Original Article

Anti-cancer activities of pure curry feeding in cancer cell-transplanted mouse

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To confirm the cytotoxic effect of instant curry containing combined spices on cancer cells *in vivo*, cancer was induced by transplanting cancer cells to mice, and the development of cancer upon feeding pure curry were examined. The concentration of lipid peroxide in the groups transplanted with cancer cells which were fed with normal feed was 19.6 nM, and it was increased as the amount of pure curry was increased. The concentration of cytochrome P-450 was decreased in the group transplanted with cancer cells which were fed with pure curry and the group without the transplant which were fed with pure curry when compared with the groups which were fed with normal feed. The activity of cytochrome P-450 was decreased as the concentration of cytochrome P-450 was decreased in the groups transplanted with cancer cells. However, it was increased in the groups without cancer cell transplant when over 2% of pure curry was fed. The amount of glutathione was increased in the groups transplanted with cancer cells when over 2% of pure curry was fed. The activities of glutathione peroxidase and glutathione *S*-transferase were decreased in the groups transplanted with cancer cells which were fed with over 1% of pure curry, and were restored to the level of the group without cancer cell transplant which were fed with normal feed. The superoxide dismutase activity in the groups transplanted with cancer cells was restored to the level of the group without cancer cell transplant which was fed with normal feed when over 1% of pure curry was fed.

Key Words: pure curry, lipid peroxide, cytochrome P-450, glutathione peroxidase, superoxide dismutase

Introduction

As diet habits become westernized and dining out industries develop, the consumption of instant curry and other foods containing various spices such as pizza and spaghetti is increasing in South Korea. The primary purpose of the use of spices in food is to stimulate appetite as well as to eliminate off-taste and off-flavor, to add aroma, and to dye foods. Nowadays, spices are also used to develop commercial disease-preventing foods by utilizing physiological activities such as anticancer activity, antibiotic activity and physiological-pharmaceutical activity.[1,2]

Superoxide anion radical, which is generated during the physiological metabolism related to the aging in human body, is known to toxic to cells and tissues, and thus stimulates tumors. It is also known to induce duodental ulcer, diabetes, arthritis, Alzheimer disease and the aging of the skin.[3-6] The compounds with superoxide dismutase (SOD)-like activity and low molecular weight are belongs to phytochemicals. It is known that they protect cells from superoxide toxicity by inhibiting the superoxide anion radical reaction. Exposure to environmental factors as well as chemicals and radiation cause an estimated over 90% of all cancers. Researches show that about one-third of all cancer deaths are related to dietary factors which are related to the initiation and proliferation step of cancer development.[7]

Many foods contain nitrates or nitrites which are used as preservatives.[8,9] A portion of the nitrate, which is not harmful to human health, is reduced to nitrite by nitrate reductase in the saliva and stomach. Nitrites can form nitrosating agents, and these reagent in turn can react with amines naturally present in food or in the human stomach to form carcinogenic nitrosamine.[10,11] It is known that compounds with reducing activity such as Vitamin C, α-tocopherol, sulfur dioxide and polyphenols have an ability to suppress the generation of nitrosamine and to destroy nitrosating agents or to convert them to inactive substances, and thus to decrease the generation of nitrosamine.[12-14]

The effects of pure curry on the elimination of superoxide as well as the degradation of nitrite have been reported by evaluating 10 instant commercial curry products and 26 spices that are used for curry.[15] They reported that clove, cassia and rosemary showed the strong scavenging activity on superoxide anion radical that attacks DNA in normal

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Table 1. Anticarcinogenic effects of herbs and spices

