J Food Sci. Nutr.
Vol. 2, No 3, p 241~245(1997)

Inhibitory Effects of Kimchi Extracts on Carcinogen-induced Cytotoxicity and Transformation in C3H/10T1/2 Cells

Moung-Won Choi, Kwang-Hyuk Kim*, So-Hee Kim** and Kun-Young Park†

Dept. of Food Science and Nutrition and Kimchi Research Institute, Pusan National University, Pusan 609-735, Korea
**Dept. of Microbiology, Kosin Medical College, Pusan 602-702, Korea*
***Dept. of Food and Nutrition, Dong-Ju Women's Junior College, Pusan 604-080, Korea*

Abstract

Inhibitory effects of kimchi extracts on carcinogen-induced cytotoxicity and transformation in C3H/10T1/2 cells were studied. The methanol extract(500μg/ml) of fresh(unfermented kimchi), and 3-week-fermented kimchi(properly ripened kimchi at 5°C) inhibited 3-methylcholanthrene(MCA)-induced cytotoxicity in C3H/10T1/2 cells by 84 and 99%, respectively. The inhibitory effect of 3-week-fermented kimchi was higher than that of the fresh kimchi at same test condition. The methanol soluble fraction, and haxane extract of 3-week fermented kimchi also surpressed the cytotoxicity of C3H/10T1/2 cells mediated by 7,12-dimethylbenz[a] anthracene(DMBA) and N-methyl-N'-nitro-N-nitrosoguanidine(MNNG). Furthermore, MCA-induced transformation of C3H/10T/1/2 cells was significantly inhibited by the methanol soluble fraction of 3-week fermented kimchi. With these results, we suggest that kimchi might have anticarcinogenic effect in part due to inhibition of carcinogen-induced cytotoxicity and transformation of C3H/10T/1/2 cells.

Key words: kimchi, C3H/10T1/2 cells, cytotoxicity, transformation

INTRODUCTION

Kimchi is a traditional, fermented Korean food which is prepared with various vegetables such as Chinese cabbage, radish, spices, and other seasonings. There are many types of kimchi, depending on the raw ingredient and preparation methods used.

Kimchi is fermented by the microoganisms, mainly the lactic acid bacteria, naturally present in the raw vegetable substances. Numerous physicochemical and biological factors affect the growth and sequential appearance of principal microorganisms involved in the fermentation. Salt concentration, temperature, and pH greatly influence the rate and extent of the fermentation of kimchi (1). Nutritionally, kimchi 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(2,3). And considerable amounts of amino acids are present in kimchi compared to some other fermented vegetables(1).

Kimchi contains large amount of ascorbic acid(4,5), carotenoids(6), dietary fiber, and flavonoids(7) which are known to suppress the formation of carcinogenic or mutagenic compounds, and to inhibit mutagenicity induced by several carcinogens. The extracts of red pepper(8) and garlic(9) used as kimchi ingredient, and lactic acid bacteria(10) dominating the fermentation of kimchi are believed to have antimutagenic and anticarcinogenic effects. It was also reported that the properly ripened kimchi with 3% salt concentration itself 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(1,12).

In this study, in order to better understand the protective action of kimchi against cancer, we investigated the effects of kimchi extracts on the cytotoxicity and cell transformation induced by carcinogen, using C3H/10T1/2 cell(mouse embryo fibroblast cell) system.

MATERIALS AND METHODS

Preparation of kimchi

Garack baechu, a kind of Chinese cabbage grown in

†Corresponding author

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

Ingredient	Composition
Chinese cabbage	3000g(100%)
Red pepper powder	60g(2%)
Crushed garlic	60g(2%)
Crushed ginger	15g(0.5%)
Final salt concentration	3.0%

Kimhae, was used as major raw ingredient for kimchi. Garlic, ginger, red pepper powder and *han-ju* salt were purchased from a local market. The cabbage was cut 4 to 5cm in size, 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 weight percentage of salt in kimchi was adjusted to 3%. The unfermented fresh kimchi(fresh kimchi) and the kimchi fermented for 3 weeks at 5°C(pH 4.3, 3-week-fermented kimchi) were used as test samples.

