Inhibition of Tumor Formation and Changes in Hepatic Enzyme Activities by Kimchi Extracts in Sarcoma-180 Cell Transplanted Mice

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Abstract

Inhibitory effects of the methanol extract, hexane extract, methanol soluble fraction (MSF) and juice from 3 weeks fermented kimchi on the tumor formation in sarcoma-180 cell transplanted mice were studied. Effects of the solvent extracts and juice of the kimchi on the levels of lipid peroxide, glutathione, and the enzyme activities of the liver were also investigated in normal and sarcoma-180 cell transplanted mice. At 32 days following transplantation, MSF reduced the tumor formation by 54% compared with the control group, resulting in the smallest tumor weight. Lipid peroxide content in liver increased by the transplantation of sarcoma-180 cells. However, it decreased when MSF of kimchi was treated to the mice. MSF also suppressed xanthine oxidase activity in cytosol of the liver cells in mice transplanted by sarcoma-180 cells. Kimchi extracts had no inhibitory effect on hepatic aminopyrine-N-demethylase activity in sarcoma-180 cell transplanted or normal mice. Methanol extract and hexane extract of kimchi slightly increased hepatic glutathione contents in sarcoma-180 treated mice. The injection of MSF from kimchi markedly increased glutathione levels in the liver of sarcoma-180 treated mice compared to the controls. The MSF recovered the activities of hepatic glutathione reductase and glutathione S-transferase that decreased by the injection of sarcoma-180 cells. These results showed that MSF of kimchi could suppress the growth of tumors, inhibiting lipid peroxide production and xanthine oxidase activity, in mice. We also suggested that kimchi extract might play an important role in the prevention of cancer by enhancement of the glutathione level itself as well as via glutathione reductase and glutathione S-transferase.

Key words: kimchi, antitumor effect, hepatic enzymes, sarcoma-180 cell, lipid peroxide, glutathione

INTRODUCTION

Kimchi is a traditional fermented vegetable food in Korea. The major raw material such as Korean cabbage and radish are salted and after prebrining, blended with various spices and other minor ingredients, and then fermented. There are many types of kimchi, depending on the raw ingredient and processing methods used.

Kimchi fermentation is initiated by the microorganisms which are originally present in the raw vegetable substances, but the fermentation is gradually dominated by the lactic acid bacteria. Numerous physiochemical and biological factors affect the growth and sequential appearance of principal microorganisms involved in the fermentation (1,2). Complex biochemical changes occur depending on the environmental conditions before, during, and after fermentation (3).

Kimchi has typical biochemical and nutritional properties, and health-related functions. It is an important source of vitamins, minerals, dietary fiber, and other nutrients. The vitamin B group and ascorbic acid are already present in the raw materials and may be synthesized during the fermentation process (4,5). The minerals are definitely increased with the addition of salt-pickled fish products, fresh seafood and meats.

Therefore, kimchi also has high levels of minerals of Ca, Fe, K, and of organic acids (1).

Carotenoids (6), ascorbic acid (7,8), dietary fiber, and flavonoids (9) in yellow-green vegetables used as the major material of kimchi showed antimutagenic and anticancer activities. Those are believed to suppress the formation of carcinogenic or mutagenic compounds, the mutagenicity induced by several mutagens and to show antitumor effects in mice. Other ingredients such as red pepper (10), garlic (11), and lactic acid bacteria (12,13), dominating the fermentation of kimchi, are believed to have antimutagenic and anticarcinogenic effects. It was reported that properly ripened kimchi with 3% salt concentration itself had inhibitory effects on the growth of cancer cells and might have anticarcinogenic activities because of the bioactive components or nutrients (14,15). In our previous study (16), kimchi extracts and juices had inhibitory effects on the growth and [3H] thymidine incorporation of human cancer cells, but had no toxicity to normal cells.

In order to study the effects of kimchi on toxic or detoxification systems in liver, solvent extracts and juice supernatants from 3 weeks fermented kimchi were treated to Balb/c mice transplanted with the sarcoma 180 cells. The weight of tumor and the contents of lipid peroxide and glutathione in
liver were determined. The changes in the activities of hepatic enzymes such as xanthine oxidase, aminopyrine-N-demethylase, glutathione reductase and glutathione S-transferase were also investigated.

