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Different Modifying Responses of Capsaicin in a Wide-Spectrum Initiation Model of F344 Rat

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The modifying potential of capsaicin (CAP) on lesion development was examined in a rat multiorgan carcinogenesis model. Groups 1 and 2 were treated sequentially with diethylnitrosamine (DEN) (100 mg/kg, ip, single dose at commencement), N-methylnitrosourea (MNU) (20 mg/kg, ip, 4 doses at days 2, 5, 8, and 11), and N,N-dibutylnitrosamine (DBN) (0.05% in drinking water during weeks 3 and 4). Group 3 received vehicles without carcinogens during the initiation period. Gorup 4 served as the untreated control. After this initiating procedure, Groups 2 and 3 were administered a diet containing 0.01% CAP. All surviving animals were killed 20 weeks after the beginning of the experiment and the target organs examined histopathologically. The induction of GST-P$^+$ hepatic foci in rats treated with carcinogens was significantly inhibited by treatment with CAP. CAP treatment significantly decreased the incidence of adenoma of the lung but increased the incidence of papillary or nodular (PN) hyperplasia of the urinary bladder. The tumor incidence of other organs, such as the kidney and thyroid, was not significantly different from the corresponding controls. These results demonstrated that concurrent treatment with CAP not only can inhibit carcinogenesis but can also enhance it depending on the organ. Thus, this wide-spectrum initiation model could be used to confirm organ-specific modification potential and, in addition, demonstrate different modifying effects of CAP on liver, lung, and bladder carcinogenesis.

Key Words : *Capsaicin, Rat, Carcinogenesis, Multiorgan.*

INTRODUCTION

Epidemiological studies show that dietary factors are the most important environmental risk determinant for human cancer. (Doll and Peto, 1981). Many natural food components may influence the incidence of cancer (Sugimura and Sato, 1983). The human diet contains, in addition to a great variety of natural carcinogens, many inhibitors such as flavones, aromatic isocyanates, coumarin, retinoids, indoles, and selenium (Ames,

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Abbreviations : *CAP, capsaicin ; DEN, diethylnitrosamine ; MNU, N-methylnitrosourea ; DBN, N, N-dibutyl nitrosamine*

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1983 ; Pariza et al., 1986). Some exhibit promotional as well as inhibitory behavior (Jang et al., 1988, 1989 ; Ito and Hirose, 1987). Therefore, the modifying effects of various dietary constituents on carcinogenesis should be tested, preferably by a whole-body multiorgan carcinogenesis concept (Ito et al., 1988 ; Thamavit et al., 1989).

CAP is a homovanillic acid derivative and is the principal pungent and irritating constituent of *Capsicum* fruits. The Korean people are large consumers of chili fruit of the genus *Capsicum*. It is heavily used as a food additive, and the average daily per capita consumption of CAP may reach 50㎎ (Buck and Burks, 1983). The content of CAP in *Capsicum* is about 0.02% in fresh fruit and 0.5−1 % in dried ripe fruit. It has been suspected that spicy foods play some role in human carcinogenesis. There are conflicting data on the modulating effect of CAP ; in some studies significant tumor initiating or promoting effects were observed (Hoch-Ligetti, 1951 ; Adamia, 1971 ; Toth et al., 1984 ; Agrawal et al., 1986), whereas in others this finding was not confirmed (Hahn, 1961 ; Lee, 1963 ; Agrawal and Bhide, 1987, 1988). We also reported that CAP had a promoting effect on hepatic enzyme-altered foci (Jang and Kim, 1988) but an inhibitory effect on mouse lung tumor development (Jang et al., 1989).

The present study was conducted to clarify the organ specific modifying potential of CAP in a rat multiorgan carcinogenesis induced by DEN, MNU, and DBN.

MATERIALS AND METHODS

Animals : A total of 60 6-week-old male F344 rats (Korea Research Institute of Chemical Technology) were used. Four to 5 rats were kept in one polycarbonate cage in a room at 22± 2℃ with a 12−hr light/dark cycle. They were given tap water *ad libitum*.

Chemicals : DEN, DBN, and CAP were obtained from the Sigma Co., USA. MNU was from the Nakarai Chemical Co., Japan.

