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Inhibitory Effects of Diallyl Disulfide or Aspirin on 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine-induced Mammary Carcinogenesis in Rats

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Modifying effects of diallyl disulfide (DAD), aspirin or DL-α-difluoromethylornithine (DFMO) on 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)-induced mammary carcinogenesis in SD rats were investigated. A total of 166 female rats, 6 weeks old, were divided into 8 groups. They were fed a high fat diet throughout the experiment. Starting at 7 weeks of age, groups 1–4 were given PhIP (85 mg/kg body weight in corn oil) by gavage 8 times in 10 days, and groups 5–8 were given corn oil alone. For the beginning 4 weeks, groups 2 and 5 were given DAD at 200 ppm in diet. Similarly groups 3 and 6, and groups 4 and 7 were given aspirin (400 ppm) and DFMO (400 ppm), respectively. Mammary carcinomas were only recognized in groups 1–4 at the termination (25 weeks after the start of experiment). Multiplicity (mean number/rat) of neoplasms in group 2 (PhIP+DAD, 0.90/rat) and group 3 (PhIP+aspirin, 1.37/rat) was significantly smaller than that in group 1 (PhIP alone, 2.45/rat) ($P<0.005$ and $P<0.05$, respectively). These results indicate that dietary intake of DAD or aspirin during the time corresponding to initiation phase has chemopreventive potential on PhIP-induced mammary carcinogenesis in rats.

Key words: PhIP — Mammary carcinogenesis — Chemoprevention

Dietary factors are regarded as having a profound impact on the occurrence of human cancers.[1-3] A variety of heterocyclic amines have been identified in cooked meat. They have potent mutagenic activities and their carcinogenic potentials have been demonstrated in different organs, such as forestomach, liver and large intestine.[4,5] 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) is one of the best-known carcinogenic heterocyclic amines.[5] This compound has carcinogenic potential in the mammary gland in female rats.[6,7] Epidemiological observation has also indicated an increased risk of breast cancer associated with fried meat consumption,[8-10] suggesting the significance of PhIP as a human carcinogen for breast cancer.

Currently, anticarcinogenic activities of a large number of chemicals are being examined in animal studies.[11] However, only a few agents are known to be effective in PhIP-induced mammary carcinogenesis.[12,13]

Diallyl disulfide (DAD), an organosulfur compound, is one of the oil-soluble constituents of garlic. Aspirin is a nonsteroidal anti-inflammatory drug (NSAID), which inhibits the arachidonic acid cascade. DL-α-Difluoromethylornithine (DFMO) is an irreversible inhibitor of ornithine decarboxylase (ODC).[14] These chemicals have been reported to exhibit chemopreventive functions in various animal studies. Organosulfur compounds are blocking agents that prevent cancer-producing compounds from reaching or reacting with critical target sites.[11] Aspirin and DFMO are categorized as suppressing agents.[11] In previous studies, DAD and DFMO reduced the incidence of carcinogen-induced mammary tumors.[15-18] Furthermore, NSAIDs have been proposed to be protective against mammary carcinogenesis. The major reason for the chemopreventive effects is considered to be inhibition of cell proliferation during the post-initiation phase. However, current evidence suggests that various chemopreventive agents have inhibiting effects on cell proliferation during the initiation phase.[19,20]

A rather long exposure is necessary for the induction of tumors by PhIP.[7] Recently, it was reported that mammary tumors were developed by intragastric administration of PhIP with a high fat diet.[21,22] We also established a method based on a combination of a high fat diet and PhIP exposure, and obtained a higher incidence of mammary carcinomas in a shorter period.[23] In the present study, the modifying effects of DAD, aspirin and DFMO on PhIP-induced mammary carcinogenesis during the initiation phase were examined using this model.