MATERIALS AND METHODS

Preparation of kimchi
Korean cabbage grown in Kimhae, Kyungsan province, was used as the major raw ingredient for kimchi. Garlic, ginger and red pepper powder were purchased from a local market. Korean cabbage was divided into 8 pieces, soaked in 10% brine for 10 hours and rinsed with fresh water. Drained Korean cabbage was cut into 4 to 5 cm pieces. The ratio of ingredients for kimchi was 2 of red pepper powder, 2 of crushed garlic and 2 of crushed ginger to 100 of salted Korean cabbage. The final salt concentration of kimchi was adjusted to 3%. Kimchi was fermented for 3 weeks at 5°C and then used as the test sample (3 weeks fermented kimchi, pH 4.3).

Preparation of solvent extracts and juice supernatants from kimchi
The 3 weeks fermented kimchi was freeze-dried and minced in a blender. The mincedkimchi samples (25 g) were extracted with methanol (500 ml), three times, by shaking for 8 hours to obtain methanol extract. Other mincedkimchi samples (25 g) were extracted with hexane (500 ml) by the same method as methanol extraction. After extraction with the hexane, 500 ml of methanol was added to the residues and shaken for 16 hours, followed by reflux for 90 minutes in a 70–80°C water bath. After filtration, the methanol soluble fraction (MSF) was taken. The kimchi extracts were dried by a rotary vacuum evaporator (Buchi 011 & 461, Switzerland) and then dissolved in phosphate buffered solution (PBS).

The 3 weeks fermented kimchi was also minced by a blender and centrifuged at 10,000 rpm for 10 minutes. The supernatant was filtrated through a 0.45 µm (pore size) membrane and then used as the test sample.

Animals
4 weeks old, male Balb/c mice were supplied by the Korean Chemistry Institute (Taejon, Korea). A basal diet and drinking water were available ad libitum. Mice were housed in polycarbonate cages with a 12 hr light/dark cycle in a temperature (21 ± 2°C) controlled room.

Solid tumor formation in mice
7 day-old sarcoma-180 ascites cells were transplanted subcutaneously into the left groin of Balb/c mice at a dose of 1 × 10⁸ cells/mouse. 0.5 mg/kg of methanol extract, 2.5 mg/kg of hexane extract and MSF, and 200 µl of juice supernatant from kimchi were injected i.p. once a day for 20 days from 24 hrs after transplantation. Following 32 days, the formed tumors were dissected and weighed.

Preparation of liver homogenates, microsomal and cytosol fractions
Livers were quickly removed, weighed and homogenized in a 0.25 M sucrose buffer containing 2 mM-mercaptoethanol (1:4, v/v) using a glass teflon homogenizer. The homogenate was centrifuged at 600 × g for 20 min. Then, the supernatant was centrifuged again at 105,000 × g for 60 min to obtain the upper fraction as cytosol. The pellet was resuspended in the same volume of 0.25 M sucrose buffer and centrifuged at 105,000 × g for 60 min to obtain the microsomal fraction. The homogenate was used for the determinations of the contents of lipid peroxide and glutathione. The activities of xanthine oxidase, glutathione reductase and glutathione S-transferase in the cytosol fraction were also investigated. And the microsomal fraction was used for the measurement of the activity of aminopyrine-N-demethylase.

Determinationsof lipid peroxide and glutathione levels in the cytosol fraction of mouse liver
The content of lipid peroxide was determined by the method of Okawa et al. (17) and represented as the content of malondialdehyde (nmol) per g of tissue. The glutathione level was measured as previously described (18).

Measurements for enzyme activities in the microsomal and the cytosolic fractions of mouse liver
The activity of xanthine oxidase was determined by the method of Strip and Della (19). The enzyme activity was defined as n mol of formed uric acid per mg of protein per min at 30°C. The activities of aminopyrine N-demethylase and glutathione reductase were measured using the methods described by Nash (20), and Mize and Longdon (21), respectively. Their activities were measured with formed formaldehyde, and glutathione (nmol) per mg protein per min. Glutathione S-transferase activity was measured by the method of Habig et al. (22) and defined as n mol of formed 2,4-dinitrophenyl-glutathione per mg protein per min.