Experimental Protocol : The animals were divided into 4 groups. As shown in Fig. 1, Groups 1 and 2 were pretreated with 3 carcinogens. The animals were initially given a single ip injection of 100 ㎎/㎏ DEN dissolved in 0.9% NaCl solution. MNU was given 20 ㎎/㎏, ip, in citrate-buffered solution adjusted to pH 6.0, 4 doses on days 2, 5, 8, and

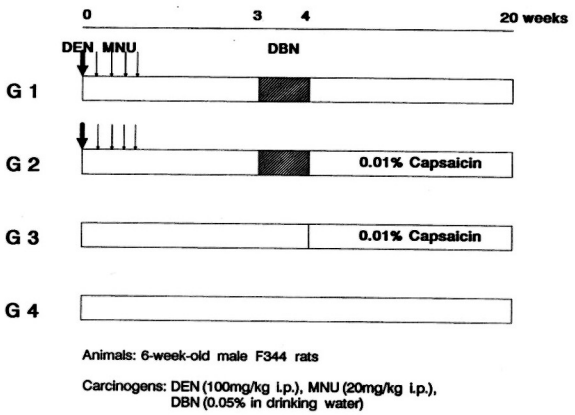

Fig. 1. Experimental Design.

11. DBN was given 0.05% in drinking water during weeks 3 and 4. Group 3 received vehicles without carcinogens, and Group 4 served as the untreated control. Subsequently, the animals in Groups 2 and 3 were given a solid pellet diet containing 0.01% CAP until week 20. All surviving animals were sacrificed, and their major organs—including the thyroid, lungs, esophagus, stomach, intestine, pancreas, liver, kidney and urinary bladder—were removed and fixed in a 10% buffered formalin solution. All organs were embedded in paraffin and stained with hematoxylin and eosin for histologic examination. Only the animals which survived until the end of week 20 were included in the effective numbers. For quantitative analysis of GST-P^+ hepatic foci, 3 slices of liver tissue were fixed in ice-cold acetone and processed for embedding in paraffin, and a subsequent immunohistochemical examination was done by the ABC method. The data were measured by a color image processor, model VIDAS (Kontron, West Germany).

Data on the incidence of lesions were analyzed for statistical significance with the two-tailed Chi-square test. Other data were analyzed with Student's t-test.

RESULTS

Data concerning mean body weights and relative liver, lung, and kidney weights in all groups are shown in Table 1. The mean body weight was lower in the carcinogen-treated group than in the carcinogen-untreated group. However, there were no significant differences in the mean body weights and relative organ weights between the corresponding controls. Table 2 shows the average daily and

Table 1. Mean Body and Organ Weights

Group Treatment	No. of Rats	Mean Body Weight(g)	Relative Organ Weight[a] (g/100 g b.w.)		
			Liver	Lungs	Kidneys
1. DEN+MNU+DBN	16	247± 78	2.9± 0.2	0.50± 0.08	0.76± 0.06
2. DEN+MNU+DBN →CAP	15	248± 78	3.1± 0.5	0.46± 0.07	0.77± 0.06
3. CAP	10	285± 78	3.1± 0.3	0.05± 0.06	0.71± 0.04
4. CONTROL	10	281± 77	3.0± 0.3	0.50± 0.03	0.70± 0.04

[a]Values represent mean± SD.

Table 2. Average Daily and Total Intake of Diet and Capsaicin

Group Treatment	Average Daily Intake[a]		Total Intake of Capsaicin (g/kg b.w/16 wks)
	Diet (g/kg b.w/day)	Capsaicin (mg/kg b.w/day)	
1. DEN+MNU+DBN	48.2± 3.2	0	0
2. DEN+MNU+DBN →CAP	45.7± 1.3	4.6± 0.1	0.52
3. CAP	44.1± 2.1	4.4± 0.1	0.49
4. CONTROL	46.9± 3.6	0	0

[a]Values represent mean± SD.

