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S-Methylcysteine and Cysteine Are Inhibitors of Induction of Glutathione S-Transferase Placental Form-positive Foci during Initiation and Promotion Phases of Rat Hepatocarcinogenesis

Nobuyasu Takada,[1,3] Yoshihisa Yano,[2] Hideki Wanibuchi,[1] Shuzo Otani[2] and Shoji Fukushima[1,4]

[1]*First Department of Pathology and* [2]*Second Department of Biochemistry, Osaka City University Medical School, 1-4-54 Asahi-machi, Abeno-ku, Osaka 545*

S-Methylcysteine (SMC) occurs in a variety of plants, including *Allium sativum*, *Phaseolus vulgaris*, and *Cruciferae*. In this study, we synthesized five organosulfur compounds (OSCs), SMC and four analogs, and examined their modifying effects on diethylnitrosamine-induced neoplasia of the liver in male F344 rats, using the medium-term bioassay system of Ito (Ito test) based on the two-step model of hepatocarcinogenesis. In addition, we investigated the modifying effects of SMC and cysteine on the initiation stage of rat hepatocarcinogenesis. Carcinogenic potential was scored by comparing the numbers and areas of induced glutathione S-transferase placental form (GST-P)-positive hepatocellular foci. All OSCs examined had a tendency to decrease the number of GST-P-positive foci when given in the promotion stage of the Ito test, and in particular SMC and cysteine exerted significant inhibitory effects. When given during the initiation stage, these two OSCs also significantly inhibited focus formation. Regarding the mechanism underlying the inhibitory effects of SMC and cysteine, measurement of ornithine decarboxylase in SMC- and cysteine-treated liver tissues after partial hepatectomy (PH) revealed a significantly reduced activity, and the proportion of hepatocytes positive for proliferating cell nuclear antigen was significantly decreased by SMC or cysteine administration. Moreover, examination of the expression of the early response proto-oncogenes, c-*fos*, c-*jun*, and c-*myc*, after PH demonstrated down-regulated induction of c-*jun* mRNA transcripts by SMC, sustained for an eight-hour period. Our results support the view that SMC and cysteine are chemopreventive agents for rat hepatocarcinogenesis and that their intake may be of importance for cancer prevention.

Key words: S-Methylcysteine — Cysteine — Rat hepatocarcinogenesis — Polyamine biosynthesis

We previously found that second stage administration of eight oil-soluble organosulfur compounds (OSCs), after initiation with diethylnitrosamine (DEN), resulted in enhanced development of putative preneoplastic lesions, glutathione S-transferase placental form (GST-P)-positive foci, in rat liver.[1,2] Moreover, this change was associated with increased cell proliferation and elevated activity of ornithine decarboxylase (ODC), a rate-limiting enzyme of polyamine biosynthesis. On the other hand, two other oil-soluble OSCs, methyl propyl disulfide and propylene sulfide, decreased DEN-induced GST-P-positive focus formation dose-dependently.[3] Thus, we demonstrated both enhancing and inhibitory effects of oil-soluble OSCs on the post-initiation stage in rat hepatocarcinogenesis.

It is well-known that there are water-soluble OSCs in garlic and onions. S-Methylcysteine (SMC) exists in various plants, including *Allium sativum*,[4] *Phaseolus vulgaris*,[5] and *Cruciferae*.[6] While its biological characteristics have not received much attention, Sumiyoshi and Wargovich.[7] reported that S-allylcysteine (SAC), an analog of SMC, can prevent 1,2-dimethylhydrazine-induced colon cancer in mice.