Statistical analysis
The statistical analysis of the test data was performed by analysis of variance. Significant differences between treatment means were determined by using Duncan’s multiple range test.

RESULTS AND DISCUSSION
Antitumor effects of kimchi extracts
After a viability test, sarcoma-180 cells were transplanted subcutaneously into the left groin of Balb/c mice. Then, 0.5 mg/kg of methanol extract, 2.5 mg/kg of hexane extract, methanol soluble fraction (MSF) and juice supernatant from the 3 weeks fermented kimchi were i.p. injected once a day for 20 days from 24 hours following transplantation. The survival times of mice treated with the kimchi extracts and juice were not significantly different compared to those of the con-
trols (data not shown). However, at 32 days following transplantation, the tumor weights of mice treated with methanol extract, hexane extract and juice from kimchi decreased compared to those of the controls (Table 1). The MSF of the kimchi reduced tumor formation significantly in the mice (p<0.05). The MSF decreased tumor growth by 54% compared with the control and resulted in the smallest tumor weight.

Effects of kimchi extracts on the value of lipid peroxide and xanthine oxidase activity

Lipid peroxides are formed from polyunsaturated fats by oxidation in mitochondria, microsome, erythrocyte and platelets. Membranes of cells are rich in phospholipids containing polyunsaturated fatty acids. Therefore, the formation of lipid peroxide can cause cell damage and tumor formation in the stomachs of experimental animals (23).

Fig. 1 shows the contents of lipid peroxide in the liver homogenate of mice treated with kimchi extracts in normal or sarcoma-180 cells transplanted mice. With transplantation of cancer cells, the content of malondialdehyde increased to 25.7 n mol per g of tissue (Control: 16.9). The hexane extract and juice of the 3 weeks fermented kimchi slightly decreased hepatic malondialdehyde. However, 5 and 10 mg/kg of MSF markedly reduced the content of malondialdehyde to 18.7 and 17.0 n mol per g of tissue, respectively.

It was believed that the activity of xanthine oxidase increased during the infectious process. Xanthine oxidase generated free radicals such as superoxide anion and hydrogen peroxide, and caused cellular deterioration (24,25). In this study, the transplantation of sarcoma-180 cells increased the activity of xanthine oxidase in the cytosol fraction of the liver (Fig. 2). However, the MSF of kimchi suppressed the activity of hepatic xanthine oxidase, being measured by the content of uric acid.

As shown in the above results, especially kimchi MSF markedly decreased lipid peroxide production in the non-microsomal system and xanthine oxidase activity in the cytosol of the liver in sarcoma-180 transplanted mice.

Table 1. Antitumor activities of methanol extract, hexane extract, methanol soluble fraction (MSF) and juice from 3 weeks fermented kimchi in tumor bearing Balb/c mice with sarcoma-180 cells)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tumor weight (g)</th>
<th>Inhibition rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sacoma-180×Control</td>
<td>4.32±1.2a</td>
<td>-</td>
</tr>
<tr>
<td>Sacoma-180×Methanol ext</td>
<td>3.40±0.8ab</td>
<td>21.3</td>
</tr>
<tr>
<td>Sacoma-180×Hexane ext</td>
<td>3.57±1.5ab</td>
<td>17.4</td>
</tr>
<tr>
<td>Sacoma-180×MSF</td>
<td>1.98±0.8a</td>
<td>54.2</td>
</tr>
<tr>
<td>Sacoma-180×Juice</td>
<td>2.80±1.3ab</td>
<td>35.2</td>
</tr>
</tbody>
</table>

a,bMeans with different letters beside symbols are significantly different at the p<0.05 level by Duncan's multiple range test.

7-days old sarcoma-180 ascites cells were s.c. transplanted into the left groin of inbred strain, male Balb/c mice. 0.5 mg/kg of methanol extract, 2.5 mg/kg of hexane extract, MSF, and juice from kimchi or the equal volume of phosphate buffered saline (control) was I.P. injected once a day for 20 days from 24 hrs following transplantation. All mice were sacrificed at 32 days following the transplantation, and tumor weight was measured.