Table 3. Quantitative Data of GST-P$^+$ Foci

Group Treatment	No. of Rats	GST-P$^+$ Foci Data[a]	
		No./cm^2	Area(mm^2)/cm^2
1. DEN+MNU+DBN	16	3.41± 2.23	0.10± 0.07
1. DEN+MNU+DBN →CAP	15	1.83± 1.38*	0.05± 0.03**
3. CAP	10	0	0
4. CONTROL	10	0	0

[a]Values represent mean± SD.
Significantly different from Group 1 at *P<0.05, **P<0.02

total intake of diet and CAP. Average food consumption was slightly less in the CAP-supplemented groups. The total intake of CAP for 16 weeks was 0.49−0.52 g/kg body weight. Several rats died of pneumonia in the initiation stage from 3 carcinogens. These animals were not included in the effective numbers.

The induction of GST-P$^+$ hepatic foci in rats treated with carcinogens was significantly inhibited by treatment with 0.01% CAP (P<0.05 in number or P<0.02 in area) (Table 3). In rats treated with a combination of DEN, MNU, and DBN, neoplastic and preneoplastic lesions were primarily induced in the lung, thyroid, kidney, and urinary bladder. CAP significantly decreased the incidence of adenoma of the lung (from 56% to 13%) but enhanced the incidence of papillary of nodular (PN) hyperplasia of the urinary bladder (from 13% to 53 %) (P<0.02). Although some other lesions were induced in the thyroid and kidney by carcinogen treatment, their incidences were not significantly different in the CAP-supplemented group. Without carcinogen pretreatment, none of neoplastic lesions were induced (Table 4).

Table 4. Incidence of Neoplastic and Preneoplastic Lesions

	Treatment			
	CAR (16)[a]	CAR+CAP (15)	CAP (10)	Control (10)
Lung				
Hyperplasia	3 (19)[b]	4 (27)	0	0
Adenoma	9 (56)	2 (13)*	0	0
Thyroid				
Hyperplasia	2 (13)	1 (17)	0	0
Kidney				
Atypical tubules	9 (56)	6 (40)	0	0
Urinary bladder				
Simple hyperplasia	6 (38)	4 (27)	0	0
PN hyperplasia	2 (13)	8 (53)*	0	0
Papilloma	0	1 (7)	0	0

[a]Number of rats examined
[b]Number of tumor-bearing rats/rats examined (%)
Significantly different from CAR group at *$p<0.02$
CAR : Carcinogens (DEN+MNU+DBN)

DISCUSSION

The present study clearly demonstrated that CAP had a different modifying potential on carcinogenesis depending on the organ. It inhibited the development of GST-P$^+$ foci in the liver and adenoma of the lung but enhanced PN hyperplasia of the urinary bladder induced by DEN, MNU, and DBN.

The fact that a single chemical can exert either enhancement or inhibition depending upon organ is well-documented. For example, butylated hydroxyanisole (BHA) significantly enhances tumor development in the forestomach and urinary bladder but inhibits the development of liver lesions (Ito and Hirose, 1987). Sodium L-ascorbate promotes carcinogenesis of the renal pelvis and bladder while inhibiting it in the liver. Butylated hydroxytoluene (BHT) similarly decreases liver and enhances bladder lesion development (Thamavit et al., 1989). Consideration of the literature and information presently available does not provide a clear explanation of the differential modification of the carcinogenic response observed with a number of chemicals including CAP.

CAP (8-methyl-N-vanillyl-6-noneamide) is the major pungent ingredient of hot peppers of the plant genus *Capsicum*. The pharmacological effects of various species of *Capsicum* are well-documented (Monsereenusorn et al., 1982 ; Buck and Burks, 1986). Data on the mutagenic activity of CAP and chili extract in bacterial systems are contradictory. Both substances have been reported to be non-mutagenic in bacteria (Buchmann et al., 1982 ; Muralidhara and Narasimhamurthy, 1988). but Toth et al. (1984) reported a low level of mutagenic activity in the Ames test, and more recently both chili extract and CAP were demonstrated to be mutagenic in the Ames test (Nagabhushan and Bhide, 1985). Chili is reported to act as a promoter of hepatocarcinogenesis (Adamia, 1971) and to produce hepatomas when fed at a 10% level in the diet (Hoch-Ligetti, 1951). Further, chili and CAP have been shown to produce cirrhosis of the liver (Agrawal and Bhide, 1988 ; Lee, 1963), damage to duodenal mucosa (Naponitaya and Nye, 1974), and gastric ulcers. Toth et al. (1984) reported an increased incidence of adenocarcinoma of the duodenum as a result of feeding 0.0625−1 % CAP in the diets of mice for 35 days. Agrawal et al. (1986) also reported the promoter role of chili in carcinogen-initiated mice. However, some investigators could not detect definite carcinogenic activity of CAP at rather low dose levels (Agrawal and Bhide, 1987, 1988). In one study, the administration of CAP inhibited tumor development in mice (Poroff, 1941). We previously reported that CAP can promote hepatic GST-P$^+$ foci and inhibit mouse lung tumor development (Jang et al., 1988, 1989). In the present study, inhibition of carcinogenesis in the lung by CAP was consistent with