MATERIALS AND METHODS

Animals, diet, water and carcinogen Weaning female SD rats were obtained from Japan SLC, Inc. (Shizuoka). PhIP (PhIP hydrochloride) was purchased from Nard Institute (Osaka). DAD was purchased from Tokyo Kasei Chemical Co. (Tokyo), aspirin was from Nacalai

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Tesque, Inc. (Kyoto), and DFMO was kindly donated by Merrell-Dow Research Institute, Cincinnati, OH. All diet ingredients were obtained from CLEA Japan, Inc. (Tokyo) and mixed in our laboratory. The experimental diet was prepared weekly and stored in a cold room. The composition of the high polyunsaturated fat diet used in this study is as follows: casein (vitamin-free), 23.5%; DL-methionine, 0.35%; corn starch, 32.9%; dextrose, 8.3%; alphacel, 5.9%; corn oil, 23.52%; mineral (AIN), 4.11%; vitamin (AIN, revised), 1.18%; choline bitartrate, 0.24%. All animals were housed in wire cages (3 or 4 rats/cage). They had free access to water and diet under controlled environmental conditions of humidity (50±10%), lighting (12 h light/dark cycle) and temperature (23±2°C).

Experimental procedure One hundred and sixty-six rats, 6 weeks old, were divided into eight groups. They were fed the high fat diet throughout the experiment. Starting at 7 weeks of age, rats in groups 1–4 were given 8 doses of PhIP (85 mg/kg body weight) in corn oil via an intragastric tube for 10 days and animals of groups 5–8 received the solvent alone. Rats in groups 2–7 were given a test chemical in the diet (groups 2 and 5; 200 ppm diallyl disulfide, groups 3 and 6; 400 ppm aspirin, groups 4 and 7; 400 ppm DFMO) starting at 6 weeks of age (Fig. 1). The concentrations of DAD, aspirin and DFMO were decided on the basis of the previous experiments.[15,24–27] From 7 weeks after the first exposure to PhIP, each rat was carefully checked for palpable mammary tumors. The experiment was terminated at 25 weeks after the start of the experiment, since the incidence of palpable tumors reached 50–60% at around 21 weeks in groups 1 and 4, and the rate of increase of the incidence appeared to become small after this time point. At the termination, complete autopsies were performed on all animals. At autopsy, the location, number and size of mammary tumors were recorded. Tissues were fixed in 10% buffered formalin, embedded in paraffin blocks, and processed for routine histological observation with hematoxylin and eosin stain. Pathological diagnosis of mammary tumors was made according to the criteria outlined by Young and Hallowes.[28]

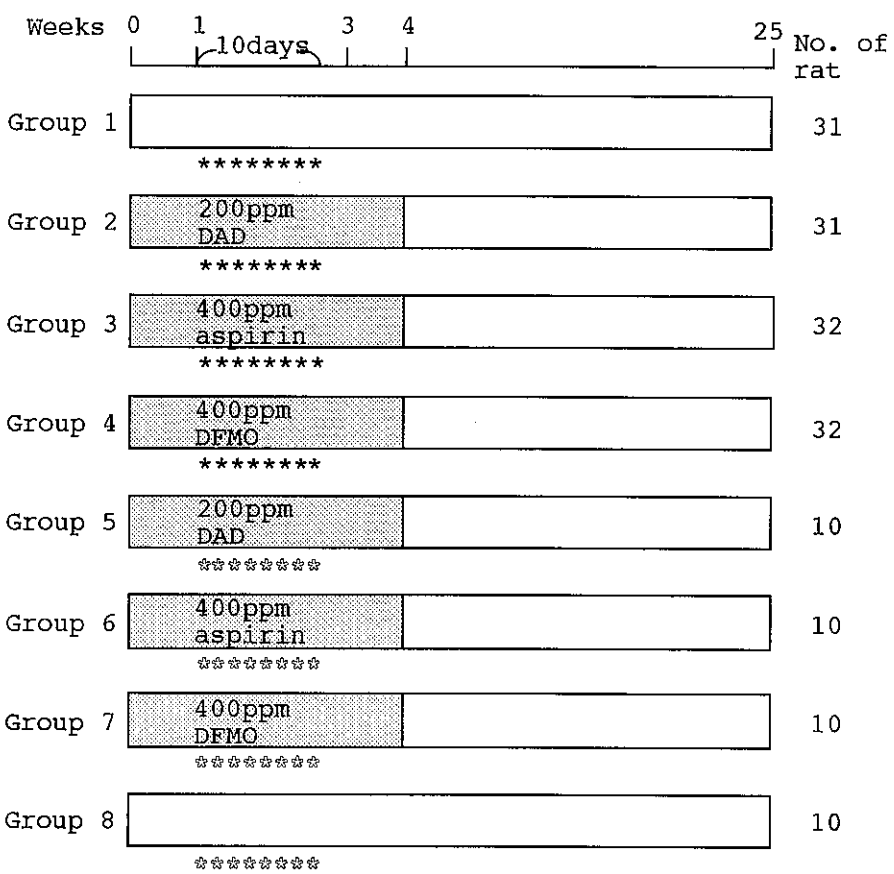

Fig. 1. Experimental protocol. □, high fat diet; ▨, high fat diet with test compound; ★, PhIP 85 mg/kg body weight in corn oil by gavage; ☆, corn oil by gavage.

