Food Sci. Technol. Res., **9** (2), 148–151, 2003

Note

6-(Methylsulfinyl)hexyl Isothiocyanate Isolated from Wasabi (*Wasabia japonica* MATSUM) Suppresses Tumor Progression in an Experimental Mouse System

Yoko FUKE,[1] Naoki HIGASHI,[1] Ikuko NAGATA,[1] Saeko SAWAKI,[1] Takahiro NOMURA[2] and Kazuo RYOYAMA[3]

[1]*Department of Food Science and Human Nutrition, Tokyo Metropolitan College, Akishima-shi, Tokyo 196-8540, Japan*
[2]*Cancer Research Institute, Kanazawa University, Kanazawa-shi, Ishikawa 920-0934, Japan*
Present address: Kanazawa Gakuin College, Kanazawa-shi, Ishikawa 920-1392, Japan
[3]*Faculty of Technology, Aomori University, Aomori-shi, Aomori 030-0943, Japan*

Received June 18, 2002; Accepted January 23, 2003

We examined the anti-metastatic effect of 6-(methylsulfinyl)hexyl isothiocyanate (6-MITC) isolated from wasabi (*Wasabia japonica* MATSUM). Pulmonary micrometastasis was quantified using a dependable method to detect the human c-Ha-ras gene, which was carried in the tumor cell line. Mice belonging to the S-1 group were administered 6-MITC continuously for 35 days from the time of tumor cell inoculation, and S-2 group mice were administered 6-MITC for 21 days from the day of amputation. Oral administration of 200 μM 6-MITC solution was effective in preventing metastasis of the experimental tumor. In the S-1 group, 7 out of ten experimental mice have lungs carrying no detectable human c-Ha-ras gene. Amplified human c-Ha-ras bands were detected in only the lungs of three mice; in these, the metastatic indexes of the lungs were respectively 0.60, 0.70 and 0.90. In the S-2 group, the bands were detected in four lungs of 5 experiments, with the metastatic indexes of the lungs in the range 0.36–0.72. Starting the treatment at the time of tumor cell inoculation was more effective in preventing metastasis than beginning the treatment on the day of amputation.

Keywords: 6-(methlysulfinyl) hexyl isothiocyanate (6-MITC), wasabi, tumor metastasis, r/mHM-SFME-1 cell line

Many food plant ingredients affect physiological functions such as activation of the immune system, suppression of tumor development, induction of cellular differentiation, and activation of cell proliferation (Osawa, 1995). Among them, wasabi (*Wasabia japonica* MATSUM), a typical pungent spice in Japan, not only affects appetite but also inhibits platelet aggregation (Kumagai *et al*., 1994) and anti-microbial activity (Isshiki & Tokuoka, 1993). Recently, wasabi has also been reported to inhibit the growth of *Helicobacter pylori* in the stomach (Kinae *et al*., 2001) and to suppress 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NKK)-induced lung tumorigenesis in mice (Yano *et al*., 2000). We have reported that a specific fraction of wasabi inhibits the growth of human stomach cancer cells *in vitro* (Fuke *et al*., 1994). The active ingredient of this fraction has been identified as 6-(methylsulfinyl)hexyl isothiocyanate (6-MITC), which is not a pungent component of wasabi but an aromatic one (Ono *et al*., 1996; Etoh *et al*., 1990a; Etoh *et al*., 1990b). Oral administration of 6-MITC also suppressed the development of experimentally-induced mouse papilloma via a two-stage process in which 9,10-dimethyl-1, 2-benzanthracene (DMBA) acts as an initiator and 2-o-tetradecanoylphorbol-13-acetate (TPA) as a promoter (Fuke *et al*., 1997). Fuke *et al*. (1997) also found that 6-MITC induces quinone oxidoreductase (QR) in murine Hepa1c1c7 cell, phase II detoxication enzymes in the liver. Furthermore, Hou *et al*. (2000) reported that 6-MITC significantly induces QR activity, and that this induction is regulated at the transcription level by the QR gene. Meanwhile, we have established a mouse cell line, r/mHM-SFME-1, which spontaneously metastasized into the lung of a syngeneic Balb/c mouse from a tumor which grew on the back or footpad where the cells were inoculated (Nomura *et al*., 1993). Using this cell line, we have reported a dependable method for quantifying the microscopically indistinguishable pulmonary micrometastasis by detecting the human c-Ha-ras gene which is carried in the tumor cell line (Matano *et al*., 1995). Also using this cell line, we have previously reported that the oral administration of 400 μM 6-MITC effectively suppresses the pulmonary metastasis of the tumor cells (Fuke *et al*., 2000). However, in the previous experiments, decreased intake of water containing 6-MITC was observed. In the current work, the reduced water intake recovered to the normal level with half the dosage that was used in previous experiments. Improving the water intake problem, the potential of 6-MITC for the chemo-prevention of tumor progression was studied. Concerning the control of tumor malignancy by a diet component, an altered administration schedule was adopted.