Solvent extraction of kimchi(13,14)

Two kinds of kimchi samples(fresh kimchi and 3 weeks-fermented kimchi) were freeze-dried and minced in a blender. The minced kimchi samples(25g) were extracted with hexane(500ml), three times, by shaking for 12 hours. After taking hexane extract, 500ml methanol was added to the residues and shaken for 16 hours, followed by reflux for 90 minutes at 70~80°C water bath. After filtration, methanol soluble fraction(MSF) was taken. The kimchi extracts were dried by rotary vaccum evaporator(Buchi 011 & 461, Switzerland) and then dissolved in dimethyl sulfoxide(DMSO).

Carcinogens/Chemicals

3-methylcholanthrene(MCA), 7,12-dimethylbenz[a]anthracene(DMBA) and Giemsa stain were obtained from Sigma Chemical Co.(St. Louis, MO, USA). N-methyl-N'-nitro-N-nitrosoguanidine(MNNG) was purchased from Aldrich Chemical Co.(Milwaukee, WI, USA). Eagle's basal medium, fetal calf serum (FCS), 0.05% trypsin-0.02 EDTA, penicillin-streptomycin were obtained from Gibco Chemical Co.(Grand Island, NY, USA).

Cells(15)

C3H/10T1/2 cells, mouse embryo cells, were obtained from Japanese Cell Line Collection(Tokyo, Japan). The medium used for the cells was Eagle's basal medium supplemented with 10% fetal calf serum(FCS) and 100unit/ml penicillin-streptomycin. Cultures were maintained in a humidified atmosphere of 5% CO_2 at 37°C. A medium change is made on the 5th day after seeding. The cells were transferred every 10 days, using phosphate buffered saline and 0.05% trypsin-0.02% EDTA, and new flasks were seeded with 5×10^4 cells in 5ml of medium each.

Cytotoxicity assay

Cytotoxicity was determined by measuring the inhibition of colony formation as described previously(16,17). C3H/10T1/2 cells were plated at 2×10^3 cells/60mm dish. Twenty-four hours at 37°C under 5% CO_2 after seeding, the test compounds were added to serum-free Eagle's basal medium. The cells were treated with MCA(5μg/ml), DMBA(1μg/ml), and MNNG(1μg/ml) in the absence or presence of kimchi extracts(hexane extract: 100μg/ml, MSF: 100, 200μg/ml). Following the treatment for a period of 48 hours, the medium was changed, and the cells were allowed to grow an additional 7 days in the medium supplemented with 10% FCS. Surviving colonies were fixed with methanol, stained with Giemsa stain and counted. Cytotoxicity was expressed as the number of surviving colonies on the treated dishes divided by the number of surviving colonies on the control dishes, and is expressed as the survival fraction.

Transformation test

Transformation experiments were performed by a modified method of Reznikoff et al.(18). 2×10^3 C3H/10T1/2 cells were seeded in 60mm dish(10dishes/group). Twenty-four hours after seeding, the cells were loaded with serum-free Eagle's basal medium containing MCA(5μg/ml) and MSF of kimchi extract(100, 200μg/ml) or DMSO(the control) for 24 hours. After 2 days, the medium was changed to complete medium supplemented with 10% FCS. Subsequently, the medium was changed twice weekly until the cells reach confluence, then once a week. At the 6 week, cells in the dishes were fixed with methanol and stained by Giemsa, and the morphologically transformed foci were counted. Transformed foci were classified as three types(type Ⅰ, Ⅱ, and Ⅲ) by the morphological criteria initially established by Reznikoff et al.(18).