Statistical analysis The significance of differences of incidence, multiplicity or mean size of pathological lesions of the mammary gland between groups was evaluated using Student's t test or Fisher's exact probability test.

RESULTS

Two rats in group 3 and one rat in group 6 or 8 died accidentally during the gavage treatment. No statistically significant differences in survival curves were observed. There are no significantly differences in the final body weights among the groups given PhIP (groups 1–4). PhIP administration reduced body weight gain and the final body weights of groups 1–3 (PhIP alone, PhIP + DAD and PhIP + aspirin) were significantly smaller than that of the vehicle control group. Liver weight of group 5 (DAD alone) was also significantly smaller than that of the non-treatment group. However, no significant differences in relative liver weight were recognized (Table I). Histologic evaluation of animals given test chemicals did not reveal any sign of toxicity.

Palpable mammary tumors in rats of groups 1 and 4 first appeared 9 weeks after the start of the experiment. Compared to the control group, tumor onset was delayed

Table I. Final Body and Liver Weights

Group no.	Treatment	No. of rats	Body weight (g)	Liver weight (g)	Relative liver weight (%)
1	PhIP alone	31	263.6±18.2[a)]	10.0±1.8	3.8±0.7
2	PhIP+DAD	30	271.4±22.7	10.6±2.3	3.9±0.7
3	PhIP+Aspirin	30	264.9±26.8	10.9±1.6	4.1±0.5
4	PhIP+DFMO	31	273.5±27.2	11.2±1.9	4.1±0.6
5	DAD alone	9	300.7±27.2	13.2±2.6	4.4±0.8
6	Aspirin alone	10	280.8±20.4	11.0±1.3	3.9±0.4
7	DFMO alone	9	292.7±32.9	11.0±2.6	3.8±0.6
8	Vehicle control	9	291.8±15.1[b, c, d)]	10.2±1.6[e)]	4.0±0.5

a) Mean±SD.
b) Significantly different from group 1 by Student's t test ($P<0.0001$).
c) Significantly different from group 2 by Student's t test ($P<0.05$).
d) Significantly different from group 3 by Student's t test ($P<0.01$).
e) Significantly different from group 5 by Student's t test ($P<0.01$).