Fig. 1. The effects of 3 weeks fermented kimchi extracts and juice on hepatic lipid peroxide content in normal and sarcoma-180 transplanted Balb/c mice).

The explanation is the same as shown in Table 1.

Fig. 2. The effects of 3 weeks fermented kimchi extracts and juice on cytosolic xanthine oxidase activity in the liver of normal and sarcoma-180 transplanted Balb/c mice.

The explanation is the same as shown in Table 1.

Effects of kimchi extracts on the value of glutathione and the activities of hepatic enzymes, detoxifying xenobiotics

Living systems have several mechanisms for metabolizing and detoxifying xenobiotics. The enzymes are responsible for the biotransformation in the biochemical pathways of the organism. The pathways of xenobiotic transformation are divided into phase I and phase II pathways (26). Phase I enzymes produced more polar and less lipophilic groups. Synthetic reaction or conjugation by phase II enzymes (such as glutathione S-transferase, UDP-glucuronyltransferase) resulted in the addition of endogenous molecules such as glucuronic acid, sulfate and glutathione (27,28).

Cytochrome P450 has received the most attention among phase I enzymes. It was divided into type I and type II,
based on drug binding type and site existence. Type I and type II catalyze aminopyrine and aniline as a substrate, and produce formaldehyde and p-aminophenol as a product, respectively, which is associated with the production of free radicals in the microsomal system (25). Table 2 shows the formaldehyde formation by microsomal aminopyrine-N-demethylase in normal and sarcoma-180 cell transplanted mice, after treatment with the extracts from the kimchi. Kimchi extracts had no inhibitory effect on hepatic aminopyrine-N-demethylase activity in sarcoma-180 cell transplanted or normal mice (p<0.05).

With these results, we suggested that the kimchi extracts might have an inhibitory action on the formation of free radicals in the non-microsomal system rather than the microsomal system in the mice.

Glutathione is believed to bind with electrophiles of various carcinogens and thus it blocks the binding of the carcinogen with DNA (29). Since almost all of the ultimate carcinogens or mutagens are electrophilic, the conjugation of glutathione with them by itself or via glutathione S-transferase is important in a mechanism for carcinogen detoxification (30). In red blood cells, glutathione protects them from oxidative damage and plays a key role in detoxification by reacting with hydrogen peroxide, organic peroxides, and the harmful by-products of aerobic life (31). In addition, glutathione is known as an antioxidant which is a possible inhibitor of chemical carcinogen (32).

As shown in Fig. 3, kimchi extracts had no effect on the hepatic glutathione content in normal mice. But the injection of sarcoma-180 cells decreased glutathione levels in the liver (control: 5.2 μmol/g vs S-180 treated control: 3.8 μmol/g). The methanol extract and hexane extract of kimchi slightly increased hepatic glutathione contents in sarcoma-180 cell treated mice. 2.5, 5 and 10 mg/kg of MSF increased the glutathione levels in those treated mice to 4.5, 4.5 and 5.0 μmol/g, respectively.

Glutathione reductase is involved in transformation of glutathione to the reduced form. Fig. 4 shows the activity of hepatic glutathione reductase in sarcoma-180 cell tested mice. The level decreased to 40.5 n mol/mg protein (vs control : 54.2 nmol/mg protein) by the injection of sarcoma-180 cells. However, 2.5, 5 and 10 mg/kg of MSF increased the glutathione reductase activity to 46.9, 50.5 and 50.0 n mol/mg protein, respectively.

Fig. 5 reveals the change of hepatic glutathione S-transferase activity by kimchi extracts and juice in normal or sarcoma-180 transplanted mice. The injection of sarcoma-180 cells decreased glutathione S-transferase activity to 102.5