In the present study, the second stage-modifying potentials of five water-soluble OSCs, SMC and four analogs, were examined in the rat liver medium-term bioassay system of Ito[8] based on the two-stage concept of carcinogenesis. The four analogs of SMC examined in this study were cysteine, SAC, S-propylcysteine (SPC), and S-ethylcysteine (SEC). In experiment 2, the modifying effects of SMC and cysteine given during the initiation stage of rat hepatocarcinogenesis were investigated.[9] In addition, to cast light on possible mechanisms that might be involved, the activities of ODC and spermidine/spermine N^1-acetyltransferase (SAT), rate-limiting enzymes of polyamine metabolism which are reported to be increased by promoters of chemical carcinogenesis,[10–12] were measured in liver of SMC- and cysteine-treated rats after partial hepatectomy (PH). In addition, expression of proliferating cell nuclear antigen (PCNA) was examined immunohistochemically in SMC- and cysteine-treated livers after PH. The influence of SMC in-

[3] Present address: Second Department of Surgery, Osaka City University Medical School.

[4] To whom requests for reprints should be addressed.

tragastric intubation prior to PH on the expression of *c-fos*, *c-jun*, and *c-myc* protooncogenes during the early cell proliferation response of rat liver was also assessed.

MATERIALS AND METHODS

Chemicals SMC, cysteine, SAC, SPC, and SEC were kindly provided by Wakunaga Pharmaceutical Co., Ltd., Osaka. The chemical structures of these compounds are shown in Fig. 1. DEN was obtained from Tokyo Chemical Industry Co., Ltd., Tokyo. [1-^{14}C]Ornithine and [acetyl-1-^{14}C]acetyl coenzyme A were obtained from Moravek Biochemicals, Inc., Brea, CA.

Animals and treatments One hundred and ten male F344 rats (Charles River Japan, Inc., Hino, Shiga) purchased at 5 weeks of age in experiments 1, 3, and 4, and 42 male rats purchased at 7 weeks of age in experiment 2, were housed in an air-conditioned room at a temperature of 23 $\pm$1°C, a relative humidity of 36$\pm$6%, with a 12 h light-12 h dark cycle, and given a pellet diet (Oriental MF; Oriental Yeast Co., Tokyo) and tap water *ad libitum* during the experimentation. They were acclimatized for 1 week before use.

S-allylcysteine $H_2C{=}CH\text{-}H_2C\text{-}S\text{-}CH_2CH(NH_2)COOH$

S-propylcysteine $H_3C\text{-}H_2C\text{-}H_2C\text{-}S\text{-}CH_2CH(NH_2)COOH$

S-ethylcysteine $H_3C\text{-}H_2C\text{-}S\text{-}CH_2CH(NH_2)COOH$

S-methylcysteine $H_3C\text{-}S\text{-}CH_2CH(NH_2)COOH$

Cysteine $H\text{-}S\text{-}CH_2CH(NH_2)COOH$

Fig. 1. Water-soluble organosulfur compounds examined in this study.

Experiment 1 was performed to investigate the modifying effect of each OSC on the second stage of hepatocarcinogenesis using the Ito test.[8] The experimental design is shown in Fig. 2. Eighty-five rats were divided into 11 groups. The rats in groups 1 to 6, 10 rats each, were given a single i.p. injection of DEN (200 mg/kg b.w.) dissolved in saline to initiate hepatocarcinogenesis. After 2 weeks, they received SAC (groups 1 and 7), SPC (groups 2 and 8), SEC (groups 3 and 9), SMC (groups 4 and 10) or cysteine (groups 5 and 11), each at a dose of 100 mg/kg b.w. dissolved in saline (4 or 8 ml/kg), by i.g. gavage 5 times per week for 6 weeks. Animals were subjected to PH at week 3 to maximize any interaction between proliferation and the effects of the compounds tested. Group 6 was given DEN and PH, followed by saline without administration of any test compound, as a control. Groups 7 to 11 (5 rats each) received saline instead of DEN solution, but were subjected to administration of test compounds and PH. Surviving rats in each group were killed for examination at the end of week 8. The livers were examined immunohistochemically for GST-P-positive focus formation.

The doses of the chemicals tested were the same as or nearly equimolar to those used in previous OSC experiments.[1,7] Each dose was determined on the basis of preliminary experiments (unpublished data).