Materials	Functions and mechanism	References
Turmeric (curcumin)	- Inhibits human colon cancer (HT-29, HCT-15) proliferation - Inhibits mouse skin cancer by inhibiting protein kinase C activity, tyrosine protein kinase activity and arachidonic metabolism - Induces apoptosis on NIH 3T3 and cancer cell line	16 17
Turmeric (curcumin)	- Has chemopreventive effect on mice skin, forestomach, colon and oral cancer - Decreases the death rate and hepatocellular carcinoma production in mouse due to the liver cancer induced by diethylnitrosamine	18
Turmeric (curcumin)	- Has cytotoxic activity which repress bladder cancer production	19
P54 (Complex of curcumin and essential oil)	- Effective on colon cancer patients by acting as a anti-inflammatory agent	20
Capsaicin, Curcumin & Ginger	- Inhibit mutation and cancer development	21
Cumin, Turmeric & Basil	- Increase the glutathione-S-transferase activity to over 78% in stomach, liver, and esophageus	22
Rosemary extract	- Inhibits DNA adduct formation induced by benzo(a)pyrene or aflatoxin B1 - Induces the expression of glutathione-S-transferase	23
Rosemary extract	- Protects liver and stomach from toxic and carcinogenic materials by stimulating the activities of detoxification enzyme, GST and QR	24
Rosemary (carnosol)	- Inhibits the mouse breast cancer induced by 7,12-dimethylbenz(a) anthracene(DMBA) and DMBA-DNA adduct formation	25
Herbs & Spices (phenols, terpenes, isothiocynates, and allyl sulphides)	- Prevent the formation of tumor in animals exposed by carcinogen - Act as inducer or inhibitor of cancer-related enzymes - Act as antioxidants or apoptosis inducer	26
Plant lectin & Saffron	- Have antitumor and anticarcinogenic effect	27
Alcohol extract of rosemary and sage	- Has antitumorgenic activities	28
Coriander seed	- Inhibits the toxic effect of lipid metabolism in colon cancer induced by 1,2-dimethyl hydrazine	29
Garlic & Olive oil	- Lower the development of stomach cancer	30

cells, induces mutations, and generates cancer cells, and such effects were also detected in 10 instant curry products. In addition, the effect of eliminating nitrite that generates the strong carcinogen nitrosamine has been proved in all spices and curry products. These results imply that intake of instant curry or its ingredients might prevent the aging and cancer development.

Studies on the anticarcinogenic effect of spices such as turmeric, rosemary extract, sage extract, ginger, coriander seed, black pepper, cumin and garlic have been carried out by many research groups as shown in Table 1.[16-30] Such spices have been known to be effective on large intestine cancer, liver cancer, stomach cancer, breast cancer, skin cancer, bladder cancer, and colon cancer. Some papers, however, reported the possible carcinogenicity of ethylene oxide which is used as a sterilizing agent of spices[31] and a epidemiologic study showed the high occurrence of esophageus cancer in immigrant Indian women in Israel where the use of spices is prevalent.[32]

In this study, cancer cells were transplanted into mice to induce cancer, and in related to the development of cancer, the production of free radicals and substances related to the elimination of toxic materials in the liver were compared and analyzed to validate the *in vitro* result of the toxicity to cancer cells of pure curry *in vivo*.

Materials and methods

Experimental protocol

The following experiments were performed to validate the effect of pure curry, a component of instant curry, which was fed to mice on the biochemical substances related to cancer which was induced on mice by injecting murine ascites (Sarcoma-180). The ingredients of pure curry were shown in Table 2 and animal experiments were performed according to the protocol shown in Tables 3.

Table 2. Recipe of pure curry

Ingredients	Contents (%)
Turmeric	27.0
Coriander seed	27.0
Cumin	12.0
Fenugreek	9.0
Fennel	6.0
Ginger	4.0
Nutmeg	3.0
Cinnamon	3.0
Celery seed	4.0
Others	5.0
Total	100.0

Maintenance of experimental animals

Three weeks old male mice (IBRS #202), of which the average body weight is 10 g, were obtained from Samtaco Co. in Korea. They were adapted to powder feed for mice (Samtaco, #31) for 1 week, and they were randomly divided into 21 animals per group, and kept 3 mice per cage. Control mice were fed with powder feed for mice and experimental mice were fed with powder feed mixed with pure curry at the concentration of 1.0, 2.0, or 5.0%. Ex-

Table 3. Group designation and supplementation dose of pure curry

Groups		Experimental feeds (EF)	Treatment during experimental periods			
			1 week	4~7 weeks	8 weeks	after 8 weeks
Cancer	A (n = 21)	ND[ψ]	Adaptation (ND)	Feeding (EF)	Sarcoma injection	Feeding (EF)
	B (n = 21)	ND + 1.0% PC[ζ]	Adaptation (ND)	Feeding (EF)	Sarcoma injection	Feeding (EF)
	C (n = 21)	ND + 2.0% PC	Adaptation (ND)	Feeding (EF)	Sarcoma injection	Feeding (EF)
	D (n = 21)	ND + 5.0% PC	Adaptation (ND)	Feeding (EF)	Sarcoma injection	Feeding (EF)
Normal	E (n = 21)	ND	Adaptation (ND)	Feeding (EF)	-	Feeding (EF)
	F (n = 21)	ND + 1.0% PC	Adaptation (ND)	Feeding (EF)	-	Feeding (EF)
	G (n = 21)	ND + 2.0% PC	Adaptation (ND)	Feeding (EF)	-	Feeding (EF)
	H (n = 21)	ND + 5.0% PC	Adaptation (ND)	Feeding (EF)	-	Feeding (EF)

[ψ] ND: normal diet (Samtaco, Feed # 31); [ζ]PC: pure curry; The age of mice were 3 weeks when the experiment began.

perimental animals were raised sufficiently with appropriate feeds for 7 weeks (11 weeks old) prior to the administration of cancer cells. During the period, diet and water were not limited, and the temperature, humidity and light were controlled 20 ± 1℃, 55 ± 10%, 12 h (8:00 AM - 20:00 PM), respectively.