RESULTS AND DISCUSSION

C3H/10T1/2 cells have been widely used to study mech-

anisms of neoplastic transformation in mammalian cells and as a target indicator cell system to screen industrial chemicals for carcinogenicity(19). Since the cytotoxicity, mutation, and transformation of chemical can be studied together in one cell system(18,20,21), C3H/10T1/2 cell is believed very effective to investigate the mechanisms of genotoxicity of the chemicals. Using this C3H/10T1/2 cell system, we studied the effects of kimchi extracts on the cytotoxicity and cell transformation induced by carcinogens.

Inhibitory effect of kimchi extracts on carcinogen-induced cytotoxicity

C3H/10T1/2 cells were treated with MCA(5μg/ml), DMBA(1μg/ml), MNNG(1μg/ml) in the absence or presence of kimchi extracts(hexane extract: 100μg/ml, MSF: 100, 200μg/ml) in serum-free Eagle's basal medium for 48 hours. Following the treatment, the cells were allowed to grow an additional 7 days in the medium supplemented with 10% FCS.

Table 2 shows an inhibitory effect of the fresh kimchi, and 3 weeks-fermented kimchi extracts on MCA-induced cytotoxicity in C3H/10T1/2 cells. 100, 200, 500μg/ml of methanol soluble fraction(MSF) from fresh kimchi and 3 weeks-fermented kimchi suppressed the MCA-induced cytotoxicity in C3H/10T1/2 cell system by 11, 34, 84% and 32, 63, 99%, respectively. Namely, increased concentrations of kimchi extracts increased the survival fractions of the cells. Since the inhibitory effect of 3-week-fermented kimchi was higher than that of the fresh kimchi, we again tested the inhibitory effects of the extracts of 3 weeks-fermented kimchi on the cytotoxicity induced by DMBA and MNNG in C3H/10T1/2 cell system. 100μg/ml of methanol soluble fraction(MSF) and hexane extract of 3 week-fermented kimchi reduced DMBA-mediated cytotoxicity by 16%, 35%, respectively(Table 3). On the cytotoxicity of MNNG, hexane extract and methanol soluble fraction of the kimchi also revealed an inhibitory effect(Table 4).

Table 2. Effect of methanol soluble fraction(MSF) from the fresh kimchi, and 3 week-fermented kimchi on 3-methylcholanthrene(MCA, 5μg/ml)-induced cytotoxicity of C3H/10T1/2 cells[1)]

Treatment		Cell colony	Survival fraction[2)]
Control(MCA)		46.3±3.2	1.00
MCA+MSF(100μg/ml)	fresh	51.3±7.2[3)]	1.11(11%)
	3wks	61.3±2.5	1.32(32%)
MCA+MSF(200μg/ml)	fresh	62.0±6.2	1.34(34%)
	3wks	75.3±4.5	1.63(63%)
MCA+MSF(500μg/ml)	fresh	85.3±5.1	1.84(84%)
	3wks	92.5±2.1	1.99(99%)

[1)]Twenty four hours after seeding, 2×10^3 C3H/10T1/2 cells/60mm dish were cultured in serum-free Eagle's basal medium in the presence of various concentration of the above kimchi extracts and MCA(5μg/ml) for 48 hours. Following the treatment, the cells were allowed to grow an additional 7 days in the medium supplemented with 10% FCS. Surviving colonies were fixed with methanol, stained, and counted

[2)]Number of surviving colonies on treated dishes / Number of surviving colonies on control dishes

[3)]Values are means±SD

Inhibitory effect of kimchi extracts on the transformation by MCA

Using the transformation test with C3H/10T1/2 cells, we investigated the protective effect of kimchi extracts aganist carcinogenicity. Transformation foci formed by MCA in these cells were separated into type Ⅰ, Ⅱ, Ⅲ foci (Fig. 1). Raznikoff et al.(18) reported that type Ⅰ was not malignant altered foci, but type Ⅱ and Ⅲ were scored as malignantly transformed because a high percentages(50% for type Ⅱ and over 85% for type Ⅲ) of these foci produced tumors after these cells are inoculated s.c. into irra-

