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Effects of Kimchi Extracts on Interleukin-2 Production and Natural Killer Cell Activity in Mice

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Abstract

To determine the immune effect of kimchi extracts in mice, 0.5 mg/day of the extracts from kimchis, which were prepared with conventionally (general kimchi) and organically (organic kimchi) cultivated ingredients, were treated orally to male BALB/c mice. Following 1, 3 and 5 weeks of treatment, the Interleukin-2 (IL-2) production in the presence (con A-stimulated) or the absence (spontaneous) of con A (5 μg/ml) and the natural killer cell (NK) activity of the splenocytes were measured. The IL-2 productions in most of treatments with methanol extract from general kimchi were significantly higher than those of control ($p<0.05$). And at the 3 weeks of treatment, the spontaneous or con A-stimulated IL-2 productions from splenocytes of mice treated with it increased more than those of control group, by 2.8 and 2.2 times, respectively. However, the longer the treatment with methanol extracts from organic kimchi showed the higher the enhancing effect on the IL-2 production. The spontaneous or con A-stimulated IL-2 productions from splenocytes of mice treated with dichloromethane fraction from general kimchi also increased at 5 weeks of teatment compared to those of control group, by 2.7 and 2.5 times, respectively. The natural killer cell activity of splenocytes from mice treated with methanol extracts from general kimchi for 1~5 weeks was significantly higher than that of control goup ($p<0.01$). The effect of methanol extracts from general kimchi was the highest at 3 weeks of treatment, as same as in the IL-2 production. The enhancing effect of methanol extracts from organic kimchi on the NK cell activity was the highest at 5 weeks of treatment. The NK cell activity of splenocytes from mice treated with dichloromethane fraction from general kimchi for 5 weeks was significantly higher than those in control and 3 weeks of treatment. These results showed that the effects of kimchi extracts on the IL-2 production and the NK cell activity in mice were profound in long term of treatment (3 and 5 weeks than 1 week). We suggest that kimchi extracts might have an immune effect in part due to its enhancing action on the IL-2 production and the NK cell activity.

Key words: kimchi, immunolgy, IL-2, NK cell, Yac-1, MTT

INTRODUCTION

Kimchi is a Korean traditional and fermented food. It is prepared using major raw material (Chinese cabbage or radish) and various other vegetables and ingredients such as onion, spices and salted fish, through the processes of grading, brining, blending, and fermentation. There are many types of kimchis, depending on the major materials and preparation methods used, and their biochemical, microbiological, and nutritional characteristics are different.

The fermentation of kimchi is carried out by various microoganisms, mainly the lactic acid bacteria, from the raw vegetable substances. Nutritionally, kimchi is an important source of vitamins, minerals, dietary fiber, and other nutrients. The vitamin B groups and ascorbic acid are already present in the raw materials and may be synthesized during the fermentation (1,2). The considerable amounts of amino acids are present in kimchi compared to other fermented vegetables (3).

Many studies have reported on the fermentations and preservations of kimchi, but a few studies on the physiological function, especially, the protective effect aganist cancer, of kimchi reported. Kimchi contains large amount of ascorbic acid (4,5), carotenoids(6), flavonoids (7) which are known to suppress the formation of carcinogenic or mutagenic compounds, and to inhibit mutagenicity induced by several carcinogens. The yellow green vegetables, the major source of kimchi, have dietary fiber and lactic acid bacteria (8) which can get positive effect on the prevention of colon cancer. The extracts of red pepper (9) and garlic (10) used as kimchi ingredient are also believed to have anti-mutagenic and anticarcinogenic effects. It was also reported that the properly ripened kimchi with 3% salt concentration had the inhibitory effects on the growth of cancer cells and might have anticarcinogenic activity (11,12). Additionally, kimchi is known to improve digestion, prevent constipation, control intestinal microflora, and have other pharmaceutical functions (3,12).

It is believed that the interleukin-2 (IL-2) is a cytokine to be essential for the long term growth of activated T cells

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(13). Natural killer (NK) cells are a immune component to mediate cytolysis of tumor cells, virus-infected targets, parasites, fungi and bacteria (14-16).

For this study, we prepared kimchis with the ingredients that were grown conventionally (general kimchi) and organically (organic kimchi). In order to understand the protective action of kimchi in immune system regarding with the cancer prevention, the effects of the extracts from general and organic kimchi for 1, 3 and 5 weeks on the IL-2 production and the NK cell activity in mice were investigated.