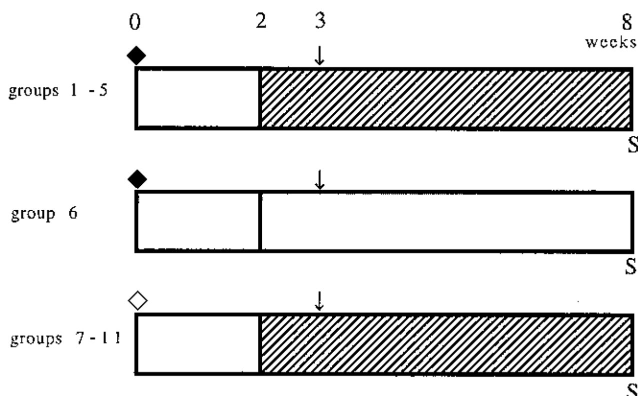

Fig. 2. Liver medium-term bioassay protocol. ◆, DEN, 200 mg/kg body weight (i.p.); ◇, saline, 5 ml/kg body weight (i.p.); ↓, partial hepatectomy; S, animal(s) killed; ▨, test chemicals (i.g.) 5 times/week; ▒, saline, 1 ml/kg body weight (i.g.).

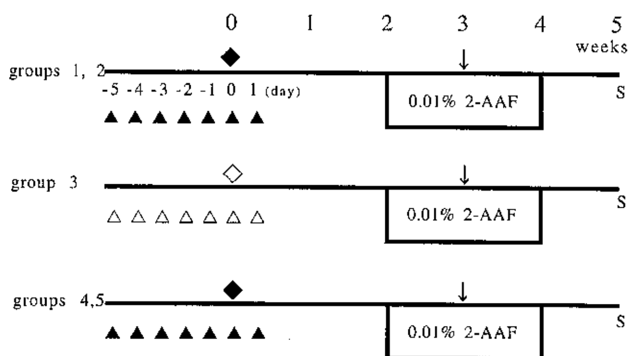

Fig. 3. Protocol for examination of modifying effects on the initiation stage. ◆, DEN, 20 mg/kg body weight (i.p.); ◇, saline, 5 ml/kg body weight (i.p.); ↓, partial hepatectomy; S, animal(s) killed; ▲, test chemicals (i.g.); △, saline, 1 ml/kg body weight (i.g.).

Experiment 2 was planned to examine the modifying effects of SMC and cysteine, which showed inhibitory effects on the second stage of hepatocarcinogenesis in experiment 1, on the initiation stage. The experimental design is shown in Fig. 3. Forty-two rats were divided into five groups, those in groups 1 to 3 being given a single i.p. injection of DEN (20 mg/kg b.w.) dissolved in saline, while groups 4 and 5 received saline instead of the DEN solution. SMC (groups 1 and 4) or cysteine (groups 2 and 5) was administered i.g. at 100 mg/kg b.w. dissolved in saline, once a day from 5 days prior to DEN or saline injection to one day after. Rats in group 3 were given saline i.g. instead of OSCs. All rats were fed 0.01% 2-acetylaminofluorene (2-AAF) in powdered diet from weeks 2 to 4 and subjected to PH at week 3. Surviving rats in each group were killed at the end of week 5. Immunohistochemical examination of GST-P in excised livers was performed as described for experiment 1.

Since cell proliferation is generally very low in the normal liver of adult rats, we also used PH models for the next three experiments.

In experiment 3, to evaluate the relation between cell proliferation and inhibitory effects of SMC and cysteine, 15 rats were used for examination of ODC and SAT activities in the liver tissues. Five rats received SMC or cysteine (each of 100 mg/kg b.w., i.g.) at 0, 24, and 48 h prior to PH. The control group (5 rats) received saline alone. All rats were killed 4 h after PH, and the ODC and SAT activities were assessed.

In experiment 4, to examine sequential changes in PCNA-positive cells due to SMC and cysteine treatment, 18 rats were used. Two groups of 6 rats received SMC or cysteine (100 mg/kg b.w., i.g.) 0, 24, 48 h prior to PH. The control group (6 rats) received saline alone prior to PH. The liver tissue removed at PH was used as material for zero time. Three rats in each group were killed at 4 h and 24 h after the final i.g., administration, and PCNA-positive cells were stained immunohistochemically and counted (total of 5000 hepatocytes examined per rat).