Materials and Methods

Materials The active ingredient from wasabi, 6-MITC, was chemically synthesized by Shiratori Pharmaceutical Co. (Chiba). The 6-MITC was supplied to mice in sterile water, even though it is soluble in organic solvents.

Cell and culture A tumor cell line, r/mHM-SFME-1, was originally derived from mouse embryonic fibroblast SFME (Loo *et al*., 1987) and established by the introduction of activated hu-

E-mail: fuke@tmca.ac.jp

man c-Ha-ras and myc genes (Nomura *et al*., 1993; Shirahata *et al*., 1990). Cells were cultured with a mixture of Hames F12 and Dulbecco's modified Eagle medium (F/D, 1 : 1) (Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS: Cosmo Bio Co., Tokyo) in 5% CO_2 at 37°C.

Detection of micrometastasis Six-week-old Balb/c female mice were subcutaneously injected with r/mHM-SFME-1 cells (5×10^5) into the right hind footpads. The tumor-bearing legs of all control, S-1 and S-2 mice were amputated 14 days after inoculation. The total DNA from the lungs was extracted 35 days after inoculation. Since mouse Ha-ras has a Hind III site in its exon-1 but the human form does not, the total DNA was at first digested completely with Hind III (Takara Co., Tokyo). Undigested human c-Ha-ras exon-1 was amplified by 30 cycles of PCR, and detected by agarose gel electrophoresis. Each band of amplified human Ha-ras exon-1 was quantified by measuring the band area. Based on a standard band with 1×10^4 tumor cells, the metastatic index was calculated by dividing the total area of each sample by that of the standard (Fuke *et al*., 2000).

Administration of 6-MITC For the oral administration to mice, 200 μM 6-MITC was prepared under sterile conditions and supplied as a beverage. S-1 group mice were continuously supplied with 6-MITC for 35 days from the time of tumor cell inoculation. S-2 group mice were supplied continuously for 21 days from the day of amputation. The control mice and the normal mice (i.e., those without tumors) were supplied with sterile water instead of the 6-MITC solution. The numbers of S-1, S-2, control and normal mice were respectively 10, 6, 6, and 3. However, DNA of one lung of a mouse in the S-2 group was not obtained, with the result that data were only available for 5 of the S-2 mice.

Results and Discussion

Administration of a lower 6-MITC concentration In the previous study, all the lung DNA samples from the tumor-bearing mice which received oral administration of 40 μM 6-MITC showed clear bands of human c-Ha-ras exon-1 with metastasized tumor cells. In contrast, only one mouse lung in the five mice supplied with 400 μM 6-MITC expressed a human c-Ha-ras band. The water intake of these treated mice, however, was 60% of that of non-treated control mice (Fuke *et al*., 2000). For this reason, in the current study, the concentration of 6-MITC orally administered to the mice was 200 μM 6-MITC. Although the reduced water intake was observed in the experimental animals, the body weights of these mice were not affected by the administration of 6-MITC at this concentration (Table 1). A reduced body weight was observed at day 18 in all tumor-bearing mice (controls, S-1 and S-2 mice), and it recovered within two weeks. This body weight alteration was observed independently of 6-MITC administration. Since the tumor-bearing right legs of all these mice were amputated 14 days after inoculation, the observed reduction of body weight is believed to have been caused by surgical stress.

Effect of oral administration of 6-MITC on tumor metastasis In the previous study, effective suppression of tumor metastasis with 400 μM 6-MITC was obtained by oral administration for 21 days, starting immediately after the amputation. In order to investigate the preventive effect of 200 μM 6-MITC on tumor progression, mice in the S-1 group were treated with 6-MITC continuously for 35 days from the time of tumor cell inoculation. Mice in the S-2 group, however, were given 6-MITC for 21 days, i.e. the same treatment as in previous experiments. The control mice were given only water and not the 6-MITC solution.

Table 1. Effect of 6-MITC against the body weight and water intake.