MATERIALS AND METHODS

Preparation of kimchi

To prepare general kimchi, Chungbang baechu, a kind of Chinese cabbage grown in Kimhae, was obtained. Garlic, ginger, red pepper powder, radish and green onion were purchased from a local market in Pusan, Korea. To prepare organic kimchi, organically cultivated Chungbang baechu, garlic, ginger, red pepper powder, radish and green onion were obtained from Kanglim Natural Farm (Milyang, Kyungnam). Fermented anchovy juice and Gueun salt (San naedle, Co, Seoul) were purchased from a local market in Pusan, Korea. The cabbage was cut into 4 to 5 cm, brined in 10% salt solution for 10 hours, rinsed with fresh water, and drained. The ingredients and their proportions for kimchi are shown in Table 1. The final salt concentration in kimchi was adjusted to 2.5%. The prepared kimchi samples were put into the pint jar, fermented for 3 weeks at 5°C (pH 4.3), and then used as test samples.

Solvent extraction of kimchi (17,18)

Two kinds of kimchi samples (general kimchi and organic kimchi) were freeze-dried and minced in a blender. The minced kimchi samples (25 g) were extracted with methanol (500 ml), three times, by shaking for 12 hours. After taking methanol extract, the residues were extracted again with dichloromethane (500 ml) by the same method described above, and dichloromethane fraction was taken. The kimchi extacts were dried by rotary vaccum evaporator (Buchi 011 & 461, Switzerland) and dissolved in dimethyl sulfoxide (DMSO).

Chemicals

Concanavalin A (Con A) were obtained from Sigma Chemical (St. Louis, USA). RPMI 1640 medium, fetal calf serum (FCS), penicillin-streptomycin was purchaced from Gibco Chemical Co. (Grand Island, NY, USA). Intertest-2XTM Mouse IL-2 ELISA kit was obtained from Genzyme Co. (Cambridge, USA).

Table 1. Ingredients and preparing composition (%) of kimchi

Ingredient	Composition
Chinese cabbage	3000 g (100%)
Red pepper powder	105 g (3.5%)
Crushed garlic	420 g (1.4%)
Crushed ginger	18 g (0.6%)
Radish	390 g (13.0%)
Green onion	60 g (2.0%)
Fermented anchovy juice	66 g (2.2%)
Sugar	30 g (1.0%)
Final salt concentration	2.5%

Animals and treatment

Male BALB/c mice, aged 3 weeks, were treated orally with 0.5 mg of kimchi extracts (methanol extracts of general kimchi and organic kimchi, and dichloromethane fraction from general kimchi) or saline solution in a day. After 1, 3 and 5 weeks, mice were killed and the spleens were dissected for the measurement of the NK cell activity and the IL-2 production.

Isolation of splenocyte

Splenocyte were isolated by a modified method of Mishell and Shiigi (19). Spleen were dissected aseptically and grounded in RPMI-1640 supplemented with 100 U/ml of penicillin-streptomycin. The cell suspension was filtered through 70 μm mesh. The splenocytes were collected by the centrifugation and resuspended in the same media. The splenocytes were isolated by centrifugation using histopaque-1077 (400 g, 30 mins, 18°C).

Measurement of the IL-2 production

Freshly prepared splenocytes were resuspended at the density of 2×10^6 cells/ml in RPMI-1640 supplemented with 10% FCS, 100 U/ml of penicillin-streptomycin and 2 mM L-glutamin (complete medium). 1 ml each of the cells was added to each well of 24 well flat-bottomed plate. 5 μg/ml of con A was used as stimulating mutagen and each splenocyte from mice treated variously was assayed in triplicate. Following 72 hours incubation at 37°C, the culture were centrifuged at 300 g for 10 mins and the resuspended culture was centifuged at 10,000 g for 30 mins, again. The IL-2 productions of the supernatants were measured using Interest-2XTM Mouse IL-2 ELISA kit. 100 μl of the supernatant was added to each well of 96 well flat-bottomed microplate attached monoclone antibody for IL-2 and then incubated at 37°C for 25 mins. After 4 times of washing with buffer, 100 μl of streptavidin peroxidase solution was added to each well. The treated microplates were left 10 mins at room temperature. The optical density (OD) was measured at a wavelength of 450 nm by ELISA reader (Bio-Rad, Richmond, USA).