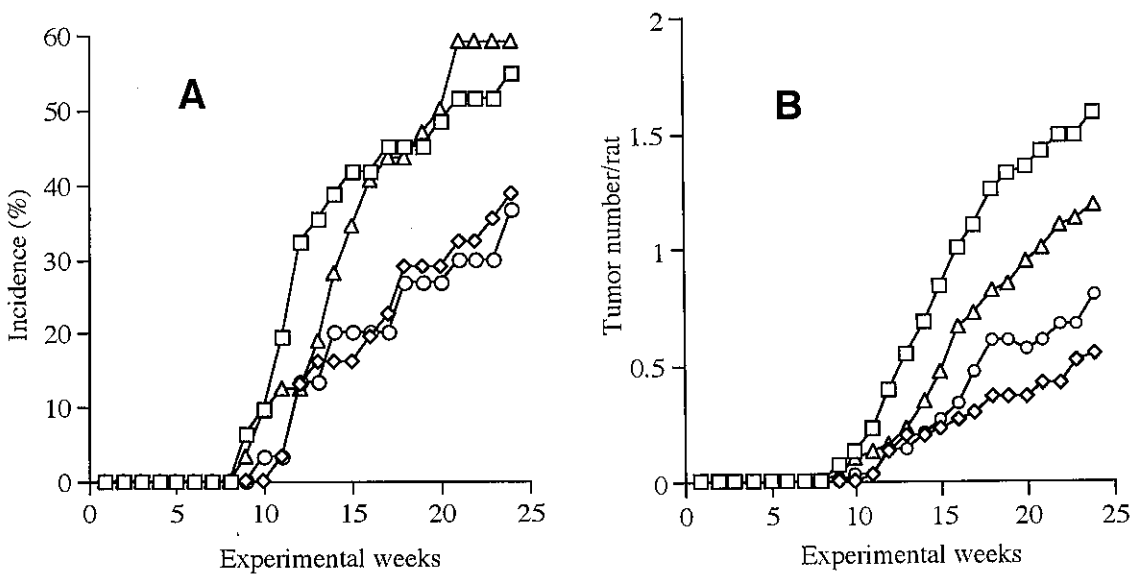

Fig. 2. Effect of dietary supplementation of DAD, aspirin or DFMO on PhIP-induced mammary tumors. A, the incidence of mammary tumors. B, the multiplicity of mammary tumors. □, PhIP alone; ◇, PhIP+DAD; ○, PhIP+aspirin; △, PhIP+DFMO.

Table II. Incidence of Mammary Tumors

Group no.	Treatment	No. of rats	Fibroadenoma (%)	Carcinoma (%)			Total tumors (%)
				Intraductal	Invasive ductal	Total	
1	PhIP alone	31	3 (9.7)	4 (12.9)	21 (67.7)	21 (67.7)	21 (67.7)
2	PhIP+DAD	31	0	2 (6.5)	16 (51.6)	16 (51.6)	16 (51.6)
3	PhIP+Aspirin	30	2 (6.7)	1 (3.3)	13 (43.3)	14 (46.7)	15 (50.0)
4	PhIP+DFMO	32	1 (3.1)	0	19 (59.4)	19 (59.4)	20 (62.5)

Table III. Multiplicity of Mammary Tumors

Group no.	Treatment	Fibroadenoma	Carcinoma			Total tumors
			Intraductal	Invasive ductal	Total	
1	PhIP alone	0.13±0.42[a)]	0.16±0.45	2.16±2.27	2.32±2.47	2.45±2.61
2	PhIP+DAD	0	0.06±0.25	0.84±0.99[b)]	0.90±1.12[c)]	0.90±1.12[b)]
3	PhIP+Aspirin	0.10±0.40	0.03±0.18	1.23±1.93	1.27±1.91	1.37±1.94[d)]
4	PhIP+DFMO	0.03±0.17	0	1.44±1.71	1.44±1.71	1.47±1.70

a) Mean±SD.
Significantly different from PhIP alone group by Student's *t* test. *b*) $P<0.05$, *c*) $P<0.01$, *d*) $P<0.05$.

1 or 2 weeks in rats fed diet supplemented with aspirin or DAD. Incidence of palpable tumors reached 50% in the group with PhIP alone at the 21st experimental week (Fig. 2A). Additional non-palpable tumors were also detected at termination. Most of the tumors were histologically invasive ductal carcinomas, and others were fibroadenomas and intraductal carcinomas. Final incidences of total tumors were 67.7, 51.6, 50.0 and 62.5% in groups 1–4, respectively. Although the incidences of groups 2 and 3 were rather lower than that of group 1, the difference was not significant (Table II). There was a tendency that DAD, aspirin and DFMO all decreased the multiplicity of palpable tumors during the experiment (Fig. 2B). At the end of the experiment, the average numbers of total tumors were 2.45, 0.90, 1.37 and 1.47 in group 1–4, respectively (Table III). The multiplicity of total tumors was significantly decreased by DAD ($P<0.005$) or aspirin ($P<0.05$). DAD also reduced the multiplicity of invasive ductal carcinoma ($P<0.005$) or total carcinoma ($P<0.01$). The mean values of the diameter of tumors were 8.33±2.47, 7.27±3.75, 6.41±3.18 and 8.46±5.13 (mean±SD) in groups 1–4, respectively. No clear differences were recognized in the mean size of tumors among these groups.

No mammary tumors were detected in groups 4–8 and neoplastic lesions other than mammary gland lesions were not found.