the results of our previous experiment, but the effect on the liver was not consistent. This discrepancy between the results may be due to the difference of dose levels and treatment duration of CAP and the initiating carcinogens used. Our previous study adopted lower doses (0.002%) of CAP and a shorter promotional phase (6 weeks) than the present study.

The exact mechanism of CAP action has not yet been elucidated. Recently, Miller et al. (1983) demonstrates that CAP competitively inhibits ethylmorhpine-N--demethylase activity in vitro in rat hepatic microsomes. CAP has been shown to interact with hepatic drug metabolizing systems in a high-affinity, concentration-dependent manner. This high-affinity interaction suggests that CAP, and its analogs, may be potent inhibitors of biotransformation systems. CAP was also found to be a potent in vitro inhibitor of human and murine epidermal metabolism of benzo(α) pyrene (BP) and enzyme-mediated binding of BP metabolite to DNA (Modly et al., 1986). CAP profoundly decreases the substance P in the urinary bladder, and there is associated urine retention and reduced motility (Monsereenusorn et al., 1982 ; Nutrition Reviews, 1986). We observed that CAP increased the incidence of PN hyperplasia, a well-known preneoplastic lesion, of the bladder. It is not known whether this lesion was the result of a direct effect of CAP on that tissue or a secondary effect caused by the partial chemical denervation of the bladder.

Many carcinogens, including DEN, MNU, and DBN, are known to cause cancer in experimental animals and in humans after metabolic activation. This activation process involves a cytochrome P—450-dependent enzyme which converts proximate carcinogens to electrophilic metabolites subsequently binding macromolecules such as DNA to the target tissue. Like many other naturally occurring and synthetic compounds, CAP can also inhibit carcinogen metabolism. Inhibition of carcinogenic metabolite-DNA binding may be an effect of the decrease in the generation of active carcinogenic metabolites as supported by inhibition of aryl hydrocarbon hydroxylase activity and/or by inactivation of the reactive metabolites (Modly et al., 1986).

Sequential treatment with potent carcinogens having different wide-spectrum initiating activities was used as the initiation step in this study. DEN is well-known to be a strong initiator of hepatocarcinogenesis, whereas MNU initiates the thyroid, urinary bladder, and hematopoietic systems. DBN is cacinogenic to the esophagus, forestomach, liver, and urinary bladder of rats. The results of the present experiment demonstrated that those 3 carcinogens can induce preneoplastic or neoplastic lesions of various organs. Although more experiments are necessary to optimize the observation period and dose of the initiating carcinogens, they do suggest that this model is very useful in detecting the modifying effects of the test chemicals on multiple organs.

Recently, attention has been focused on the identification of naturally occurring dietary constituents as antimutagens and anti-carcinogens. Our data showing the inhibitory effect of CAP on liver and lung carcinogenesis induced by DEN, MNU, and DBN suggest that this substance might have anti-carcinogenic effects as well The potential anti-carcinogenicity of CAP adds yet another dimension to the potential pharmacologic usefulness of this class of compounds. Humans are repeatedly exposed to complex and diverse mixtures that contain both initiators and promoters. Therefore, the impact of such inhibitory effects on these multiple carcinogens could be important, but further research would be required to understand the underlying mechanisms for such inhibitory effects.

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