Table 2. The effects of various 3 weeks fermented kimchi extracts on hepatic microsomal aminopyrine-N-demethylase activity in sarcoma-180 transplanted or not transplanted Balb/c mice.1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Activity</th>
<th>Formaldehyde formed</th>
<th>nmoles/mg protein/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>2.74 ± 0.46*</td>
<td></td>
</tr>
<tr>
<td>Methanol ext.</td>
<td></td>
<td>2.79 ± 0.57*</td>
<td></td>
</tr>
<tr>
<td>Hexane ext.</td>
<td></td>
<td>3.00 ± 1.08*</td>
<td></td>
</tr>
<tr>
<td>Juice</td>
<td></td>
<td>2.69 ± 0.32*</td>
<td></td>
</tr>
<tr>
<td>MSF (2.5 mg/kg)</td>
<td></td>
<td>2.83 ± 0.69*</td>
<td></td>
</tr>
<tr>
<td>MSF (5 mg/kg)</td>
<td></td>
<td>2.86 ± 1.53*</td>
<td></td>
</tr>
<tr>
<td>MSF (10 mg/kg)</td>
<td></td>
<td>2.77 ± 0.55*</td>
<td></td>
</tr>
<tr>
<td>S-180+control</td>
<td></td>
<td>2.80 ± 0.38*</td>
<td></td>
</tr>
<tr>
<td>S-180+Methanol ext.</td>
<td></td>
<td>2.83 ± 1.06*</td>
<td></td>
</tr>
<tr>
<td>S-180+Hexane ext.</td>
<td></td>
<td>2.70 ± 0.77*</td>
<td></td>
</tr>
<tr>
<td>S-180+Juice</td>
<td></td>
<td>2.65 ± 0.68*</td>
<td></td>
</tr>
<tr>
<td>S-180+MSF (2.5 mg/kg)</td>
<td></td>
<td>2.92 ± 0.54*</td>
<td></td>
</tr>
<tr>
<td>S-180+MSF (5 mg/kg)</td>
<td></td>
<td>2.88 ± 1.67*</td>
<td></td>
</tr>
<tr>
<td>S-180+MSF (10 mg/kg)</td>
<td></td>
<td>3.13 ± 0.81*</td>
<td></td>
</tr>
</tbody>
</table>

1The explanation is the same as shown in Table 1.
2Not significantly different at the p<0.05 level by Duncan's multiple range test.
Fig. 5. The effect of 3 weeks fermented kimchi extracts and juice on cytosolic glutathione S-transferase activity in the liver of normal and sarcoma-180 transplanted Balb/c mice.

The explanation is the same as shown in Table 1.

$n$ mol/mg protein from a control value of 155.0 $n$ mol/mg protein. 5 and 10 mg/kg of MSF recovered the glutathione S-transferase activity to 125.0 and 140.0 $n$ mol/mg protein, respectively, in sarcoma-180 cell transplanted mice.

From these results, the tumor cells in the mice decreased the hepatic glutathione level, the activities of glutathione reductase and glutathione S-transferase. However, MSF from kimchi seemed to recover the detoxifying systems.

In addition, it was known that glutathione S-transferase was involved in the detoxification of a large group of hydrophobic compounds bearing an electrophilic center by the conjugation with the thiol group of glutathione (33). A few studies have been carried out on compounds that inhibited carcinogenesis by increasing the glutathione S-transferase activity. The compounds of benzyl isothiocyanate, $\beta$-naphthoflavone, coumarin, $\alpha$-angelicalactone, disulfiram, indole-3-carbinol, indole-3-acetonitrile, and diets containing dried powdered preparations of brussels sprouts, cabbage, coffee beans, or tea leaves, increased the activity of glutathione S-transferase in the liver and small intestine of mice (33).

Therefore the increase of glutathione level and glutathione S-transferase activity by the MSF of kimchi in this study seems to be very beneficial in the prevention of cancer.

It was thought that, especially, the MSF of kimchi greatly suppressed the growth of tumors induced by the inhibition of lipid peroxide production and xanthine oxidase activity. It also seems that kimchi extract might play an important role in the prevention of cancer by glutathione itself as well as via glutathione reductase and glutathione S-transferase.

Based on our studies of kimchi, we suggested that 3 weeks fermented kimchi, properly ripened kimchi, could have an antitumor effect in vivo. But the question about how kimchi enhanced the hepatic enzyme activities and the glutathione level in mice still remains, and will be investigated continuously. And we expect that the active compounds of MSF from kimchi for antitumor effects will be identified in future investigations.

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