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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 teated 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 fermention is initiated by the microoganisms 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 [^{3}H] 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

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liver were determined. The changes in the activities of hepatic enzymes such as xanthine oxidase, aminopyrine-N-demethylase, glutathione reductase and glutathione *S*-trasferase were also investigated.

MATERIALS AND METHODS

Preparation of *kimchi*

Korean cabbage grown in *Kimhae, Kyungnam* 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 sizes. 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 minced *kimchi* samples (25 g) were extracted with methanol (500 ml), three times, by shaking for 8 hours to obtain methanol extract. Other minced *kimchi* 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 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 polycarbonare 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 1×10^6 cells/mouse. 0.5 mg/kg of methanol extracts, 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, g/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 activitiy of aminopyrine-N-demethylase.

Determinations of 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 (*n* mol) 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 (*n* mol) 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-dinitrobenzene-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 malnondiadehyde 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 malnondiadehyde. However, 5 and 10 mg/kg of MSF markedly reduced the content of malnondiadehyde 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* supressed 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[1].

Treatment	Tumor weight (g)	Inhibition rate (%)
Sacoma-180+Control	4.32 ± 1.2^{a}	-
Sacoma-180+Methanol ext.	3.40 ± 0.8^{ab}	21.3
Sacoma-180+Hexane ext.	3.57 ± 1.5^{ab}	17.4
Sacoma-180+MSF	1.98 ± 0.8^{b}	54.2
Sacoma-180+Juice	2.80 ± 1.3^{ab}	35.2

[a,b]Means with different letters beside symbols are significantly different at the $p<0.05$ level by Duncan's multiple range test.

[1)]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 sacrified at 32 days following the transplantation, and tumor weight was measured.

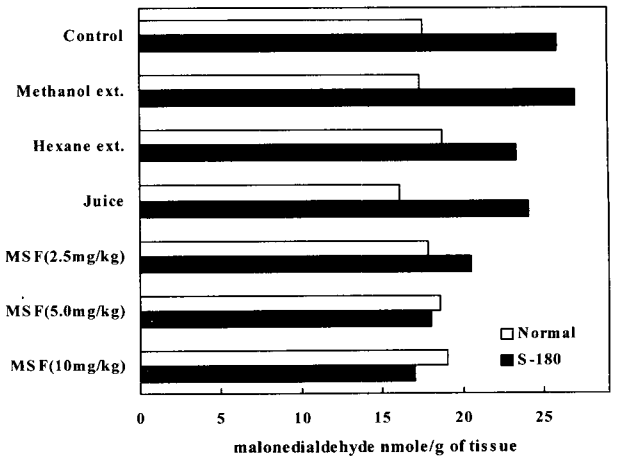

Fig. 1. The effects of 3 weeks fermented *kimchi* extracts and juice on hepatic lipid peroxide content in normal and sarcoma-180 transplanted Balb/c mice[1)].
[1)]The explanation is the same as shown in Table 1.

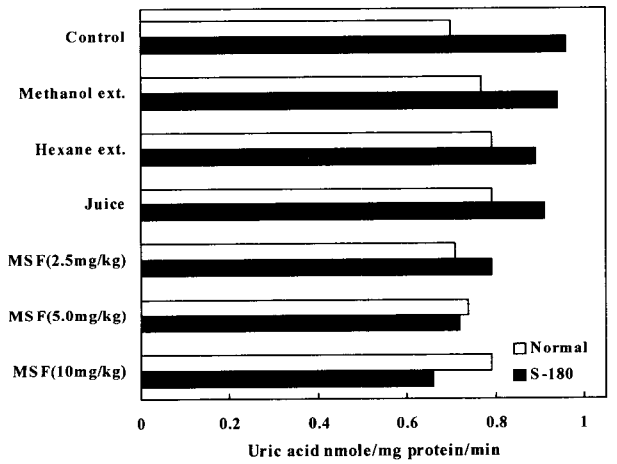

Fig. 2. The effects of 3 weeks fermented *kimchi* extracts and juice on cytosolic xanthine oxidase activity in the liver of normal and sarcoma-180 transplanted Balb/c mice[1)].
[1)]The explanation is the same as shown in Table 1.

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 P_{450} 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 eletrophilic, 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

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)].