Table 3. Effect of methanol soluble fraction(MSF) and hexane extract(HE) from 3 week-fermented kimchi on 7,12-dimethylbenz[a]anthracene(DMBA, 1μg/ml)-induced cytotoxicity of C3H/10T1/2 cells[1)]

Treatment	Cell colony	Survival fraction[2)]
Control(DMBA)	53.7±0.5	1.00
DMBA+MSF(100μg/ml)	62.3±3.3[3)]	1.16(16%)
DMBA+MSF(200μg/ml)	75.3±3.7	1.40(40%)
DMBA+HE(100μg/ml)	72.7±3.4	1.35(35%)

[1-3)]The explanation is the same as shown in Table 2

Table 4. Effect of methanol soluble fraction(MSF) and hexane extract(HE) from 3 week-fermented kimchi on N-methyl-N'-nitro-N-nitroso-guanidine(MNNG, 1μg/ml)-induced cytotoxicity of C3H/10T1/2 cells[1)]

Treatment	Cell colony	Survival fraction[2)]
Control(MNNG)	36.0±4.4	1.00
MNNG+MSF(100μg/ml)	42.0±3.4[3)]	1.17(17%)
MNNG+MSF(200μg/ml)	55.3±3.2	1.53(53%)
MNNG+HE(100μg/ml)	50.7±4.2	1.42(42%)

[1-3)]The explanation is the same as shown in Table 2

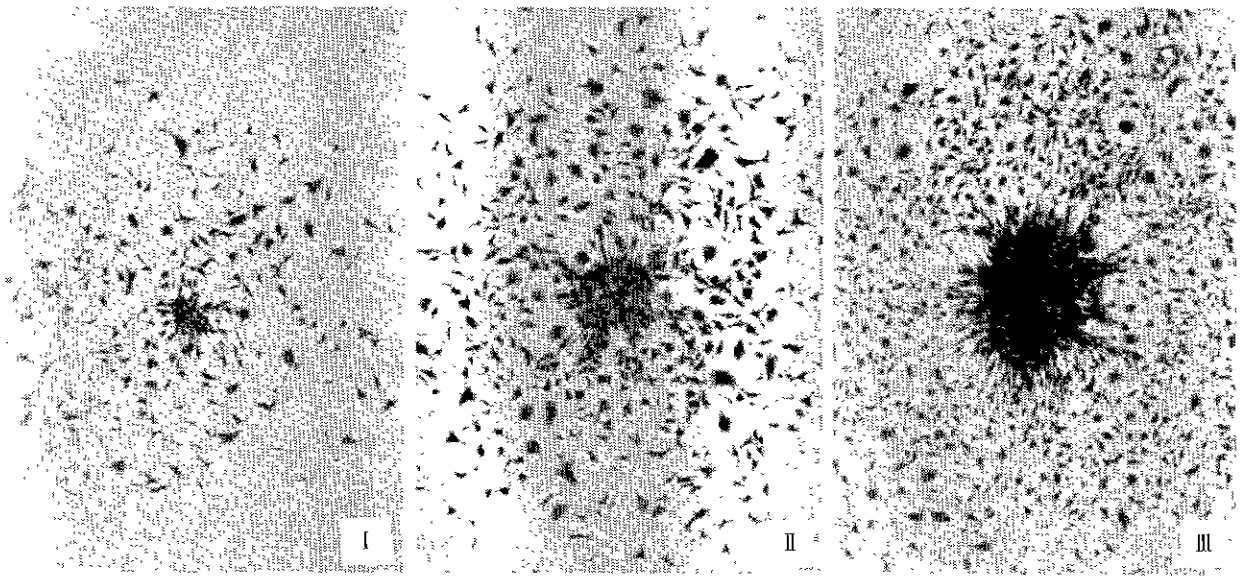

Fig. 1. Photomicrographs of various types of foci formed in the transformation test on C3H/10T1/2 cells treated 5μg/ml of 3-methylcholanthrene(×40).
The experimental procedure is the same as shown in Table 5.