Measurement of NK cell activity

Preparation of effector cells

The splenocytes were resuspended in the complete media. The cells allowed to adhere in a culture flask for 1 hour at 37°C in a 5% CO_2 atmosphere. Non-adherent NK cells used as the effector cells were collected by centrifugation and resuspended. The cell viability was more than 90%.

The NK cell activity assay

The assay for NK cell activity was adapted from the

method described previously (15), using the dye, 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl-tetrazolium 5-diphenyl-tetra zolium bromide(MTT). The YAC-1 murine lymphoma cell line was obtained from American Type Culture Collection (Rockville, Md., USA) and used as target cell. Yac-1 cells were maintained at a density of 2×10^5 cells/ml in complete medium. The cells were collected by centrifugation and resuspended at a concentration of 5×10^4 cells/ml. For the NK cell activity assay, 50 ul each of effector cells and Yac-1 cells (5×10^4 cells/ml)were added to each well of a 96-well flat-bottomed microplate. Each splenocyte from mice treated variously was assayed in triplicate at each effector/target ratio(200 : 1, 100 : 1). After 3 days culture at 37°C, the cells were loaded 10 μl of freshly prepared MTT (5 g/ml) and in cubated for a further 4 hours at 37°C. 25 μl of sodium dodecyl sulfate (SDS, 10% in 0.02 N HCl) was added to each well and the microplates were left 30 mins at room temperature for colour development. The optical density (OD) was measured at a wavelength of nm by ELISA reader. The percentage cell cytolysis was calculated as follow:

$$1 - \frac{\text{OD of non-lysed target cells} - \text{OD of effect}}{\text{OD of target cell alone}}$$

Statistic anlysis

Data were presented means±SD after one-way ANOVA anlysis. Comparisons between groups or treatment were computed using Student's t-test.

RESULTS AND DISCUSSION

In immune system, it is believed that IL-2 is a cytokine to activate T-lymphocyte and stimulate the final differentiation and proliferation of cytotoxic lymphocytes by induction with MHC (Major Histocompatibility Complex) (20). Whereas, killing by NK cells has a natural immunity which is not induced by a specific antigen or MHC (21).

To determine the function of kimchi extracts in the immune system, 0.5 mg/day of kimchi extracts for treated groups or saline solution for control group was injected orally to male BALB/c mice. Following 1, 3 and 5 weeks of treatment, the spleens of mice dissected and then the IL-2 production in the presence (con A-stimulated) or absence (spontaneous) of con A (5 μg/ml) and the natural killer cell activity of the splenocytes were measured.

Effect of kimchi extracts on the IL-2 production

As shown in Fig. 1, the IL-2 productions of splenocytes from mice with methanol extract from general kimchi for 1, 3 and 5 weeks were significantly higher than those in control ($p<0.05$). Especially, following 3 weeks of treatment, the IL-2 productions from splenocytes of mice were higher than in 1, 5 weeks of treatment. The spontaneous or con A-stimulated IL-2 productions from splenocytes of mice treated for 3 weeks increased compared to those of control group, by 2.8 and 2.2 times, respectively.

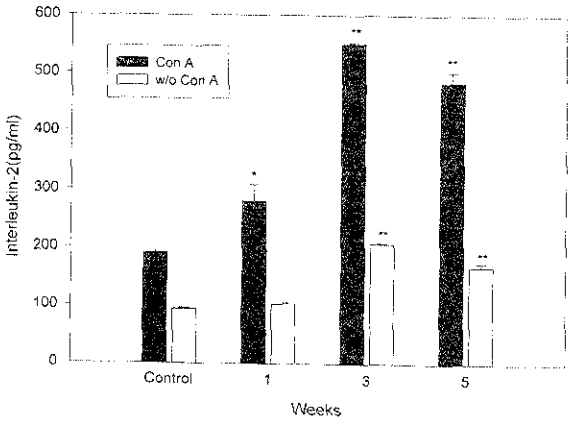

Fig. 1. IL-2 production from splenocytes of BALB/c mice treated orally with methanol extract of general kimchi. The splenocytes were isolated from mice treated orally with 0.5 mg/day of kimchi extracts or saline solution as control in a day for 1, 3 and 5 weeks. 5 μg/ml of concanavalin (con A) was used as stimulating mutagen and each splenocyte from mice treated variously was assayed in triplicate. Following 72 hours incubation at 37°C, the IL-2 productions of the supernatants were measured using Interest-2XTM Mouse IL-2 ELISA kit. *,**Significantly different from control by Student's t test (*$p<0.05$, **$p<0.01$).

