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Anticancer Effects of Organic Chinese Cabbage *Kimchi*

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Abstract

The anticancer effect of methanol extracts from common Chinese cabbage *kimchi* (CC *kimchi*) and organically cultivated Chinese cabbage *kimchi* (OC *kimchi*) was studied on the cell growth, MTT assay and SRB assay using AGS human gastric cancer cells. Methanol extracts from CC *kimchi* and OC *kimchi* exhibited the anticancer activities *in vitro* and *in vivo*. Methanol extract from 6 day-fermented CC *kimchi* and OC *kimchi* inhibited the growth of AGS cells by 55.2% and 60.7%, respectively. At MTT assay and SRB assay, 6 day-fermented OC *kimchi* showed higher inhibition rate (MTT: 42%, SRB: 61%) than 6 day-fermented CC *kimchi* (MTT: 33%, SRB: 52%). Methanol extracts from 6-day fermented CC *kimchi* and OC *kimchi* reduced the tumor formation and prolonged the life span of sarcoma-180 cell injected Balb/c mouse. OC *kimchi* treated group resulted in the smaller tumor weight of 4.58 ± 0.32 g compared to the CC *kimchi* group of 5.40 ± 0.78 g and the control group of 7.50 ± 0.54 g and OC *kimchi* treated group (25.3 days) lived longest among control (20.2 days) and CC *kimchi* (23.5 days) treated groups.

Key words: organic Chinese cabbage, *kimchi*, AGS human gastric cancer cell, sarcoma 180 cell, anticancer effect

INTRODUCTION

The gastric cancer is one of leading causes of cancer deaths in Korea, because of Korean traditional dietary habit. *Kimchi* is a major korean traditional fermented food, though, salts for *kimchi* storage and NO_3 in chinese cabbage were suspected as the causes of gastric cancer. But, a recent study showed that optimally ripened *kimchi* with 3% salt concentration had the inhibitory effects on the growth of cancer cells and might have anticarcinogenic activity (1).

Because its major ingredients are vegetables, *kimchi* has abundant active compounds such as ascorbic acid, carotenoids, dietary fiber, chlorophylls and flavonoids which are known to suppress the formation of carcinogenic or mutagenic compounds, and to inhibit mutagenicities induced by several carcinogens (2-5). *Kimchi* contains high levels of lactic acid bacteria ($1 \times 10^{7\sim8}$/ml) when optimally ripened (6). Son (7) reported that the lactic acid bacteria isolated from *kimchi* showed antimutagenecities. The extract from red pepper and garlic used as *kimchi* ingredients showed antimutagenic and anticarcinogenic effect (8,9).

Vegetables are major ingredients of *kimchi* and their farming methods are classified into two ways, conventional and organic. Conventional method uses manufactured or chemical fertilizers which are easy and cheap to handle, but in its soil the contents of humus is very little. Then, the role of humus is to hold nutrients needed by plants and to serve as an effective buffer regulating the balance between acid and base in the soil solution. So, the nutrient and water holding capacity of soil with chemical fertilizers may be less than that of soil with organic fertilizers (10,11). Whereas, organic agricultural method generally rejects chemical pesticides and emphasize building healthy soil having abundant humus and ions, an important measure of fertility. Thus, consumer health is achieved by eating whole, fresh and organically raised food (10).

In this study, to develop functional *kimchi* that shows anticancer effects, organic Chinese cabbage having high-contents of active compounds such as chlorophyll, carotenoids, ascorbate and dietary fiber (12) and strong texture (13) was used. The anticancer effects of *kimchi* were studied *in vitro* and *in vivo*. The growth inhibitory effects, MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolin bromide) assay and SRB (sulfohodamine B) assay of methanol extracts from CC *kimchi* and OC *kimchi* were evaluated using AGS human gastric cancer cells. The life span elongation rate and tumor growth were measured in Balb/c mouse by injecting the sarcoma-180 cells.