Table 5. Inhibitory effects of the methanol soluble fraction(MSF) of 3 week-fermented kimchi on the transformation of C3H/10T1/2 cells treated with 3-methylcholanthrene(MCA, 5μg/ml)[1]

Kimchi extract	Total number(foci)			
	Type I	Type II	TypeIII	Type II + III
Control(MCA)	2.4±0.9[2]	3.2±0.8[a,3]	4.2±1.1[a]	7.4
MCA+MSF(100μg/ml)	4.2±1.3	2.8±1.5[a,b]	2.2±1.5[a,b]	5.0
MCA+MSF(200μg/ml)	1.8±0.8	0.6±0.6[b]	0.2±0.5[b]	0.8

[1]Twenty four hours after seeding, C3H/10T1/2 cells were treated with MCA(5μg/ml) and MSF(100, 200μg) from 3 weeks-fermented kimchi for 48 hours, at which time a medium change was made. Medium with 10% FCS was changed twice weekly until the cells reached confluence, then once weekly. At 6 weeks, the transformation frequency was calculated.
[2]Values are means±SD
[3]The different letters are significantly different at the $p<0.05$ level of significance as determined by Duncan's multiple range test

diated C3H mice. As shown in Table 5, treatment with MCA(5μg/ml) produced 7.4 foci of type II + III in C3H/10T1/2 cell system. However, when 200μg/ml of methanol soluble fraction(MSF) from the 3-week-fermented kimchi were added to the test system, only 0.8 foci of type II + III were observed($p<0.05$). Thus the methanol soluble fraction(MSF) of 3 weeks-fermented kimchi significantly reduced the transformation frequency(Type II or III) produced by MCA.

Ascorbic acid, carotene, and dietary fiber in kimchi are already believed to be compounds which have antimutagenic and anticarcinogenic effects(4-7). The extracts of red pepper, and the extracts of garlic used as kimchi ingredients also suppressed the growth of cancer cells in the previous reports(8,9).

We previously reported that kimchi extracts exhibited a direct cytotoxic effect on tumor cells *in vitro* and increased the phagocytic cell activities(22). Based on our studies, we suggest that kimchi might have an anticarcinogenic effect by inhibiting the carcinogen-mediated cytotoxicity and neoplastic transformation in the cells and enhancing the immune response related to antitumor activity.

But the question how kimchi extracts inhibited the cytotoxicity and transformation induced by carcinogen from this study remained to be investigated continuously. More research regarding anticarcinogenic effect of kimchi *in vivo* on various types of cancer will have to be continued.

ACKNOWLEDGEMENTS

This research was funded by the MAF-SGRP(Ministry of Agriculture and Forestry-Special Grants Rese-

arch Program) in Korea.