Sarcoma 180 culture and injection

Sarcoma-180 cells stored in a nitrogen tank were cultured in RPMI medium supplemented with penicillin-streptomycin at 37℃, 5% CO_2 incubator. Cultures were refed twice, and cells were washed with PBS after 4 days. The adherent cells were detached with 0.05% trypsin-0.02% EDTA, centrifuged at 1,000 rpm for 10 min. The pelleted cells were counted using a hemacytometer and diluted to 10^7/mL with RPMI medium. The cultured sarcoma 180 cells were activated *in vivo* by injecting to the left peritoneal cavity of mice approximately 10 days prior to the transplantation of cancer cells to mice. Ten milliliter PBS was injected into the peritoneal cavity of mice anaesthetized with ether, shaken sufficiently and sarcoma 180 cells were collected with PBS using a 10 mL syringe. This procedure was repeated 3 times.

Half milliliter of cancer cells collected were mixed with 0.5 mL tryptophan at the ratio of 1:1, counted using a hemocytometer, diluted to 10^7/mL with PBS, and 0.1 mL cancer cells were injected into the left peritoneal cavity of cancer cell transplant groups.

Collection of organs and pretreatment with the agents

Experimental animals were weighted immediately after anesthetized and their liver and kidney were collected. The collected organs were rinsed with cold saline, and the moisture was removed completely with absorbing papers, weighted, and stored in a -70℃ liquid tank until used. For the liver tissues, 4 volumes of 0.1 M potassium phosphate buffer (pH 7.4) was added to 1 g tissues, and homogenized with a glass teflon homogenizer. The homogenized tissues were centrifuged at 600 × *g* for 10 min to remove nucleus and nonhomogenized tissues, and the supernatant was centrifuged again at 10,000 × *g* for 20 min. When the supernatant was centrifuged at 105,000 × *g* for 1 h, the supernatant was considered as cytosol fraction, and the pellet dissolved in 0.1 M potassium phosphate buffer (pH 7.4) was considered as microsome fractionation. The concentration of cytochrome P-450 and cytochrome P-450 activity were measured from microsome fraction, and cytosol fraction was used for the measurement of glutathione peroxidase and glutathione-*S*-transferase. For the assessment of lipid peroxidase, 9 volumes of 1.15% KCl buffer was added to 1 g liver tissues and the samples were prepared by the homogenization. All procedures were performed at 4℃ unless described otherwise.

Measurement of lipid peroxidase concentration in mouse liver

The concentration of lipid peroxidase was measured according to the method described by Ohkawa *et al.*[33] Two hundred microliters of 8.1% sodium dodecyl sulfate and 1.5 mL 20 % acetic acid buffer (pH 3.5) were added to 0.2 mL liver homogenizer. Then, 1.5 mL 0.67% thiobarbituric acid was added for staining, incubated in a 100℃ water bath for 1 h, and cooled immediately after the incubation. One milliliter of distilled water and 5 mL *n*-butanol were added to the above reaction mixture and centrifuged at 3,000 × *g* for 10 min. The supernatant was collected and the optical density at 532 nm was measured. The concentration of lipid peroxidase was calculated from the standard curve. For the standard curve, 1,1,3,3-tetraethoxypropane (TEA) was dissolved in 1.15 % KCl and prepared 200, 100, 50, 25, and 12.5 μM solution immediately prior to use.