Group	Body weight (g)[a]							Water intake[b] (ml/day/mouse)
	0	6	12	18	24	30	35 days	
Control (n=6)	19.6±0.8	20.4±0.7	20.7±0.9	18.7±0.8	19.1±0.9	19.8±0.6	20.2±0.9	2.0
S-1 (35 days) (n=10)	19.8±0.9	20.4±0.8	20.8±0.9	18.7±0.8	19.7±1.1	20.2±1.1	20.2±1.1	2.1
S-2 (21 days) (n=6)	19.7±0.9	20.5±1.1	20.9±1.4	18.6±0.9	19.3±0.7	20.4±1.0	20.3±0.7	2.2
Normal (n=3)	19.5±3.2	20.6±2.6	21.1±3.3	21.0±2.0	20.7±2.2	21.1±2.1	21.0±1.5	2.9

[a]Mean±SD, [b]Mean

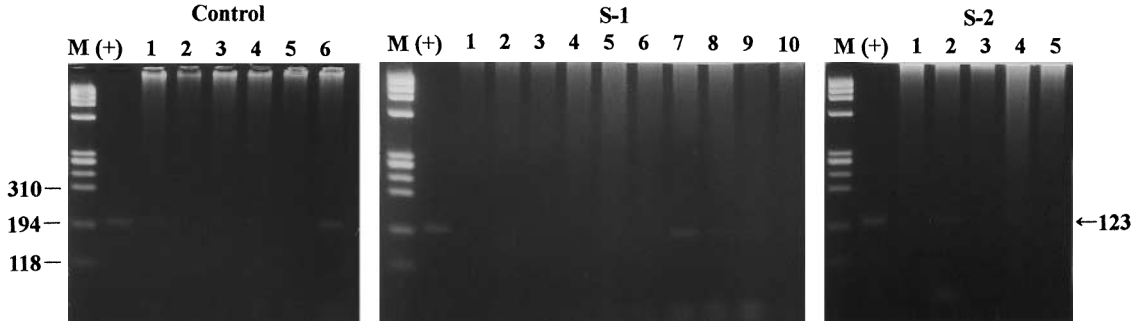

Fig. 1. Pulmonary metastasis detected by tumor-carrying genes. Human c-Ha-ras exon-1 (123 bp, indicated by arrowhead) detected in PCR (30 cycles) amplified by 1 μl of Hind III digested mouse lung (tumor cell) DNA as template. Mice were inoculated with 5×10^5 r/mHM-SFME-1 tumor cells on their right hind footpads. M=ΦX174-HaeIII; (+)=1×10^4 r/mHM-SFME-1 DNA; Cntrol Lane 1–6=DNA samples from the lungs of control mice; S-1 lanes 1–10=DNA samples from the lungs of S-1 group mice; S-2 lanes 1–5=DNA samples from the lungs of S-2 group mice.

Table 2. Effect of 6-MITC against the pulmonary metastasis of experimental tumor cell line.

Group	Lane number	Metastatic index
Control mice	1	0.82
	2	0.64
	3	0.73
	4	0.82
	5	0.64
	6	1.00
S-1 mice	1	(−)
6-MITC (200 μM)	2	(−)
administered 35 days	3	(−)
after amputation	4	(−)
	5	(−)
	6	(−)
	7	0.90
	8	0.70
	9	0.60
	10	(−)
S-2 mice	1	(−)
6-MITC (200 μM)	2	0.72
administered 21 days	3	0.36
after amputation	4	0.50
	5	0.36

The amplified human c-Ha-ras band was detectable in every lung sample from each of the control mice 35 days after inoculation with the tumor cells (Fig. 1, Table 2); this represents pulmonary metastasis of tumor cells from the footpad (Matano *et al*., 1995). The range of metastatic indexes of these control mouse lungs was 0.64–1.00. In the S-1 group, the bands were detected only in three out of ten mice lungs, with the metastatic indexes of these lungs equal to 0.60, 0.70 and 0.90 respectively. The lungs of the other seven mice showed no evidence of human genes following PCR with 30 cycles of amplification. In the S-2 group, the bands were detected in four mice lungs out of the 5, with the metastatic indexes of these lungs ranging from 0.36 to 0.72, i.e. somewhat lower than for the controls. There were no differences between the samples, including controls, in the results of PCR for the housekeeping GAPDH (glyceraldehyde-3-phosphate dehydrogenase) gene (data not shown). The above results suggest that oral administration of 200 μM 6-MITC is effective in preventing metastasis of the experimental tumor.