REFERENCES

1. Cheigh, H. S. and Park, K. Y. : Biochemical, microbiological and nutritional aspects of kimchi(Korean fermented vegetable products) *Crit. Rev. Food Sci. Nutr.*, **34**, 175(1994)
2. Oh, Y. J., Hwang, I. J. and Claus, L ' Nutritional evalution of kimchi Symposium Presentation Reports of Korean Soc. of Food Sience & Technology, p 226(1994)
3. Gu, Y. J. and Choi, S. Y : Scientific technology of kimchi. Korean Food Reseach Institute, p 137(1990)
4. Park, K. Y., Kweon, M. H, Baik, H. S. and Cheigh, H. S ' Effect of L-ascorbic acid of the mutagenicity of aflatoxin B_1 in the *Salmonella* assay system. *Environ. Muta. Carcino*, **8**, 13(1988)
5. Bright-See, E : Vitamin C and cancer prevention. *Oncology*, **10**, 294(1983)
6. Mathew-Roth, M. M. · Carotenoids and cancer prevention experimental and epidemiological studies. *Pure Appl. Chem.*, **57**, 717(1985)
7. Hertog, M. G L, Hollman, P. C. H. and Ketan, M B. : Content of potentially anticarcinogenic flavonoids of 28 vegetables 9 fruits commonly consumed in the Netherlands. *J. Agric. Food Chem.*, **40**, 2379(1992)
8. Kim, S H, Park, K. Y. and Suh, M. J. : Inhibitory effects of aflatoxin B_1 mediated mutagenicity by red pepper powder in the *Sallmonella* assay system. *J. Korean Soc Food Nutr.*, **20**, 156(1991)
9. Park, K. Y., Kim, S H., Suh, M. J. and Chung, H. Y. : Inhibition effects of garlic on the mutagenicity in *Sallmonella* assay system and on the growth of HT-29 human colon carcinoma cells. *J. Korean Food Sci. Technol.*, **23**, 370(1991)
10 Fernandes, C. F. and Shahani, K. M. : Anticarcinogenic and immunological properties of dietary Lactobacilli. *J. Food Prot.*, **53**, 704(1990)
11 Park, K. Y., Baek, K. A., Rhee, S. H. and Cheigh, H. S : Antimutagenic effect of kimchi. *Foods Biotech.*, **4**, 141 (1995)
12 Park, K. Y. · The nutritional evaluation, and antimutagenic and anticancer effects of kimchi. *J. Korean Soc. Food Nutr.*, **24**, 169(1995)
13. Hammerschmidt, P. A. and Pratt, D. E : Phenolic antioxidants of dried soybeans, *J. Food Sci.*, **43**, 556(1978)
14. Pratt, D. E. and Birac, P. M. : Source of antioxidant activity of soybeans and soy products. *J. Food Sci.*, **44**, 1720 (1979)
15. Jones, P. A., Laug, W. E., Cardner, A., Nye, C. A., Fink, L M. and Benedict, W. F. : *In vitro* correlates of transformation in C3H/10T1/2 clone 8 mouse cell. *Cancer Res.*, **36**, 2863(1976)
16. Biling, P. C , Uwakfo, A. O. and Heidelberger, C. : Influence of benzoflavine of aflatoxin B_1-induced cytotoxicity, mutation and transformation of C3H/10T1/2 cells. *Cancer Res.*, **43**, 2659(1983)
17. Bovoiko, C. J., Abernethy, D. J. and Stedman, D. B. : Alteration of intercellular communication associated with the transformation of C3H/10T1/2 cells. *Carcinogenesis*, **8**, 321(1987)
18. Raznikoff, C. A , Bertram, J S , Brankow, D. W. and Heidelberger, C. L. : Quantitative and qualitative studies of chemical transformation of cloned C3H mouse embryo cells sensitive to postconfluence inhibition of cell division. *Cancer Res.*, **33**, 3239(1973)
19. Billings, P. C., Heidelberger, C. and Londolph, J. R. : S-9 metabolic activation enhances aflatoxin-mediated transformation C3H/10T1/2 cells *Toxi. Appl. Pharm.*, **77**, 58 (1985)
20. Landolph, J. R. and Heidelberger, C. · Chemical carcinogens produce mutations to obtain resistance in transformable C3H/10T1/2 cells. *Proc. Natl. Acad. Sci. U. S. A.*, **76**, 930(1979)
21. Nennow, S., Garland, H. and Curtis, G. · Improved transformation of C3H/10T1/2 cells by direct and indirect acting carcinogens. *Carcinogenesis*, **3**, 377(1980)
22. Choi, M W., Kim, K H and Park, K Y : Effects of kimchi extracts on the growth of sarcoma-180 cells and phagocytic activity of mice. *J. Korean Soc. Food Sci. Nutr.*, **26**, 254(1997)

(Received September 18, 1997)