In experiment 5, to evaluate changes of *c-fos*, *c-jun*, and *c-myc* in the livers of rats treated with SMC, 10 rats were divided into 2 groups and mRNA expression of those early response genes was assessed. All rats underwent PH. SMC was administered i.g. at 100 mg/kg b.w. at 0, 24 and 48 h prior to PH in group 1 (five rats), and in group 2 (five rats), saline was administered in the same manner. The liver tissue removed at PH was used as the control and single rats in each group were killed at 2, 4, 8, 12 and 24 h after the final i.g. administration. The livers were removed and total RNA was prepared by the method of Chirgwin *et al.*[13]

Tissue processing At autopsy, livers were excised and weighed, and sections 2 to 3 mm thick were cut with a razor blade. Three slices, one each from the right posterior, anterior, and caudate lobes, were fixed in ice-cold acetone for immunohistochemical examination of GST-P in experiments 1 and 2. To measure ODC and SAT activities in experiment 3, rat liver samples were frozen in liquid nitrogen. For immunohistochemical examination of PCNA, they were fixed in buffered formalin, embedded in paraffin wax, and sections 3 μm thick were cut and deparaffinized with xylene.

Immunohistochemical staining of GST-P and PCNA The avidin-biotin-peroxidase complex (ABC) method described by Hsu *et al.*[14] was used. After hydration, liver sections were treated sequentially with normal goat or horse serum, anti-rat GST-P antibody[15] (1 : 8000) (provided by Dr. K. Satoh, Hirosaki University) or anti-PCNA antibody (DAKO Japan Co., Ltd., Kyoto), biotin-labeled goat anti-rabbit or mouse IgG (1 : 400) and ABC (Elite Vectastain, Vector Labs, Burlingame, CA). The sites of peroxidase binding were demonstrated by the diaminobenzidine method. Sections were then counterstained with hematoxylin for microscopic examination.

The numbers and the areas of GST-P-positive foci >0.2 mm in diameter and the total areas of the liver sections examined were measured using a color video image processor (VIP-21C, Olympus-Ikegami, Tokyo). The numbers of PCNA-positive cells were measured in 5000 cells at random per rat, and the average was calculated.

Measurement of ODC and SAT activities ODC and SAT activities were measured by the methods of Otani *et al.*[16] and Matsui *et al.*,[17] respectively. Frozen pieces of rat liver were suspended in 0.5 ml of 50 m*M* Tris (pH 7.5) containing 0.25 *M* sucrose and disrupted in a homogenizer for one minute. The homogenized suspensions were centrifuged at 100,000*g* for thirty minutes, and the supernatant was assayed for ODC and SAT activities by measurement of the amount of radioactive CO_2 produced from [1-^{14}C]ornithine and the amount of acetyl moiety transferred from [acetyl-1-^{14}C]acetyl coenzyme A to spermidine, respectively.

Northern blotting of early response genes in rat liver Twenty micrograms of total RNA from each sample was fractionated on 1% agarose gel and transferred to Hybond nylon membrane. The membrane was prehybridized and hybridized with [^{32}P]dCTP-labeled probes for *c-fos, c-jun, c-myc* using *redi*prime DNA labelling system (Amersham International plc, Little Chalfont, Buckinghamshire, England), according to the manufacturer's instructions. After hybridization, the membranes were washed, and signals were detected by autoradiography and quantified using an image analyzer (BAS 2000 II, Fujix, Tokyo).

Statistical analysis Statistical analysis of data was performed using Student's *t* test.

RESULTS

Final body and relative liver weights in experiment 1 are shown in Table I. No significant intergroup variation was found, with or without DEN initiation.