DISCUSSION

Our results demonstrate the ability of DAD or aspirin to inhibit PhIP-induced mammary carcinogenesis. This is the first report to show that these compounds have chemopreventive activities on heterocyclic amine-induced mammary carcinogenesis. It is well known that garlic (*Allium sativum L.*) contains a complex mixture of organosulfur compounds.[29] Of them, DAD, a lipid-soluble sulfur compound, has been reported to be effective in preventing the formation of rodent neoplasms[24, 30, 31] including *N*-methyl-*N*-nitrosourea-induced carcinogenesis in mammary glands.[15] In the present study, dietary exposure to DAD decreased the multiplicity of the mammary cancers induced by PhIP. The mechanisms of the inhibitory effect of DAD on the mammary carcinogenesis are not clear, but several factors may be involved. Like other carcinogens, PhIP needs to be activated to the *N*-hydroxy derivative by cytochrome P450 1A2.[32, 33] The *N*-hydroxy-PhIP can be activated to highly reactive *N*-acetoxy or *N*-sulfonyloxy derivatives.[34] DAD was reported to be effective in reducing the formation of MNU-induced O^6-methylguanine and N^7-methylguanine adducts in mammary tissues.[15] It is possible that DAD suppresses the activity of cytochrome P450 and decreases the formation of the ultimate carcinogen(s) of PhIP. Several organosulfur compounds are known to inhibit carcinogenesis by increasing the carcinogen-detoxifying

capability via induction of phase II enzymes. DAD is known to induce glutathione *S*-transferase (GST),[24] which plays an important role in detoxification of many xenobiotics.[35] GST is reported to inhibit covalent DNA binding of *N*-acetoxy-PhIP *in vitro*.[36] Thus, it is reasonable to assume that DAD reduces PhIP-induced adduct formation and inhibits carcinogenesis in the mammary gland by suppression of phase I enzymes and activation of phase II enzymes.

NSAIDs including aspirin are also known to be chemopreventive in the development of colon tumors.[25, 37–40] Epidemiological studies on NSAIDs are in agreement with the tumorigenesis data in animal studies.[41–44] Furthermore, some studies have suggested inhibitory effects of these agents on mammary cancer induction.[45–49] In this study, dietary exposure to aspirin inhibited PhIP-induced mammary carcinogenesis. NSAIDs are inhibitors of cyclooxygenases, which are prostaglandin synthetases. Prostaglandin biosynthesis is considered to be associated with mutagenesis, cell growth and tumor promotion.[50] The aspirin metabolite salicylate has been reported to induce cell cycle arrest and apoptosis, particularly in cells at the later stages of neoplastic progression.[51] Although the mechanisms of suppression of mammary carcinogenesis by aspirin are also not clear, the present results suggest that arachidonate cascade modifiers can be effective even during the initiation phase.

In the present study, DFMO showed a tendency to suppress PhIP-induced mammary carcinogenesis. The tendency may be associated with the inhibitory effect on ODC, the rate-limiting enzyme in the polyamine biosynthetic pathway.[14] The ODC inhibition may be related to modification of the cell cycle in mammary epithelium exposed to PhIP.[52]

In conclusion, we have clarified that PhIP-induced mammary carcinogenesis is inhibited by dietary exposure to DAD or aspirin. These findings represent a new chemopreventive characteristic of DAD and aspirin, and may have implications for the prevention of human mammary cancers.