The treatment of mice with methanol extract from organic kimchi for 3 and 5 weeks enhanced the con A-stimulated IL-2 production of splenocytes than control group's by 1.3 and 1.5 times, respectively (Fig. 2). The spontaneous IL-2 production of splenocytes treated for 5 weeks was also significantly higher than controls ($p<0.01$).

Fig. 3 shows the spontaneous or con A (5 μg/ml)-stimulated IL-2 production from the splenocytes of mice treated orally with dichrolomethane fraction of general kimchi. When mice were treated with dichrolomethane fraction of general kimchi for 1 and 3 weeks, the IL-2 productions of the splenocytes were lower than those of control group. However, compared to those of control group, the spontaneous or con A-stimulated IL-2 productions from splerenocytes of mice treated

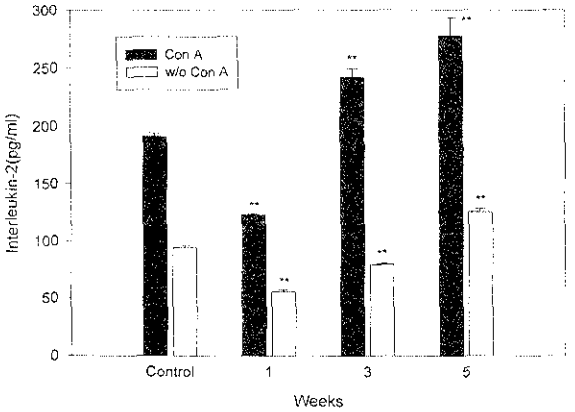

Fig. 2. IL-2 production from splenocytes of BALB/c mice treated orally with methanol extract of organic kimchi. The explanation is the same as shown in Fig. 1.

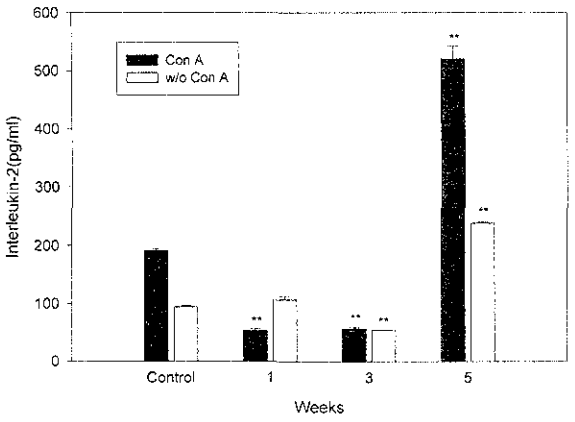

Fig. 3. IL-2 production from splenocytes of BALB/c mice treated orally with dichloromethane fraction of general kimchi. The explanation is the same as shown in Fig. 1.

for 5 weeks increased by 2.7 and 2.5 times, respectively.

Cho et al. (22) already reported that dichrolomethane fraction of kimchi exhibited strong antimutagenic activities compared to orther fractionated samples. In this study, the effect of methanol extract of general kimchi on the IL-2 production was the highest at 3 weeks of treatment and then decreased at 5 weeks of treatment. But in treatment with dichrolomethane fraction of general kimchi, the effect of that fraction on the IL-2 production increased most at 5 weeks.

Effect of kimchi extracts on the NK cell activity

The NK cells were believed to be large lymphocytes with numerous cytoplasmic granules. To study kimchi's function in immune system, the NK cell activity of splenocytes from mice treated with kimchi extracts (0.5 mg/day) for 1, 3 and 5 weeks was measured, using Yac-1 murine lymohoma cell line as target cell. The effector/target ratios were 200 : 1 and 100 : 1. The higher the effector/target ratios were, the better the cytolysis of NK cell was.

Table 2 shows the NK cell activity of splenocyte from mice treated with 0.5 mg/day of kimchi extracts or saline solution (control group) for 1 week (Table 2). All of kimchi extracts treated orally to mice significantly enhanced the NK cell activity of splenocyte ($p<0.01$). Especially, the effect of methanol extract from general kimchi was the highest and the NK cell activity of splenocyte from mice treated with it was enhanced by 1.6 times compared to control's (effector/ target ratio ; 200 : 1).