MATERIALS AND METHODS

Preparation and solvent extraction of *kimchi*

The Chinese cabbage were cut into 8 pieces and soaked in 10% brine at 10°C and then rinsed with tap water. The standardized ratios of ingredients for *kimchi* were 13.0 of radish, 2.0 of green onion, 2.5 of red pepper powder, 1.4 of garlic, 0.6 of ginger, 2.2 of anchovy juice, 1.0 of sugar and final salt concentration 2.5 in the proportion of 100 salted Chinese cabbage (14). Chungbang Chinese cabbage (from Kimhae, Korea), garlic, radish, spring onion, ginger, red pepper powder, anchovy juice (Miwon, Co.) and salt (Chunil salt for CC *kimchi*, Gueun salt from Sannaedle, Co, Seoul for OC

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kimchi) were purchased from Bujeon market in Pusan, Korea. Chinese cabbage, garlic, radish, green onion and red pepper powder were organically cultivated at Kanglim Natural Farm (Milyang, Kyungnam) for OC *kimchi*. Organic Chinese cabbage is harvested using fertilizer whose raw material of high-quality organic substance burned at 60~80°C and fermented for more than 90 days.

The prepared *kimchi* (0 day) was put into the pint jars and then fermented for 0, 3, 6, and 9 days at 15°C. After fermentation at 15°C for 0, 3, 6, and 9 days respectively, *kimchi* samples were freeze dried. 20 folds of methanol was added to the dried and powdered samples and extracted 3 times. The methanol extracts were concentrated under a vacuum rotary evaporator and then dissolved into dimethyl sulfoxide (DMSO).

Anticancer effects *in vitro*

Cell culture : RPMI-1640, fecal calf serum (FCS), trypsin-EDTA and penicillin-streptomycin were purchased from GIBCO Co. (Gaithersburg, MD, USA). The AGS human gastric adenocarcinoma cell was obtained from Korea Cell Line Bank (KCLB). The cell was cultured in RPMI-1640 supplemented with 10% FCS and 1% penicillin-streptomycin.

Growth inhibition assay : The cultured cells were dissociated with 0.05% trypsin-0.02% EDTA and seeded 2×10^4 cells/ml for the growth inhibition assay. The cells were incubated in 5% CO_2 incubator at 37°C for 24 hrs, and then the media supplemented with methanol extracts of CC *kimchi* and OC *kimchi* were changed every two days. In the control experiment, the cells were treated with DMSO. After 6 days, the cells were washed with phosphate buffer, treated with trypsin-EDTA and then counted by hemocytometer.

MTT Assay : The MTT assay was based on metabolic reduction of 3-(4,5-dimethyl-thiazol-2-yl)-2,5- diphenyltetrazolin bromide (MTT). A 50 ml aliquot of MTT solution (1 mg/ml) in RPMI-1640 medium, with no serum or glutamine was added directly to all of the appropriate microtiter plate wells containing cells (1×10^4 cells/ml), complete growth medium, and test agents. The culture was then incubated for 4 hours to allow for MTT metabolism to formazan. After this, the supernatant was aspirated and 150 μl of DMSO was added to dissolve the formazan. Plates were agitated on a plate shaker to ensure a homogeneous solution, and the optical densities were read on an automated spectrophotometric plate reader at a single wavelength of 540 nm (15-17).

SRB Assay : A cancer cell was seeded at a plating density of 1×10^4 cells/ml in 96-well plates and cultured for 24 hrs, and then the cells were incubated in 5% CO_2 incubator at 37°C for 48 hrs, changing the media supplemented with *kimchi* samples. Adherent cell cultures were fixed in well by adding 50 μl of cold 50% (wt/vol) trichloroacetic acid (TCA)(final concentration, 10% TCA) and incubating for 60 minutes at 4°C. The supernatant was then discarded, and the plates were washed five times with deionized water and then dried. 100 μl of SRB solution (0.4% wt/vol in 1% acetic acid) was added to each microtiter well, and the culture was incubated for 10 minutes at room temperature. Unbound SRB was removed by being washed five times with 1% acetic acid. Then the plates were air-dried. Bound stain was solubilized with Tris buffer, and the optical densities were read on an automated spectrophotometric plate reader at a single wavelength of 515 nm (16,17).

Anticancer effects *in vivo*

Animals : 6-week old, male balb/c mice were supplied by the Korean chemistry institute (Taejun, Korea). They were housed in an air-conditioned room with a 12 hr light/dark cycle. Basal diets and drinking water were available ad libitum.

Antitumor test : 7 day-old sarcoma 180 ascites cells were transplanted subcutaneously into the left groin of balb/c mouse, at a dose of 6×10^6cells/mouse. Methanol extracts from 6-day fermented CC *kimchi* and OC *kimchi* (0.75 mg/kg) and common volumn of phosphate buffered saline (control) were injected intrapeneally (I.P.) once a day for 20 days from 24 hrs following the transplantation. All mice were sacrificed at 32 days after the transplantation, and tumor weights were measured (18).