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REFERENCES

1) Doll, R. Epidemiology and the prevention of cancer: some recent developments. *J. Cancer Res. Clin. Oncol.,* **114,** 447–458 (1988).
2) Sugimura, T. Multistep carcinogenesis: a 1992 perspective. *Science,* **258,** 603–607 (1992).
3) Weisburger, J. H. Current view on mechanisms concerned with the etiology of cancers in the digestive tract. *In* "Pathophysiology of Carcinogenesis in Digestive Organs," ed. E. Farber, pp. 1–20 (1977). Univ. of Tokyo Press, Tokyo.
4) Felton, J. S. and Knize, M. G. Occurrence, identification, and potential mutagenicity of heterocyclic amines in cooked food. *Mutat. Res.,* **259,** 205–217 (1991).
5) Wakabayashi, K., Nagao, M., Esumi, H. and Sugimura, T. Food-derived mutagens and carcinogens. *Cancer Res.,* **52,** 2092s–2098s (1992).
6) Ito, N., Hasegawa, R., Sano, M., Tamano, S., Esumi, H., Takayama, S. and Sugimura, T. A new colon and mammary carcinogen in cooked food, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). *Carcinogenesis,* **12,** 1503–1506 (1991).
7) Hasegawa, R., Sano, M., Tamano, S., Imaida, K., Shirai, T., Nagao, M., Sugimura, T. and Ito, N. Dose-dependence of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) carcinogenicity in rats. *Carcinogenesis,* **14,** 2553–2557 (1993).
8) Matos, E. L., Thomas, D. B., Sobel, N. and Vuoto, D. Breast cancer in Argentina: case-control study with special reference to meat-eating habits. *Neoplasma,* **38,** 357–366 (1991).
9) Knekt, P., Steineck, G., Jarvinen, R., Hakulinen, T. and Aaroma, A. Intake of fried meat and risk of cancer: a follow-up study in Finland. *Int. J. Cancer,* **59,** 756–760 (1994).
10) Ronco, A., Stefani, E. D., Mendilaharsu, M. and Deneo-Pellegrini, H. Meat, fat and risk of breast cancer: a case-control study from Uruguay. *Int. J. Cancer,* **65,** 328–331 (1996).
11) Wattenberg, L. W. Prevention-therapy-basic science and the resolution of the cancer problem: presidential address. *Cancer Res.,* **53,** 5890–5896 (1993).
12) Hirose, M., Akagi, K., Hasegawa, R., Yaono, M., Satoh, T., Hara, Y., Wakabayashi, K. and Ito, N. Chemoprevention of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)-induced mammary gland carcinogenesis by antioxidants in F344 female rats. *Carcinogenesis,* **16,** 217–221 (1995).
13) Hasegawa, R., Hirose, M., Kato, T., Hagiwara, A., Boonyaphiphat, P., Nagao, M., Ito, N. and Shirai, T. Inhibitory effect of chlorophyllin on PhIP-induced mammary carcinogenesis in female F344 rats. *Carcinogenesis,* **16,** 2243–2246 (1995).
14) Metcalf, B. W., Bey, P., Danzin, C., Jung, M. J., Casara, P. and Vevert, J. P. Catalytic irreversible inhibition of