Following 3 weeks of treatment with kimchi extracts, the NK cell activity of splenocyte from mice treated with methanol extract from general kimchi was also the highest among kimchi extracts (Table 3). However, in mice treated orally with dichloromethane fraction from general kimchi for 3 weeks, the NK cell activity of splenocyte was decreased than that of 1 week treated group.

As shown in Table 4, after 5 weeks of treatment, the NK cell activity of splenocytes from mice treated with methnaol extract from organic kimchi was the highest and it(effector/ target ratio; 200 : 1) was enhanced by 2 times compared to that of control. The effect of dichloromethan fraction of general kimchi in mice treated for 5 weeks was also higher than in mice treated for 3 weeks. However, NK cell activity of splenocytes of mice treated with metanol extract of general kimchi for 5 weeks decreased slightly than that of mice treated for 3 week.

These results showed that the effects of kimchi extracts on the IL-2 production and the NK cell activity in mice were profound in long term of treatment (3 and 5 weeks than 1 week).

Table 2. Effect of kimchi extracts on the NK cell activity of splenocyte[1] in mice treated orally for 1 week

Treatment	%Cytolysis (Effector/Target ratio)	
	200 : 1	100 : 1
Control	33.81±0.90[2]	18.49±2.46
Methanol ext. from general kimchi	52.54±0.68**	18.75±0.34
Methanol ext. from organic kimchi	42.06±2.02**	23.89±2.14
Dichloromethane fr. from general kimchi	46.75±2.36**	29.13±0.24**

[1]Male BALB/c mice were treated orally with 0.5 mg/day of kimchi extracts (treated group) or saline solution (control group) for 1 week. The cells allowed to adhere in a culture flask for 1 hour at 37°C in a 5% CO_2 atmosphere. Non-adherent NK cells used as the effector cells. 50 ul each of effector cells and Yac-1 murine lymphoma cells(target cell, 10^3 cells/ml)were added to each well of a 96 well flatbottomed microplate. Following 4 days incubation at 37°C, the survival cells were measured using 3-(4, 5-dimethylthiazol-2-yl)2, 5-diphenyl-tetrazolium 5-diphenyl-tetra zolium bromide (MTT). Results are expressed as percent of cytolysis

[2]Means±SD

**Significantly different from control by Student's t test ($p<0.01$)

Table 3. Effect of kimchi extracts on the NK cell activity of splenocyte[1] in mice treated orally for 3 weeks

Treatment	%Cytolysis (Effector/Target ratio)	
	200 : 1	100 : 1
Control	30.77±1.01[2]	16.45±2.12
Methanol ext. from general kimchi	66.39±0.97**	43.80±1.69**
Methanol ext. from organic kimchi	58.50±1.51**	28.41±0.39**
Dichloromethan fr. from general kimchi	15.93±0.44**	3.98±0.15**

[1,2)**]The explanation is the same as shown in Table 2

Table 4. Effect of kimchi extracts on the NK cell activity of splenocyte[1] in mice treated orally for 5 weeks

Treatment	%Cytolysis (Effector/Target ratio)	
	200 : 1	100 : 1
Control	32.55±1.19[2]	21.09±2.06
Methanol ext. from general kimchi	56.75±1.79**	40.94±0.77**
Methanol ext. from organic kimchi	65.24±5.46**	49.37±1.20**
Dichloromethan fr. from general kimchi	38.55±0.10**	22.75±0.56

[1,2)**]The explanation is the same as shown in Table 2

IL-2 is known to be produced by helper T lymphocytes in response to antigen exposure and stimulate lymphokine-activated killer cells or NK cells (23,24). And NK cells are a subset of lymphocytes that are derived from the bone marrow and found mainly in the blood and the spleen (25). They are believed to have cytotoxic effects to various malignant cells and normal cells of the infected host, playing an important role in the first-line defense aganist viral diseased and cancer (26,27). NK cells are neither T-cells nor B cells and do not undergo thymic maturation or T cell receptor rearangement.

Based on our studies, it was thought that kimchi might have a immue function, enhancing both the IL-2 production by induction with MHC and the NK cell activity, the spontaneous cytotoxicity.

But the question how kimchi extracts enhanced the IL-2 production and NK cell activity remained to be investigated. More research regarding the immune funtion of kimchi *in vivo* and *in vitro* will have to be continued.

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