Treatment	Activity: Formaldehyde formed nmole/mg protein/min
Control	2.74±0.46[a]
Methanol ext.	2.79±0.57[a]
Hexane ext.	3.00±1.08[a]
Juice	2.69±0.32[a]
MSF (2.5 mg/kg)	2.83±0.69[a]
MSF (5 mg/kg)	2.86±1.53[a]
MSF (10 mg/kg)	2.77±0.55[a]
S-180+control	2.80±0.38[a]
S-180+Methanol ext.	2.83±1.06[a]
S-180+Hexane ext.	2.70±0.77[a]
S-180+Juice	2.65±0.68[a]
S-180+MSF (2.5 mg/kg)	2.92±0.54[a]
S-180+MSF (5 mg/kg)	2.88±1.67[a]
S-180+MSF (10 mg/kg)	3.13±0.81[a]

[1)]The explanation is the same as shown in Table 1.
[a]Not significantly different at the $p<0.05$ level by Duncan's multiple range test.

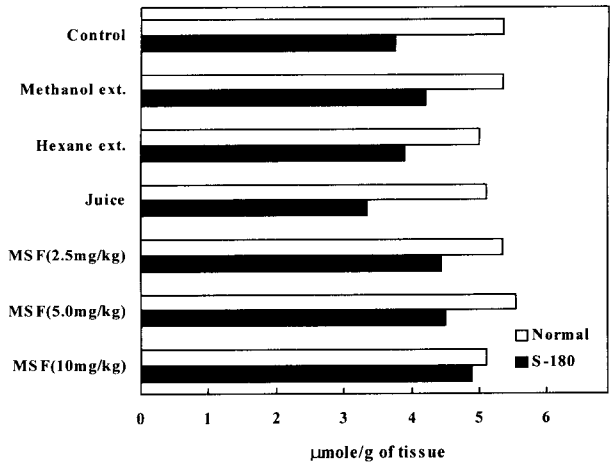

Fig. 3. The effects of 3 weeks fermented *kimchi* extracts and juice on cytosolic glutathione content in the liver of normal and sarcoma-180 transplanted Balb/c mice[1)].
[1)]The explanation is the same as shown in Table 1.

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 sartoma-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

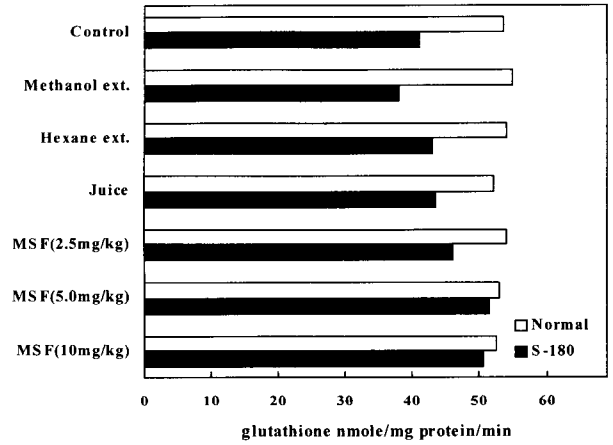

Fig. 4. The effects of 3 weeks fermented *kimchi* extracts and juice on cytosolic glutathione reductase in the liver of normal and sarcoma-180 transplanted Balb/c mice[1)].
[1)]The explanation is the same as shown in Table 1.

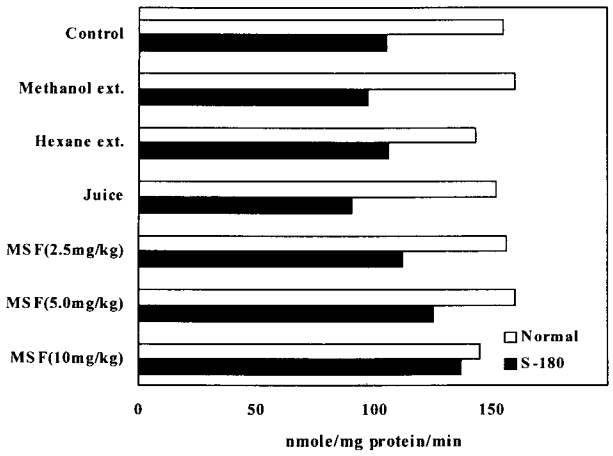

Fig. 5. The effect of 3 weeks fermented *kimchi* extracts and juice on cytosolic glutathione *S*-transferase activity in the liver of normal and sarcoma-180 transplanted Balb/c mice[1)].
[1)]The explanation is the same as shown in Table 1.

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 detoxyfying 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, β-naphthoflavone, coumarin, α-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 supressed 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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