr>
</tbody>
</table>
### Treatment Relative organ weights

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Liver (g)</th>
<th>Spleen (g)</th>
<th>Heart (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCI-H460 cell alone</td>
<td>26.64 ± 1.60</td>
<td>6.61 ± 0.22</td>
<td>1.27 ± 0.22</td>
<td>0.58 ± 0.03</td>
</tr>
<tr>
<td>MGP intraperitoneal injection G.</td>
<td>27.50 ± 2.15</td>
<td>5.73 ± 0.20**</td>
<td>1.37 ± 0.37</td>
<td>0.59 ± 0.03</td>
</tr>
<tr>
<td>MGP intravenous injection G.</td>
<td>31.18 ± 2.02</td>
<td>5.17 ± 0.51**</td>
<td>0.80 ± 0.71</td>
<td>0.53 ± 0.05*</td>
</tr>
</tbody>
</table>

### Treatment Relative organ weights

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Kidney (g)</th>
<th>Testis (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>R</td>
</tr>
<tr>
<td>NCI-H460 cell alone</td>
<td>0.82 ± 0.22</td>
<td>0.80 ± 0.19</td>
</tr>
<tr>
<td>MGP intraperitoneal injection G.</td>
<td>0.83 ± 0.06</td>
<td>0.89 ± 0.10</td>
</tr>
<tr>
<td>MGP intravenous injection G.</td>
<td>0.79 ± 0.03</td>
<td>0.82 ± 0.05</td>
</tr>
</tbody>
</table>

Significant difference from positive control (NCI-H460 cell alone) group at *p<0.05, **p<0.01.

### Table 5. Blood chemistry levels, on the final day, of NCI-H460 tumor-bearing mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ALP IU/U</th>
<th>CA mg/dl</th>
<th>CRE mg/dl</th>
<th>ALT IU/L</th>
<th>AST IU/L</th>
<th>PHOS mg/dl</th>
<th>UN mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCI-H460 cell only</td>
<td>67.62 ± 7.27</td>
<td>10.18 ± 0.44</td>
<td>0.35 ± 0.05</td>
<td>184.08 ± 43.85</td>
<td>25.75 ± 4.62</td>
<td>9.27 ± 0.99</td>
<td>29.38 ± 3.68</td>
</tr>
<tr>
<td>MGP intraperitoneal injection G.</td>
<td>67.23 ± 9.33</td>
<td>9.92 ± 0.59</td>
<td>0.32 ± 0.08</td>
<td>143.30 ± 47.50</td>
<td>21.32 ± 7.82</td>
<td>7.98 ± 0.75</td>
<td>22.02 ± 2.26**</td>
</tr>
<tr>
<td>MGP intravenous injection G.</td>
<td>214.99 ± 94.89**</td>
<td>7.97 ± 2.76**</td>
<td>0.25 ± 0.09*</td>
<td>87.45 ± 35.82**</td>
<td>47.05 ± 25.77</td>
<td>7.70 ± 2.56</td>
<td>19.79 ± 5.37**</td>
</tr>
</tbody>
</table>

Results are mean ± S. D.
* Significant difference from positive control (NCI-H460 cell alone) group at p<0.05.
** Significant difference from positive control (NCI-H460 cell alone) group at p<0.01.