Measurement of the concentration of cytochrome P-450 in mouse liver

The concentration of cytochrome P-450 in the microsome of liver tissues was measured according to the method described by Omura and Sate.[34] Microsomal suspension was diluted with phosphate buffer (pH 7.4) to the 1 mg/mL of protein concentration and added 2 mg sodium dithionite. Subsequently, the samples were flushed with carbon monoxide (CO) gas for 30 sec (1 bubble/sec) and

the optical densities of the reduced carbon monoxide complex at 450 nm and 490 nm were measured using a UV spectrophotometer. The molar extinction coefficient was used as 91 m^{-1} M cm^{-1}, and the concentration of cytochrome P-450 (nmole/mg) was calculated by applying the following formula.

cytochrome P-450 (nmole/mg) =
⊿A(450~490)×1,000/91×V mL (microsome mL + buffer mL) / V mL (microsome buffer mL)

Measurement of cytochrome P-450 activity in mouse liver

Cytochrome P-450 activity in microsome fraction was measured according to the method described by Ding *et al.*[35] Hundred microliters of 1 mM ascorbic acid, 100 μL of 0.1 mM *p*-nitrophenol, and 600 μL of 0.1M potassium phosphate buffer were added to 100 mL microsome fraction diluted to the protein concentration of 1 - 1.5 mg/mL, and adjusted the final voule to 900 μL. The samples were preincubated for 2 min at room temperature and 100 μL of 10 mM NADPH was added. The samples were incubated at room temperature for 3 min and 500 μL of cold 10% perchloric acid was added. Subsequently, the samples were centrifuged at 3,000 × *g* for 5 min, 1 mL of supernatant was collected, 100 μl of 10 N NaOH was added to the supernatant, and the optical density at 546 nm was measured. The cytochrome P-450 activity was calculated referring to the standard curve that was obtained by preparing 0, 2.5, 5.0, 7.5, 10 and 15 μM *p*-nitrocatechol.

Measurement of the glutathione concentration in mouse liver

The concentration of glutathione was measured according to the method described by Ellma.[36] Half milliliters of 4% sulfosacylic acid was added to 0.5 mL cytosol fraction, centrifuged at 2,500 × *g* for 10 min, and protein was removed. To the 0.3 mL supernatant, 2.7 mL of 0.1 mM disulfide reagent (5.5‘-dithiobis-2-nitro benzoic acid dissolved in 0.1 M sodium phosphate buffer, pH 8.0) was added, and the blue color formed was measured at 412 nm. The glutathione concentration was calculated from the standard curve, and presented as glutathione mole per 1 g tissue.

The measurement of the glutathione peroxidase activity

To measure the glutathione peroxidase (GSHPx) activity, glutathione is oxidized by peroxidase and the reduction of NADPH concentration during the process was measured by the method of Paglia and Valentine.[37] Five hundred microliters of buffer (100 mM potassium phosphate buffer containing 2 mM EDTA and 2 mM NaN_3) was added to 100 μL cytosol and 100 μL of 0.2 mM NADPH was added. In addition, 100 uL of 10 EU/mL GSSG-reductase was added, mixed sufficiently, and incubated for 5 min at room temperature. Subsequently, 100 μL of 2.5 mM H_2O_2 was added, and the optical density at 340 nm was measured. The unit of glutathione peroxidase activity was presented as nmole that is the amount of oxidized NADPH by the reaction of 1 mg protein for 1 min. The enzyme activity was calculated by the angle of the optical density that represents the degree of the oxidization of NADPH to NADP and the optical density coefficient $E^{mM} = 6.22\ M^{-1}$ was used.

Measurement of glutathione-S-transferase activity

According to the method described by Habig,[38] 500 uL of 0.1 M potassium phosphate buffer (pH 6.5) was added to 100 μL cytolol and 75 μL of 0.04 M reduced glutathione (dissolved in 0.1 M potassium phosphate buffer) was added. After incubating at 25℃ for 5 min, 325 μL of 0.12 M 1-chloro-2,4-dinitrochlorobezene was added as a matrix, and the change of the optical density at 340 nm was measured for 1 min. The activity was measured using 9.6 $mM^{-1}cm^{-1}$ as a mole light absorbance coefficient and 20% trichloroacetic acid was used as a blank.

Measurement of superoxide dismutase (SOD) activity

SOD activity was measured by applying the principle that nitroblue tetrazolium (NBT) is reduced and becomes blue color by O_2^- generated during the oxidization of hypoxanthine by xanthine oxidase (XOD), and SOD suppresses the generation of O_2^- resulting in the suppression of the reduction of NBT.[39] Five microliters of 5 mM Xanthine (pH 7.4), 5 μL of 10 mM EDTA, 5 μL of 2 mM NBT, 430 uL of 50 mM phosphate buffer (pH 7.8), and 5 μL 3 × xanthine oxidase were added sequentially to a disposable cuvette containing 50 ~ 150 μL sample solutions diluted according to their titration, and the final volume was adjusted to 500 μL. Two hundred microliters of xanthin oxidase was added to a microtube and centrifuged at 13,000 × *g* for 10 min, the supernant was removed, and diluted with 600 μL of 50 mM phosphate buffer. The change of the optical density at 560 nm was measured for 1 minute, and as control, phosphate buffer was added instead of samples. Prior to main experiments, R-value was calculated by dividing the sample optical density value by the control optical density value and prepared the standard curve to determine the amount and concentration of sample inhibiting 50% of the reduction rate of NBT. Then the amount of the sample was measured when the R-value equals to 2. One unit of SOD activity was defined as the amount suppressing 50% reduction rate of NBT in 1 min and SOD was calculated according to the following formula.