Table II summarizes data for numbers and areas of GST-P-positive foci per unit area of liver section after DEN initiation in experiment 1. Values for numbers and areas in groups 1 to 3 treated with test chemicals tended to be decreased, as compared to those in group 6. Values for both parameters in the groups given SMC (group 4) or cysteine (group 5) were significantly decreased. In particular, the group 4 values were less than half of those in the control group. In the livers of the groups without DEN, GST-P-positive foci were not seen and histological examination of the livers revealed normal architecture.

Table III shows final body and relative liver weights in experiment 2; values for groups 1 and 2 are not significantly different from those of group 3, the control data. Numbers and areas of GST-P-positive foci in experiment 2 are shown in Table IV. Values for both parameters in groups 1 and 2 were decreased, as compared to those in group 3, and cysteine (group 2) significantly inhibited development in terms of both numbers and areas. GST-P-positive foci occurred sporadically in groups 4 and 5, without DEN and with 2-AAF.

The results for ODC and SAT activities in rat liver tissues after PH in experiment 3 are summarized in Table V. ODC activities 4 h after PH were significantly decreased in SMC- or cysteine-treated liver tissues, as compared to the control group. SAT activities were also decreased by SMC or cysteine treatment, although not significantly.

Table VI shows the proportions of PCNA-positive cells in rat livers at 4 and 24 h after PH. PCNA-positive cells at the 4 h time point were significantly decreased in SMC- or cysteine-treated liver tissues, as compared to the control group. Moreover, even after 24 h, some reduction was still apparent.

Fig. 4 illustrates the effects of SMC on the induction of c-*fos*, c-*jun*, c-*myc* mRNA transcripts in rat liver during 24 h after PH. Data from image analyzer quantification are given in Fig. 5. Maximum expression of c-*fos*, c-*jun*, c-*myc* in the rat liver without SMC treatment was observed at 2, 8 and 8 h after PH, respectively. Northern blot analysis revealed that only the expression of c-*jun* in the rat liver was obviously altered by SMC treatment. For the first 8 h after PH down-regulation of c-*jun* was observed, followed by an increase at the 12 h time point.

DISCUSSION

The present study has clearly demonstrated that treatment with SMC and cysteine, particularly SMC, in both

Table II. Numbers and Areas of GST-P-positive Foci in the Livers of Rats in Experiment 1 Initiated with DEN Followed by Treatment with Various Water-soluble OSCs

Group	Test chemical	Effective no. of rats	No./cm^2	Area (mm^2/cm^2)
1	SAC	8	9.94±0.94[a]	1.37±0.58
2	SPC	9	11.41±3.11	1.58±0.53
3	SEC	9	10.93±3.00	1.47±0.37
4	SMC	10	6.53±1.81[b]	0.72±0.23[b]
5	Cysteine	10	8.45±3.01[c]	1.06±0.50[c]
6	—	9	14.34±4.47	1.55±0.56

a) Mean±SD.
b) Significantly different from group 6 at $P<0.01$.
c) Significantly different from group 6 at $P<0.05$.

Table I. Final Body and Relative Liver Weights in Experiment 1, with or without DEN Initiation Followed by Treatment with Various Water-soluble OSCs

Group	Test chemical	DEN	Effective no. of rats	Final body wt. (g)	Relative liver wt. (g/100 g body wt.)
1	SAC	+	8	252±10[a]	3.0±0.3
2	SPC	+	9	240±11	2.9±0.3
3	SEC	+	9	242±9	3.1±0.3
4	SMC	+	10	236±6	3.1±0.4
5	Cysteine	+	10	236±7	2.9±0.2
6	—	+	9	244±9	3.1±0.4
7	SAC	—	4	260±13	3.3±0.5
8	SPC	—	4	256±10	2.9±0.5
9	SEC	—	5	249±11	2.7±0.2
10	SMC	—	4	254±4	2.9±0.3
11	Cysteine	—	4	257±7	3.2±0.3

a) Mean±SD.