Survival test : The balb/c mice were injected I.P. 0.05 ml (1×10^6 cells) of cells 7 day-old sarcoma-180 ascites. Methanol extracts from *kimchi* were injected I.P. Once a day, 24 hrs after the injection, survival times of the mice were recorded (19).

Statistical analysis

Statistical analysis was performed by Student's *t*-test and analysis of variance. Significant differences between treatment means were determined by Duncan's multiple range test.

RESULTS AND DISCUSSION

Inhibitory effects of *kimchi* extracts on growth of human cancer cells

OC *kimchi* showed higher inhibitory effects compared to CC *kimchi* on the growth of AGS human gastric adenocarcinoma cells, MTT assay and SRB assay. When AGS cells were treated with methanol extracts from CC and OC *kimchi* for 6 days, samples showed fermentation had time-dependent anticancer effects. 6 day-fermented CC and OC *kimchi* (optimally ripened) showed 51% and 61% of growth inhibition, respectively (Table 1).

Table 2 showed that OC *kimchi* had higher inhibitory effect than CC *kimchi*. The inhibition rate of 0, 3, 6, 9 day-fermented OC *kimchi* showed 20, 42, 42, 30%, while those of CC *kimchi* were 26, 27, 33, 21%, respectively. Also, SRB assay, 0, 3, 6, 9 day-fermented OC *kimchi* showed higher inhibition rate of 60, 60, 61, 54%, respectively, regardless of fermentation time, while CC *kimchi* showed 52% inhibition rate only at 6 day-fermented *kimchi* (Table 3). Park (1) reported that optimally ripened *kimchi* has the strongest anticancer effect among fresh and over-ripened *kimchi*. *Kimchi* fermentation time was changed due to the temperature of storage. 3 week-fermented *kimchi* at 5°C and 6 day-fermented *kimchi* at 15°C were optimally ripened then pHs of *kimchis* were

Table 1. Inhibitory effects of common Chinese cabbage (CC) *kimchi* and organic Chinese cabbage (OC) *kimchi* on the growth of AGS human gastric adenocarcinoma cells after 6 days of incubation at 37°C

Sample	Cell number ($\times 10^4$)	Inhibition rate (%)
Control	73.0 ± 3.00^{a}	0
CC *kimchi*		
0day	52.3 ± 1.15^{b}	28
3day	48.7 ± 1.53^{bc}	33
6day	32.7 ± 1.52^{e}	55
9day	50.3 ± 1.15^{bc}	31
OC *kimchi*		
0day	50.3 ± 3.21^{bc}	31
3day	38.7 ± 3.05^{d}	47
6day	28.7 ± 1.53^{f}	61
9day	46.7 ± 0.58^{c}	36

[a-f]Means with the different letters beside symbols are significantly different at the 0.05 level of significance as determined by Duncan's multiple range test.

Table 2. 3-(4,5-dimethyl-thiazol)-2,5-diphenyltetrazolium bomide (MTT) assay of methanol extract from common Chinese cabbage (CC) *kimchi* and organic Chinese cabbage (OC) *kimchi* (5 μl/assay) against AGS human gastric cancer cell

Treatment	Absorbance at 550	Inhibition rate
Control	0.929 ± 0.210^{a}	0
CC *kimchi*		
0day	0.687 ± 0.203^{ab}	26
3day	0.677 ± 0.052^{ab}	27
6day	0.619 ± 0.096^{b}	33
9day	0.733 ± 0.154^{ab}	21
OC *kimchi*		
0day	0.748 ± 0.124^{ab}	20
3day	0.540 ± 0.103^{b}	42
6day	0.535 ± 0.045^{b}	42
9day	0.652 ± 0.147^{b}	30

[a,b]Means with the different letters beside symbols are significantly different at the 0.05 level of significance as determined by Duncan's multiple range test.

Table 3. Inhibitory effect of methanol extract of common Chinese cabbage (CC) *kimchi* and organic Chinese cabbage (OC) *kimchi* (5 μl/assay) on the growth of AGS human gastric cancer cells in sulforhodamine B (SRB) assay

Sample (mg/ml)	O.D. at 510 nm	Inhibition rate (%)
Control	0.468 ± 0.016^{a}	0
CC *kimchi*		
0day	0.289 ± 0.017^{b}	38
3day	0.303 ± 0.026^{b}	35
6day	0.225 ± 0.019^{c}	52
9day	0.295 ± 0.021^{b}	37
OC *kimchi*		
0day	0.189 ± 0.010^{c}	60
3day	0.188 ± 0.006^{c}	60
6day	0.185 ± 0.015^{c}	61
9day	0.215 ± 0.027^{c}	54

[a~c]Means with the different letters beside symbols are significantly different at the 0.05 level of significance as determined by Duncan's multiple range test.

same as 4.3. Table 1 showed that inhibitory effect of 9-day fermented *kimchi* on the growth of AGS cell was weaker than that of extracts from 6 day-fermented *kimchi*, but stronger than those from fresh *kimchi*. But with MTT and SRB assay, inhibition rates of samples fermentated at different days didn't make much difference (Table 2, 3).