It is possible that tea polyphenols (including catechins) suppress tumor metastasis (Taniguchi *et al*., 1992; Menon *et al*., 1999). However, little is known about whether food ingredients can inhibit tumor progression. When investigating such effects, one experimental cell line (r/mHM-SFME-1) has marked advantages (Nomura *et al*., 1993; Matano *et al*., 1995; Fuke *et al*., 2000). First, non-athymic syngeneic Balb/c mice with this cell line are available. Since the immune system of this mouse is entirely intact, this system reflects human tumor progression much more closely than other animal models. Second, metastasized cells are quantitatively detectable in many organs by PCR amplification of the human c-Ha-ras exon-1 oncogene in the tumor cell. This system is thus extremely useful because it is able to detect very small tumors or tumor cells which are undetectable with conventional microscopes. For these reasons, we selected r/mHM-SFME-1 cells as the means to evaluate the effect of 6-MITC in Balb/c mice. Tumors develop into a metastatic disaster via four stages. This tumor progression includes growth of the tumor at the original site (the inoculated site); detachment of the cells from the original tumor and invasion via the circulatory system; lodgment of the detached cells in neighboring tissues; and outgrowth of the tumor into the invaded organs (completion of metastasis). Amputation of the tumor-bearing leg cuts off the invasive cell supply from the original tumor. Thus, administration of 6-MITC at the time of amputation means that it is only acting against the metastasized cells. However, when 6-MITC is administered at the time of cell inoculation, it is active in all the stages involved in tumor development and not only in the tumor progression that involves the growth of metastasized cells.

In this experiment, metastasis was effectively suppressed by 200 μM 6-MITC, although the effect of this dosage was less clear than in the previous study using a concentration of 400 μM. However, the reduction of water intake recovered to the control level, with the result that body weight was not affected. With regard to tumor progression, a continuous supply of 200 μM 6-MITC for 35 days from the time of tumor cell inoculation was much more effective than administration for 21 days from the time of amputation (14 days after inoculation). It is, however, necessary to determine the optimal timing more precisely. Nevertheless, the previous and present results suggest that the oral administration of 6-MITC is effective in preventing the progression of the experimental tumor.

Using this system for detection, Hatayama *et al*. (2001) have reported that apple-derived polyphenol suppresses tumor metastasis. After 2 weeks of pre-administration of the apple polyphenol in drinking water (0.05%), mice were inoculated with r/mHM-SFME-1 cells on their left footpads. The polyphenol was continuously supplied in drinking water for another 4 weeks, and the mice lungs were then examined for signs of metastasis. This treatment was found to be effective in suppressing the metastasis.

Other researchers have also examined the anti-metastatic effects of food ingredients *in vivo* (Taniguchi *et al*., 1992; Menon *et al*., 1999). One of the ingredients, epigallocatechin gallate, effectively reduced the spontaneous metastasis of B16 melanoma following 21 days of continuous oral administration after the amputation (Taniguchi *et al*., 1992). Crucumin combined with catechin also reduced the artificial metastasis of B16 melanoma following by 10 days' continuous oral administration (Menon *et al*., 1999). We found that administration from an early stage of tumor development was more effective than administration at a more advanced phase (such as at the time of amputation). The low dosage of 6-MITC was sufficient to suppress tumor progression. Since cancers are not necessarily fatal if tumor progression can be prevented, our results strongly suggest that 6-MITC is a candidate for inclusion in a diet aimed at effectively controlling tumor progression.

References

Etoh, H., Nishimura, A., Takasawa, R., Yagi, A., Saito, K., Sakata, K., Kishima, I., Shibata, M. and Ina, K. (1990a). Stabilization of flavor in wasabi and horse radish. *Nippon Shokuhin Kogyo Gakkaishi,* **37**, 953–958 (in Japanese).

Etoh, H., Nishimura, A., Takasawa, R., Yagi, A., Saito, K., Sakata, K., Kishima, I. and Ina, K. (1990b). ω-Methylsulfinylalkyl isothiocyanates in wasabi, *Wasabi japonica* Matsum. *Agric. Biol. Chem.*, **54**, 1587–1589.

Fuke, Y., Ohishi. Y., Iwashita, K., Ono, H. and Shinohara, K. (1994). Growth suppression of MKN-28 human stomach cancer cells by wasabi. *Nippon Shokuhin Kogyo Gakkaishi,* **41**, 709–711 (in Japanese).