$$\text{SOD activity (unit/g)} = \frac{1}{\text{The amount of sample at R=2 (mL)}} \times 20$$

Statistical analysis

The mean and standard deviation of each experimental group was calculated by applying STATISTIX,[40] and the significant difference between experimental groups was assessed by ANOVA test.

Results

Content of lipid peroxide

The concentration of lipid peroxide in the liver was measured according to the experimental method in this study and the results were presented in Table 4. When the groups with cancer cell transplant fed with normal feed, concentration of lipid peroxide was 19.6, and the concentration of lipid peroxide was increased to 22.7, 25.8, 27.9 nM as the addition of pure curry was increased. In addi-

Table 4. The effect of pure curry on hepatic lipid peroxide content in normal and Sarcoma-180 treated IBRS mice

Experimental Design		Content (nM/g, Mean ± SD[ξ])
Treatment	Group	
Cancer	A (ND[Ψ])	19.6 ± 3.43
	B (ND + 1% PC[ζ])	22.7 ± 2.58*
	C (ND + 2% PC)	25.8 ± 4.08**
	D (ND + 5% PC)	27.9 ± 4.93**
Normal	E (ND)	16.3 ± 2.53
	F (ND + 1% PC)	19.1 ± 3.41
	G (ND + 2% PC)	20.8 ± 4.07*
	H (ND + 5% PC)	26.0 ± 4.35**

[Ψ] ND: normal diet (Samtaco, Feed # 31); [ζ] PC: pure curry; [ξ] SD: standard deviation; * significantly different at $p< 0.05$ level by ANOVA test; ** significantly different at $p< 0.01$ level by ANOVA test

tion, in the groups without cancer cell transplant, the content of lipid peroxide was significantly increased with increasing the amount of pure curry. This result shows that as the addition of pure curry was increased, it damaged the liver, and the addition of pure curry up to 1% did not harm the liver in normal mice without cancer cell transplant.

When the concentration of lipid peroxide according to with or without cancer cell transplant was measured, the significant difference was not detected in the cases fed with normal feed or the feed containing 5% pure curry, but the significant difference was detected in the groups fed with 1% and 2% pure curry.

Concentration of cytochrome P-450 and the activity of cytochrome P-450

Table 5 shows the concentration and the activity of cytochrome P-450 in the groups transplanted with cancer and the groups without transplant. There were significant ($p< 0.05$) differences between the groups with cancer fed with normal diet and fed with the diet supplemented with pure curry. When the consumption of pure curry was 1 % in the groups without cancer cell transplant, the difference was not significant. When the consumption of pure curry was over 2 %, the significant reduction at $p< 0.05$ was detected, which may be due to the reaction of antioxidant substances in pure curry. The consumption of pure curry exerts the effect of restoring the concentration of cytochrome P-450 that was increased by the development of cancer to the normal level. In addition, the consumption of pure curry in the groups without cancer cell transplant was shown to reduce the concentration of cytochrome P-450, and thus to increase the antioxidant activity in the liver.

In the groups transplanted with cancer cells, the significant difference in the activity of the cytochrome P-450 was not detected, but it has the tendency to be reduced similar to the change of the concentration of cytochrome P-450. However, the activity of the cytochrome P-450 in the groups without cancer cell transplant and fed with 1% pure curry was significantly reduced ($p< 0.05$), and in the groups fed with over 2%, it was significantly increased.