mammalian ornithine decarboxylase (EC 4.1.1.17) by substrate and product analogs. *J. Am. Chem. Soc.*, **100**, 2551–2553 (1978).
15) Schaffer, E. M., Liu, J.-Z., Green, J., Dangler, C. A. and Milner, J. A. Garlic and associated allyl sulfur components inhibit *N*-methyl-*N*-nitrosourea induced rat mammary carcinogenesis. *Cancer Lett.*, **102**, 199–204 (1996).
16) Fozard, J. R. and Prakash, N. J. Effects of DL-alpha-difluoromethylornithine, an irreversible inhibitor of ornithine decarboxylase, on the rat mammary tumour induced by 7,12-dimethylbenz[*a*]anthracene. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **320**, 72–77 (1982).
17) Abou-el-Ela, S. H., Prasse, K. W., Farrell, R. L., Carroll, R. W., Wade, A. E. and Bunce, O. R. Effects of D,L-2-difluoromethylornithine and indomethacin on mammary tumor promotion in rats fed high n-3 and/or n-6 fat diets. *Cancer Res.*, **49**, 1434–1440 (1989).
18) Thompson, H. J., Meeker, L. D., Herbst, E. J., Ronan, A. M. and Minocha, R. Effect of concentration of D,L-2-difluoromethylornithine on murine mammary carcinogenesis. *Cancer Res.*, **45**, 1170–1173 (1985).
19) Mori, H., Tanaka, T., Sugie, S. and Yoshimi, N. Chemopreventive effects of plant derived phenolic, organosulfur and other compounds on carcinogenesis in digestive organs. *Environ. Mutagen Res. Commun.*, **17**, 127–133 (1995).
20) Mori, H., Yoshimi, N., Tanaka, T. and Hirose, Y. Experimental colorectal carcinogenesis; role of cell proliferation. *In* "Recent Advances in Gastroenterological Carcinogenesis I," ed. E. Tahara, K. Sugimachi and T. Oohara, pp. 249–254 (1996). Monduzzi Editore, Bologna.
21) Ghoshal, A., Preisegger, K.-H., Takayama, S., Thorgeirsson, S. S. and Snyderwine, E. G. Induction of mammary tumors in female Sprague-Dawley rats by the food-derived carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine and effect of dietary fat. *Carcinogenesis*, **15**, 2429–2433 (1994).
22) El-Bayoumy, K., Chae, Y.-H., Upadhyaya, P., Rivenson, A., Kurtzke, C., Reddy, B. S. and Hecht, S. S. Comparative tumorigenicity of benzo[*a*]pyrene, 1-nitropyrene and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine administered by gavage to female CD rats. *Carcinogenesis*, **16**, 447–458 (1995).
23) Ino, N., Sugie, S., Ohnishi, M. and Mori, H. Lack of inhibitory effect of benzyl isothiocyanate on 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)-induced mammary carcinogenesis in rats. *J. Toxicol. Sci.*, **21**, 189–194 (1996).
24) Reddy, B. S., Rao, C. V., Rivenson, A. and Kelloff, G. Chemoprevention of colon carcinogenesis by organosulfur compounds. *Cancer Res.*, **53**, 3493–3498 (1993).
25) Reddy, B. S., Rao, C. V., Rivenson, A. and Kelloff, G. Inhibitory effect of aspirin on azoxymethane-induced colon carcinogenesis in F344 rats. *Carcinogenesis*, **14**, 1493–1497 (1993).
26) Tanaka, T., Kojima, T., Hara, A., Sawada, H. and Mori, H. Chemoprevention of oral carcinogenesis by DL-α-difluoromethylornithine, an ornithine decarboxylase inhibitor: dose-dependent reduction in 4-nitroquinoline 1-oxide-induced tongue neoplasms in rats. *Cancer Res.*, **53**, 772–776 (1993).
27) Kojima, T., Tanaka, T., Kawamori, T., Hara, A. and Mori, H. Chemopreventive effects of dietary D,L-α-difluoromethylornithine, an ornithine decarboxylase inhibitor, on initiation and postinitiation stages of diethylnitrosamine-induced rat hepatocarcinogenesis. *Cancer Res.*, **53**, 3903–3907 (1993).
28) Young, S. and Hallowes, R. C. Tumors of mammary gland. *In* "Pathology of Tumors in Laboratory Animals," ed. V. S. Tursov, pp. 31–74 (1973). International Agency for Research on Cancer, Lyons.
29) Block, E. The organosulfur chemistry of the genus *Allium*-implications for the organic chemistry of sulfur. *Angew. Chem. Int. Ed. Engl.*, **31**, 1135–1178 (1992).
30) Takahashi, S., Hakoi, K., Yada, H., Hirose, M., Ito, N. and Fukushima, S. Enhancing effects of diallyl sulfide on hepatocarcinogenesis and inhibitory actions of the related diallyl disulfide on colon and renal carcinogenesis in rats. *Carcinogenesis*, **13**, 1513–1518 (1992).
31) Wattenberg, L. W., Sparnins, V. L. and Barany, G. Inhibition of *N*-nitrosodiethylamine carcinogenesis in mice by naturally occurring organosulfur compounds and monoterpenes. *Cancer Res.*, **49**, 2689–2692 (1989).
32) Boobis, A. R., Lynch, A. M., Murray, S., de la Torre, R., Solsans, A., Farreé, M., Segura, J., Gooderham, N. J. and Davis, D. S. CYP1A2-catalyzed conversion of dietary heterocyclic amines to their proximate carcinogens is their major route of metabolism in humans. *Cancer Res.*, **54**, 89–94 (1994).
33) Turtletaub, K. W., Knize, M. G., Buonarati, M. H., McManus, M. E., Veronese, M. E., Mazrimas, J. A. and Felton, J. S. Metabolism of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) by liver microsomes and isolated rabbit cytochrome P450 isozymes *Carcinogenesis*, **11**, 941–946 (1990).
34) Malfatti, M. A., Buonorati, M. H., Turtetaub, K. W., Shen, N. H. and Felton, J. S. The role of sulfation and/or acetylation in the metabolism of the cooked-food mutagen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine in *Salmonella typhimurium* and isolated rat hepatocytes. *Chem. Res. Toxicol.*, **7**, 139–147 (1994).
35) Coles, B. and Ketterer, B. The role of glutathione and glutathione transferases in chemical carcinogenesis. *Crit. Rev. Biochem. Mol. Biol.*, **25**, 47–70 (1990).
36) Lin, D., Meyer, D. J., Ketterer, B., Lang, N. P. and Kadlubar, F. F. Effects of human and rat glutathione *S*-transferases on the covalent DNA binding of the *N*-acetoxy derivatives of heterocyclic amine carcinogens *in vitro*: a possible mechanism of organ specificity in their carcinogenesis. *Cancer Res.*, **54**, 4920–4926 (1994).
37) Pollard, M. and Luckert, P. H. Effect of indomethacin on intestinal tumors induced in rats by acetate derivative of