Table III. Final Body and Relative Liver Weights in Experiment 2, with or without DEN Initiation Following Treatment with SMC or Cysteine

Group	Test chemical	DEN	Effective no. of rats	Final body wt. (g)	Relative liver wt. (g/100 g body wt.)
1	SMC	+	9	251±2[a]	2.9±0.2
2	Cysteine	+	9	253±15	2.9±0.3
3	—	+	10	255±6	2.8±0.2
4	SMC	−	6	245±8	2.5±0.2
5	Cysteine	−	6	263±5	2.8±0.1

a) Mean±SD.

Table IV. Numbers and Areas of GST-P-positive Foci in the Livers of Rats in Experment 2, with or without DEN Initiation Following Treatment with SMC or Cysteine

Group	Test chemical	DEN	Effective no. of rats	No./cm²	Area (mm²/cm²)
1	SMC	+	9	2.58±1.98[a, b]	0.29±0.32
2	Cysteine	+	9	3.19±1.06[b]	0.26±0.15[b]
3	—	+	10	6.64±4.27	0.73±0.61
4	SMC	−	6	0.40±0.35	0.02±0.02
5	Cysteine	−	6	0.25±0.29	0.03±0.04

a) Mean±SD.
b) Significantly different from group 3 at $P<0.05$.

Table V. ODC and SAT Activities in Rat Liver, 4 h after PH

	Effective no. of rats	ODC activity (pmol/h/mg protein)	SAT activity (pmol/10 min /mg protein)
Control	5	756±244[a]	1.6±2.1
SMC	5	334±86[b]	0.9±1.1
Cysteine	5	391±94[b]	0.9±0.8

a) Mean±SD.
b) Significantly different from the control group at $P<0.02$.

Table VI. PCNA-positive Cells in Rat Liver, 0, 4, and 24 h after PH

	Effective no. of rats	Proportion (%) of positive cells		
		0 h	4 h	24 h
Control	6	1.3±1.8[a]	9.1±3.2	38.2±7.9
SMC	6	0.7±1.1	1.1±1.1[b]	30.6±6.6
Cysteine	6	1.2±1.4	2.1±1.3[b]	35.1±8.0

a) Mean±SD.
b) Significantly different from the control group at $P<0.05$.

the initiation and promotion stages of rat hepatocarcinogenesis inhibited GST-P-positive focus formation. Moreover, a single administration of SMC or cysteine reduced the elevation of ODC and SAT activities in the liver tissues, PCNA-positive cells were significantly decreased, and the liver regeneration-associated expression of c-*jun* mRNA was markedly down-regulated.

Several researchers have investigated the modifying effects of OSCs, especially oil-soluble forms, on the initiation or promotion stages of chemical carcinogenesis. Allyl methyl trisulfide (AMT), allyl methyl disulfide (AMD), diallyl trisulfide (DAT), and diallyl sulfide (DAS) administered 96 and 48 h prior to carcinogen exposure inhibited the development of benzopyrene-induced tumors of the forestomach in female A/J mice.[18] DAS and AMD also inhibited pulmonary adenoma formation, while neither DAT nor AMT had any effect on lung tumorigenesis.[17] Wargovich *et al.*[19, 20] reported that DAS inhibited 1,2-dimethylhydrazine (DMH)-induced colonic cancer development in mice and *N*-nitrosobenzylamine-induced esophageal tumors in rats. We also demonstrated that eight oil-soluble OSCs, DAS, DAT, allyl methyl sulfide, AMT, dipropyl trisulfide, dipropyl sulfide, allyl mercapton, and isothiocyanate acid isobutyl ester, enhanced, while two other analogs, methyl propyl disulfide and propylene sulfide, inhibited the promotion stage