Glutathione concentration, Glutathione peroxidase activity, and Glutathione S-transferase activity

The change of the concentration of glutathione among the treatment groups was shown in Table 6. Glutathione concentration was reduced to 0.14 mol/g in the groups transplanted with cancer cells and fed with normal feed, and it was significantly reduced to 0.10 mol/g ($p< 0.001$) when fed with 1% pure curry. It was increased to over 0.15 mol/g when fed with over 2% pure curry, and thus restored to the level identical to the group without cancer cell transplant and fed with normal feed. Furthermore, the

Table 5. The effect of pure curry on hepatic cytochrome P-450 content and activity in normal and Sarcoma-180 treated IBRS mice

Experimental Design		Cytochrome P-450 content (nmol/mg, Mean ± SD[ξ])	Cytochrome P-450 activity (Mean ± SD)
Treatment	Group		
Cancer	A (ND[Ψ])	5.71 ± 1.79	1.83 ± 0.28
	B (ND + 1% PC[ζ])	3.06 ± 0.94*	1.66 ± 0.79
	C (ND + 2% PC)	2.85 ± 1.21*	1.68 ± 0.49
	D (ND + 5% PC)	3.36 ± 0.53*	1.57 ± 0.58
Normal	E (ND)	3.49 ± 1.28	1.32 ± 0.25
	F (ND + 1% PC)	3.82 ± 0.81	1.15 ± 0.22*
	G (ND + 2% PC)	2.92 ± 1.36*	1.53 ± 0.33*
	H (ND + 5% PC)	2.53 ± 0.85*	1.66 ± 0.42*

[Ψ] ND: normal diet (Samtaco, Feed # 31); [ζ] PC: pure curry; [ξ] SD: standard deviation; * significantly different at $p< 0.05$ level by ANOVA test

Table 6. The effect of pure curry on hepatic glutathione content, glutathione peroxidase activity and glutathione *S*-transferase activity in normal and Sarcoma-180 treated IBRS mice

Experimental Design		Glutathione content (mol/g, Mean ± SD[ξ])	Glutathione peroxidase activity (nmol/mg protein·min Mean ± SD)	Glutathione *S*-transferase activity (Mean ± SD)
Treatment	Group			
Cancer	A (ND[ψ])	0.14 ± 0.01	178 ± 38.2	3.13 ± 1.64
	B (ND + 1% PC[ζ])	0.10 ± 0.02**	51.0 ± 16.8**	1.88 ± 1.04
	C (ND + 2% PC)	0.15 ± 0.04	25.7 ± 44.1**	1.61 ± 0.78
	D (ND + 5% PC)	0.17 ± 0.02	43.3 ± 18.4**	0.77 ± 0.56**
Nomal	E (ND)	0.17 ± 0.02	63.6 ± 21.3	0.74 ± 0.43
	F (ND + 1% PC)	0.18 ± 0.03	24.9 ± 9.4**	1.52 ± 0.53**
	G (ND + 2% PC)	0.19 ± 0.02	33.5 ± 16.1**	0.76 ± 0.39
	H (ND + 5% PC)	0.17 ± 0.01	28.7 ± 14.7**	0.85 ± 0.38

[ψ] ND: normal diet (Samtaco, Feed # 31); [ζ] PC: pure curry; [ξ] SD: standard deviation; ** significantly different at $p< 0.01$ level by ANOVA test

tendency of the increase of the concentration of glutathione was detected in the groups without cancer cell transplant, although the difference was not significant, hence it was confirmed that the detoxification ability was increased.

Glutathione peroxidase activity of the groups transplanted with cancer cells and fed with normal feed was 178 nmol/mg protein·min, which was increased approximately 3 times in comparison with the group without cancer cell transplant. However, it was less than 50 nmol/mg protein·min in the groups consumed pure curry higher than 1 %, and the difference was significant ($p< 0.01$). In addition, in the groups without cancer cell transplant, it was detected that the consumption of pure curry induced the decrease of glutathione peroxidase activity significantly ($p<0.01$), and thus the toxicity of cancer cells was decreased.

The glutathione *S*-transferase activity of the groups with cancer cell transplants fed with normal feed was 3.13 nmol/mg protein·min, which is the approximately 4 times higher than the group without cancer cell transplant. However, glutathione *S*-transferase activity was reduced approximately to 40% in the group fed 1% pure curry, although it was not significant, to 50% in the group fed 2% pure curry, and glutathione *S*-transferase activity in the group fed 5% pure curry was reduced significantly ($p < 0.01$) and restored to the level similar to the group without cancer cell transplant fed with normal feed. In the groups without cancer cell transplant, the consumption of 1% pure curry significantly increased the activity approximately 2 times in comparison with the group fed with normal feed, and when 2% was consumed, the level identical to the groups without cancer cell transplant was maintained.