MTT assay is based on metabolic reduction of MTT by enzymatic reduction of viable cancer cells. SRB assay indirectly calculates the number of viable cancer cells by measuring a dye with SRB that binds to amino acid of cellular macromolecules (16). Because MTT and SRB assay was carried out for 2 days while growth inhibition was for 6 days, growth inhibitory effects could show the growth curve of cancer cells. Therefore, growth inhibitory effects of the cells with sample treated for 6 days showed time-dependent manners, while the effects of SRB and MTT assay with 2 days' sample treatment did not show the difference according to the *kimchi* fermentation time.

Hur (20) reported that optimally ripened *kimchi* showed no toxicity to the normal cells of Ac2F liver cells (0.5~2.0%). However, marked decrease in the growth of human AGS gastric cancer cells was observed. Also Choi et al. (21) showed the *kimchi* extracts exhibited a direct cytotoxic effect on tumor cells *in vitro* and increased the phagocytic cell activities.

Anticancer effects *in vivo*

In our studies, sarcoma 180 cells were transplanted to the Balb/c mouse and then the *kimchi* samples were treated. The concentration of samples were determined on 1.0 mg/ml after viability test. Table 4 shows the tumor weights after the treatment. OC *kimchi* treated group resulted in the smaller tumor weight of 4.58 ± 0.32 g compared to the CC *kimchi* group of 5.40 ± 0.78 g and the control group of 7.50 ± 0.54 g.

After sarcoma 180 cell and *kimchi* samples were injected to the mice, the survival time was recorded. This result also showed that OC *kimchi* treated group (25.3 days) lived longest among the control group (20.2 days) and CC *kimchi* (23.5 days) groups (Table 5). Spleen and liver weights of mice were changed. The spleen and the liver weights of mice were

Table 4. Effect of methanol extract from 6 days fermented common Chinese cabbage (CC) *kimchi* and organic Chinese cabbage (OC) *kimchi* in tumor bearing Balb/c mice with sarcoma-180 cells

Treatment	Tumor weight	Inhibition rate (%)
S-180 + Control	7.50 ± 0.54	-
S-180 + CC *kimchi*	5.40 ± 0.78*	28
S-180 + OC *kimchi*	4.58 ± 0.32*	39

*Significantly different at the $p<0.05$ level by Student's *t*-test

Table 5. Effect of methanol extract from 6 days fermented common Chinese cabbage (CC) *kimchi* and organic Chinese cabbage (OC) *kimchi* on life span of Balb/c mice with sarcoma-180 cells

Treatment	Survival time (day)	Prolongation rate (%)
S-180 + Control	20.2 ± 4.4	-
S-180 + CC *kimchi*	23.5 ± 4.0	14
S-180 + OC *kimchi*	25.3 ± 3.7	21

increased in the OC *kimchi* treated group comparing to the control group. Hur (20) reported that *kimchi* had a antitumor activity. Though life span elongation was not different from control and CC *kimchi* samples, the growth of sarcoma 180 cell induced tumor were greatly suppressed and weights of spleen and liver were increased by the MSF of *kimchi* treatment. Because kuffer cell in liver and splenic macrophage were in charge of immune response to foreign substances in human body, weights of spleen and liver were increased in CC *kimchi* and OC *kimchi* treated groups, but this was not significantly different.

The result came from the fact that OC *kimchi* was made of organically cultivated ingredients which had higher contents of active compounds such as chlorophylls, carotenoids, ascorbate and dietary fiber, which have been reported to have antimutagenic and anticarcinogenic effect (22). The extracts from red pepper, and the extracts from garlic used as *kimchi* ingredients also showed growth inhibitory effect on cancer cells in the previous reports (8,9).

Thus, we can develop a functional *kimchi* that shows antimutagenic and anticancer activities by using high-quality materials such as organically cultivated ingredients. Further studies about the identification and purification of active compounds in *kimchi* are still needed.

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