Fuke, Y., Haga, Y., Ono, H., Nomura, T. and Ryoyama, K. (1997). Anti-carcinogenic activity of 6-methylsulfinylhexyl isothiocyanate, an active anti-proliferative principal of wasabi (*Eutrema wasabi* Maxim). *Cytotechnology*, **25**, 197–203.

Fuke, Y., Sawaki, S., Nomura, T. and Ryoyama, K. (2000). Suppressive effect on tumor metastasis by 6-(methylsulfinyl)hexyl isothiocyanates from wasabi. *Nippon Shokuhin Kagaku Kogaku Kaishi,* **47**, 760–766 (in Japanese).

Hatayama, I., Miura, H., Kidachi Y., Yamaguchi, H. and Ryoyama, K. (2001). Suppression of tumor metastasis and hepatocarcinogenesis in mouse by apple polyphenol. Presented at 60th Annual Meeting of the Japanese Cancer Association, Yokohama, September 26–28, p. 476

Hou, D.-X., Fukuda, M., Fujii, M. and Fuke, Y. (2000). Transcriptional regulation of nicotinamide adenine dinucleotide phosphate: quinine oxidoreductase in murine hepatoma cells by 6-(methylsulfinyl)hexyl isothiocyanate, an active principle of wasabi (*Eutrema wasabi* Maxim). *Cancer Lett.,* **161**, 195–200.

Isshiki, K. and Tokuoka, K. (1993). Allyl isothiocyanate and wholesomeness of food. *Jpn. J. Food Microbiol.,* **12**, 1–6.

Kinae, N., Furugori, M. and Masuda, H. (2001). Studies on functional properties of Sawa-wasabi (*Wasabia japonica*). *Food&Food Ingredients J. Jpn*., **192**, 27–33 (in Japanese).

Kumagai, H., Kashima, N., Seki, T., Sakurai, H., Ishii, K. and Ariga, T. (1994). Analysis of components in essential oil of upland wasabi and their inhibitory effects on platelet aggregation. *Biosci. Biotechnol. Biochem.,* **58**, 2131–2135.

Loo, D.T., Fuquay, J.I., Rawson, C.L. and Barnes, D.W. (1987). Extended culture of mouse embryo cells without senescence: Inhibition by serum. *Science*, **236**, 200–202.

Matano, S., Ryoyama, K., Nakamura, S., Okada, G. and Nomura, T. (1995). Application of the polymerase chain reaction (PCR) to quantify micro-metastasis in an experimental animal. *Cancer Lett.,* **91**, 93–99.

Menon, L.G., Kuttan, R. and Kuttan, G. (1999). Anti-metastatic activity of curcumin and catechin. *Cancer Lett.,* **141**, 159–165.

Nomura, T., Matano, S., Okada, G., Tokuyama, H., Hori, I., Nakamura, S., Kameyama, T. and Ryoyama, K. (1993). Establishment of a metastatic murine cell line carrying the human c-Ha-ras. *In Vitro Cell. Dev. Biol*., **29A**, 614–616.

Ono, H., Adachi, K., Fuke, Y. and Shinohara, K. (1996). Purification and structural analysis of substances in Wasabi (*Eutrema wasabi* Maxim.) that suppress the growth of MKN-28 human stomach cancer cells. *Nippon Shokuhin Kagaku Kogaku Kaishi,* **43**, 1092–1097 (in Japanese).

Osawa, T. (1995). Recent development of food photochemical for cancer prevention. CMC Co., Ltd., Tokyo.

Shirahata, S., Rawson, C., Loo, D.T., Chang, Y.J. and Barnes, D. (1990). Ras and neu oncogenes reverse serum inhibition and epidermal growth factor dependence of serum-free mouse embryo cells. *J. Cell. Physiol*., **144**, 69–76.

Taniguchi, S., Fujiki, H., Kobayasi, H., Go, H., Miyado, K., Sadano, H. and Shimokawa, R. (1992). Effect of (−)-epigallocatechin gallate, the main constituent of green tea, on lung metastasis with mouse B16 melanoma cell lines. *Cancer Lett.,* **65**, 51–54.

Yano, T., Yajima, S., Virgona, N., Yano, Y., Otani, S., Kumagai, H., Sakurai, H., Kishimoto, M. and Ichikawa, T. (2000). The effect of 6-methylsulfinylhexyl isothiocyanate isolated from *Wasabi japonica* (wasabi) on 4-(methylnitros amino)-1-(3-pyridyl)-1-buatnone-induced lung tumorigenesis in mice. *Cancer Lett.,* **155**, 115–120.