Superoxide dismutase activity

SOD activity was reduced mostly to 584 unit/g in the groups with cancer cell transplant fed with normal feed, and the consumption of 1% ($p< 0.05$) and 5% ($p< 0.01$) pure curry increased SOD activity significantly (Table 7). When 2% pure curry was fed, it was shown to increase SOD activity, although it was not significant. In the groups without the development of cancer fed 1% pure curry, SOD activity was detected to be higher than the group fed with normal feed with no significant difference. Similarly, when consumed 2%, there is no significant difference in the change of SOD activity.

Table 7. The effect of pure curry on hepatic superoxide dismutase content in normal and Sarcoma-180 treated IBRS mice

Experimental Designs		Content
Treatment	Group	(unit/g, Mean ± SD[ξ])
Cancer	A (ND[ψ])	584 ± 46.1
	B (ND + 1% PC[ζ])	693 ± 126*
	C (ND + 2% PC)	610 ± 54.2
	D (ND + 5% PC)	698 ± 87.6**
Normal	E (ND)	1019 ± 335
	F (ND + 1% PC)	1128 ± 197
	G (ND + 2% PC)	900 ± 274
	H (ND + 5% PC)	961 ± 141

[ψ] ND: normal diet (Samtaco, Feed # 31); [ζ] PC: pure curry; [ξ] SD: standard deviation; * significantly different at $p< 0.05$ level by ANOVA test; ** significantly different at $p< 0.01$ level by ANOVA test

Discussion

Lipid peroxide is the lipid peroxidized by the addition of oxygen to unsaturated fatty acid. It is generated by the reaction of reactive oxygen with unsaturated fatty acid that consists of the important component of cell membrane phospholipid, and it occurs readily on cell membrane of mitochondria, microsome, erythrocyte, platelet, *etc*. where unsaturated fatty acid and phospholipids are abundant. In addition, the generation of peroxidized lipid is dependent on a series of chain reactions initiated from the biosynthesis of the active form of oxygen superoxide radicals. The toxicity study of such dissociated products have been shown that these products suppress the activity of various enzymes, induces the necrosis of cells, and causes the cirrhosis of the microvasculature, *etc*.[41] The generation of lipid peroxide is a marker representing the pathophysiological phenomenon or the degree of tissue

damage, and the peroxidation of unsaturated fatty acid that is the phospholipids of biological membrane is initiated by activated oxygen species such as free radical and proceeded continuously.[42]

The results of the assessment of the content of lipid peroxide that represents the degree of tissue damage demonstrated that there is no significant difference in the content of lipid peroxide in the normal group fed with 1% pure curry. But when the concentration of spices increased to 2% or 5%, the content of lipid peroxide was increased, which suggests that the long-term uptake of high concentration of pure curry, which consists of more than ten spices, induces hepatotoxicity and therefore injures liver tissues. In case of the groups transplanted with cancer, the content of lipid peroxide was increased when mice were fed with normal diet presumably due to the oxidation stress from the growth of cancer cells.

The substances in therapeutic drugs or the components of food that induce tissue damage or have adverse effects on the function of cells are metabolized by cytochrome P-450 containing heme in the mitochondria. These substances require NADPH as a reducing cofactor and also require oxygen for the oxidization of the substrates, and thus cytochrome P-450 is referred to as mixed function oxidase.[43,44] The substances that can be metabolized by this enzyme are various drugs, chemicals, solutes, carcinogens, pesticides, *etc*. that are not only exogenous substances which we can be exposed readily, but also what are involved in the metabolism of the endogenous substances such as vitamin, steroid, and arachinoic acid. In most cases, biological activity or toxicity of metabolites that underwent such oxidization process is reduced by the second reaction with the phase II conjugate enzymes and these metabolites are excreted externally. When the amount of cytochrome P-450 increased, toxic microsubstances that are not harmful under normal condition undergo metabolic processes and become more harmful than the original substances.[45-47]

The result in this study shows that at the time of the development of cancer, the concentration of cytochrome p-450 increased substantially to detoxify and excrete peroxides such as free radicals, and, the level of cytochrome p-450 in all the groups fed with pure curry was same as the group without cancer and fed with general feed. This is in agreement with the report that the administration of antioxidants reduces cytochrome P-450,[47] and it shows that when the consumption of pure curry was over 1%, the change of the level of cytochrome P-450 is not dependent on the concentration.

When the consumption of pure curry was 1% in the groups without cancer cell transplant, the difference was not significant. As the concentration of pure curry increased, the activity was increased due to that spices react as strong antioxidants.