dimethylnitrosamine. *Science,* **214,** 558–559 (1981).
38) Reddy, B. S., Maruyama, H. and Kelloff, G. Dose-related inhibition of colon carcinogenesis by dietary piroxicam, a nonsteroidal antiinflammatory drug, during different stages of rat colon tumor development. *Cancer Res.,* **47,** 5340–5346 (1987).
39) Pollard, M. and Luckert, P. H. Indomethacin treatment of rats with dimethylhydrazine-induced intestinal tumors. *Cancer Treat. Rep.,* **64,** 1323–1327 (1980).
40) Narisawa, T., Sato, M., Tani, M., Kudo, T., Takahashi, T. and Goto, A. Inhibition of development of methylnitrosourea-induced rat colon tumors by indomethacin treatment. *Cancer Res.,* **41,** 1954–1957 (1981).
41) Thun, M. J., Namboodiri, M. M. and Heath, C. W., Jr. Aspirin use and reduced risk of fetal colon cancer. *N. Engl. J. Med.,* **325,** 1593–1596 (1991).
42) Kune, G. A., Kune, S. and Watson, L. F. Colorectal cancer risk, chronic illnesses, operation, and medications: case control results from the Melbourne colorectal cancer study. *Cancer Res.,* **48,** 4399–4404 (1988).
43) Rosenberg, L., Palmer, J. R., Zauber, A. G., Warshauer, M. E., Stolley, P. D. and Shapiro, S. A hypothesis: nonsteroidal anti-inflammatory drugs reduce the incidence of large-bowel cancer. *J. Natl. Cancer Inst.,* **85,** 1182–1183 (1991).
44) Paganini-Hill, A., Hsu, G., Ross, R. K. and Henderson, B. E. Aspirin use and incidence of large-bowel cancer in a California retirement community. *J. Natl. Cancer Inst.,* **83,** 355–358 (1991).
45) McCormick, D. L., Madigan, M. J. and Moon, R. C. Modulation of rat mammary carcinogenesis by indomethacin. *Cancer Res.,* **45,** 1803–1808 (1985).
46) Carter, C. A., Milholland, R. J., Shea, W. and Ip, M. M. Effect of the prostaglandin synthetase inhibitor indomethacin on 7, 12-dimethylbenz(*a*)anthracene-induced mammary tumorigenesis in rats fed different levels of fat. *Cancer Res.,* **43,** 3559–3562 (1983).
47) Mehta, R. G. and Moon, R. C. Characterization of effective chemopreventive agents in mammary gland *in vitro* using an initiation-promotion protocol. *Anticancer Res.,* **11,** 593–596 (1991).
48) McCormick, D. L. and Wilson, A. M. Combination chemoprevention of rat mammary carcinogenesis by indomethacin and butylated hydroxytoluene. *Cancer Res.,* **46,** 3907–3911 (1986).
49) Thompson, H. J., Briggs, S., Paranka, N. S., Piazza, G. A., Brendel, K., Gross, P. H., Sperl, G. J., Pamukcu, R. and Ahnen, D. J. Inhibition of mammary carcinogenesis in rats by sulfone metabolite of sulindac. *J. Natl. Cancer Inst.,* **87,** 1259–1260 (1995).
50) Marnett, L. J. Aspirin and the potential role of prostaglandins in colon cancer. *Cancer Res.,* **52,** 5575–5589 (1992).
51) Elder, D. J., Hague, A., Hicks, D. J. and Paraskeva, C. Differential growth inhibition by the aspirin metabolite salicylate in human colorectal tumor cell lines: enhanced apoptosis in carcinoma and *in vitro*-transformed adenoma relative to adenoma cell lines. *Cancer Res.,* **56,** 2273–2276 (1996).
52) Pegg, A. E. Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy. *Cancer Res.,* **48,** 759–774 (1988).