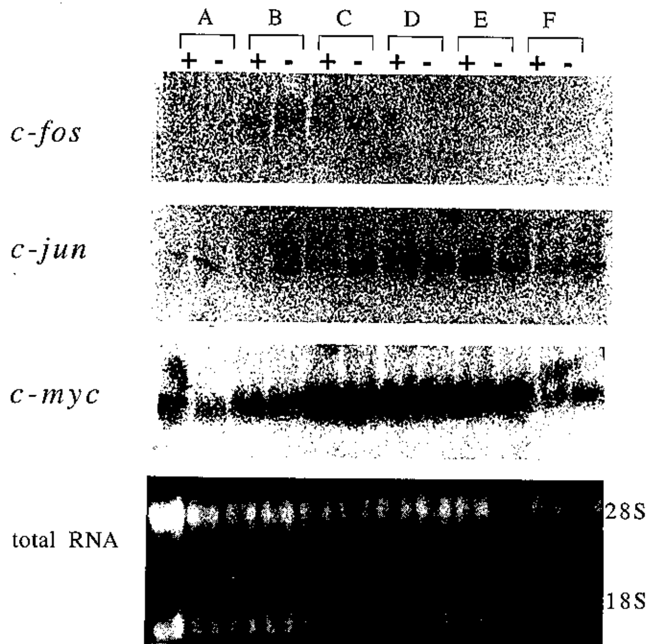

Fig. 4. Expression of c-*fos*, c-*jun*, c-*myc* mRNA in rat liver. F344 male rats underwent PH, with SMC being administered by i.g. at 100 mg/kg at 0, 24 and 48 h prior to the operation (+, SMC treatment; −, no treatment). Uniform RNA loading was confirmed by examining total RNA. Lanes A, 0 h after PH; Lanes B, 2 h after PH; Lanes C, 4 h after PH; Lanes D, 8 h after PH; Lanes E, 12 h after PH; Lanes F, 24 h after PH.

of rat liver carcinogenesis.[1-3] In addition, we proved that the enhancing action was associated with increased ODC activity in the liver.[2] This is in line with the link found in the present work between inhibition of hepatocarcinogenesis and reduction of this key enzyme of polyamine synthesis.

Cell proliferation is commonly considered capable of enhancing the frequency of initiation in chemical carcinogenesis. In addition, it appears to play an important role in the action of promoters or non-genotoxic carcinogens.[21, 22] Polyamines are involved in epithelial cell division,[12] and ODC activity increases with epithelial cell proliferation of skin or bladder when promoting agents are administered.[10, 11] In previous studies, we also demonstrated that allyl mercapton, an oil-soluble OSC, induced cell proliferation in association with elevated ODC activity.[2] SAT, a newly established rate-limiting enzyme of biodegradation of polyamines,[17] has similarly been found to be a biochemical marker for epithelial cell proliferation.[12] The fact that SMC and cysteine prevented elevation of ODC and SAT in the liver in the present case suggests that the mechanism of prevention of hepatocarcinogenesis by such OSCs might involve down-regulation of polyamine metabolism. We showed that SMC and cysteine suppressed the increases of PCNA-positive cells.

There have been only a few reports concerning the modifying effects of water-soluble OSCs on chemical carcinogenesis. In one study, the frequency of colonic tumors induced by DMH in female CF-1 mice was