Glutathione is a protective system of cells that contains a sulfhydryl radical and is used during the detoxification process of electrophilic substances, oxygen free radicals, and the lipid peroxidation metabolism. In addition, it is involved in the reduction of lipid peroxidation by which glutathione is forming a conjugation with various activity-enhancing substances, facilitating the urination by the enzyme activity such as glutathione-*S*-transferase, and thus mediates the elimination of the toxic substances in the body.[48,49] The phase II step of the metabolic enzyme system in the liver is to convert endogenous substances or the toxic substances from the outside of the body into water-soluble substances and to eliminate those substances by excreting to the outside of the body.[50,51] Glutathione *S*-transferase (Rx glutathione *R*-transferase, EC 25118, GST) is the enzyme that adds glutathione (GSH) primarily to lipophilic or electrophilic substances (Rx) and thus mediates the reaction forming thioether (R-S-G) of glutathione. It is therefore known to be the enzyme involved in the detoxification by transferring or excreting toxic materials and peroxidized materials in the body.[52] In addition, the free radical-mediated oxidation of lipid in cell membrane is proceeded by chain reactions, and antioxidants such as spices react as a chain breaking antioxidants, and thus delay the chain propagation. In other words, the antioxidant enzymes, glutathione peroxidase and catalase, are the protection system during the initial period of oxidation, and spices can be considered as the protection system during the progression steps of the oxidization.[53]

Superoxide anion radical ($O_2^-\bullet$), a substance which has influence on aging, is generated during the metabolism process in the body, has a strong activity to react and destroy during the reduction of electrons, and thus induces the potent toxicity to cells and tissues. Due to such toxicity, various aging related diseases, various diseases prevalent in adults, and arthritis are developed, and it is known that SOD is released to eliminate $O_2^-\bullet$ and involved in the conversion of $O_2^-\bullet$ to H_2O_2 and O_2 in the body. Although SOD-like active substances are not enzymes, they are low molecular weight phytochemicals with the similar functions to SOD, and it has been reported that they suppress the reaction of superoxide and protect the body from superoxide. Polyphenols, which is a kind of photochemical, were found to markedly inhibit apoptotic characteristics by reducing intracellular reactive oxygen species generation and by recovering the mitochondria membrane potential.[54]

Our result shows that SOD activity was reduced in the groups with cancer cell transplant fed with normal feed, and the consumption of 1% and 5% pure curry increased SOD activity significantly. This revealed that it has the ability to eliminate free radicals generated during the development of cancer. Also SOD activity was detected to be higher than the group fed with normal feed with no significant difference, and it was confirmed that it might improve the liver function eliminating peroxide *etc*. Therefore, it demonstrates that during the development of cancer, SOD activity was reduced severely, and the consumption of pure curry partially restored the reduced activity.

Although there is a little amount of SOD enzyme in pure curry, the SOD activity in this report would not due to the action of SOD. It is known that SOD does not easily penetrate the cells due to its molecular weight with higher than 30 kDa and loses its enzymatic activity by heating over 70℃.[54] For these reasons, SOD activity in this report might be due to the SOD-like action of curcumin, which is one of major polyphenolic substances in pure curry.

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Original Article

Anti-cancer activities of pure curry feeding in cancer cell-transplanted mouse

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餵食純咖哩對癌細胞轉殖小鼠的抗癌活性

為確認即時咖哩中的綜合香料對活體癌細胞的細胞毒素影響，以轉殖癌細胞至小鼠身上而誘導癌症發生，評估餵食純咖哩對於癌症發展的影響。轉殖癌細胞餵食正常飼料小鼠的脂質過氧化物濃度為 19.6nM，當純咖哩的量增加時其濃度也上升。與餵食正常飼料小鼠相比，轉殖癌細胞餵食純咖哩組及未轉殖癌細胞但餵食純咖哩組，其細胞色素 P-450 濃度較低。在轉殖癌細胞組，細胞色素 P-450 的活性是隨著細胞色素 P-450 的減少而降低。然而，它在沒有轉殖癌細胞但餵食超過 2%純咖哩組則是增加的。轉殖癌細胞組餵食純咖哩超過 2%時，麩胱甘肽的量會上升。轉殖癌細胞組餵食純咖哩超過 1%時，麩胱甘肽過氧化酶及麩胱甘肽 S 轉化酶活性會下降，回復到未轉殖癌細胞並餵食正常飼料組的水準。當餵食咖哩超過 1%時，轉殖癌細胞組的超氧歧化酶的活性回復到未轉殖癌細胞並餵食正常飼料組的水準。

關鍵字:純咖哩、脂質過氧化、細胞色素 P-450、麩胱甘肽過氧化酶、超氧化岐化酶。