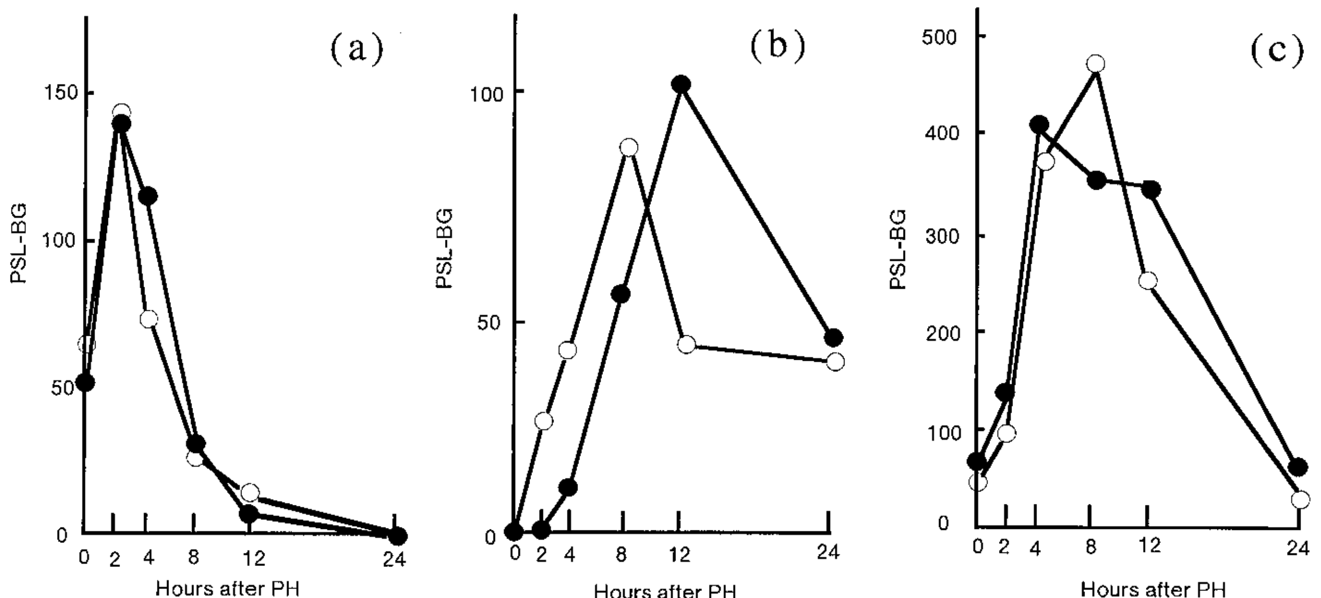

Fig. 5. Quantification of sequential changes in early response genes (a) c-*fos*, (b) c-*jun*, (c) c-*myc*. (●, SMC treatment; ○, no treatment).

significantly reduced by SAC pretreatment,[7] and in addition, SAC significantly inhibited the level of DMH-induced nuclear toxicity. In a future study, we intend to examine the differences of biological responses between oil- and water-soluble OSCs.

Though all of the OSCs used in this study were administered at doses of 100 mg/kg b.w., no effect was seen on body or relative liver weight. Accordingly, the results indicate that these OSCs are not toxic. SMC is contained at a concentration of about 40 μg per 1 g of fresh garlic (personal communication from Wakunaga Pharmaceutical Co., Ltd.). Examination of the low dose-response relationships for the inhibitory effects of SMC and cysteine appears warranted in order to evaluate the possibility of administering a practically effective level and to establish their usefulness as chemopreventive agents.

In previous studies, it was found that okadaic acid, which is a promoter for mouse skin carcinogenesis, upregulated the expression of members of the *jun* and *fos* gene families.[23, 24] Ohta *et al.*[25] reported that Nodularin, a potent inhibitor of protein phosphatases 1 and 2A, is a non-genotoxic carcinogen for the male F344 rat liver and showed that it up-regulated c-*jun*, *jun*-B, c-*fos*, *fos*-B, and *fra*-1 mRNA transcription in rat liver after i.p. administration. Thus, expression of early response genes may be very important for tumor promotion. This is the rationale for our examination of these genes after SMC treatment in our rat liver model. Expression of c-*jun* mRNA was down-regulated for the first eight hours after the final i.g. administration of SMC, when the levels of c-*fos* mRNA transcripts were unchanged compared to the control group.

The c-*jun* and c-*fos* proto-oncogenes encode proteins that form complexes which regulate transcription from promoters containing AP-1 activation elements. It was previously reported that c-Jun binds as a homodimer, while c-Fos fails to dimerize and displays no affinity for the AP-1 element.[26] Moreover, cotranslated c-Jun and c-Fos proteins bind twenty-five times more efficiently to the AP-1 site (TGACTCA) as a heterodimer than does the c-Jun homodimer.[27] Our evidence suggests that formation of Fos-Jun heterodimers, which strongly bind to AP-1 sites, is decreased in rat liver treated with SMC and that the suppressed development of preneoplastic lesions in rat liver might be directly related to this.

In conclusion, SMC and cysteine can inhibit both the initiation and promotion stages of rat hepatocarcinogenesis. Suppression of polyamine metabolism and the transitory down-regulation of induction of c-*jun* expression may play important roles in this chemopreventive action.

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