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Chemopreventive Effects of *Chelidonium majus L.*(Papaveraceae) Herb Extract on Rat Gastric Carcinogenesis Induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine(MNNG) and Hypertonic Sodium Chloride

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Abstract

The modifying effects of *Chelidonium majus L.*(Papaveracea) herb extract(CH), an analgesic traditionally prescribed for gastric and duodenal ulcer patients, on gastric tumor development given *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine(MNNG) were studied in sixty-four 6-week-old male Wistar rats. Group 1 rats were initially given MNNG(200mg/kg b.w.) by gavage at days 0 and 14 as well as saturated sodium chloride solution (S-NaCl, 1ml per rat) every 3 days during weeks 0 to 3(6 times), and then placed on basal diet containing 0.1 or 0.2% CH for 16 weeks from week 4. Rats of Groups 2 and 3 were treated with MNNG together with S-NaCl or saline(0.9% NaCl, 1ml per rat) respectively, timed as in Group 1 but without further treatment. All survival animals were killed at week 20 and histopathologically investigated. In the glandular stomach, the number of preneoplastic pepsinogen 1 altered pyloric glands(PAPGs) in the MNNG+S-NaCl→CH(0.1%) group(Group 1) was significantly smaller than in the MNNG+S-NaCl group(Group 2)($p<0.02$). The incidences of forestomach neoplastic lesions(papillomas and squamous cell carcinomas) also showed a tendency for decrease with the CH treatment. The results thus indicate that CH exerts inhibitory effects on glandular stomach carcinogenesis in the rat, so that it may have potential as a chemopreventive agent for stomach cancer in man.

Key words: chemoprevention, *Chelidonium majus L.* herb extract(CH), *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), gastric carcinogenesis

INTRODUCTION

There is a remarkable geographical variation in the prevalence of stomach cancer around world, high incidences and mortality being observed in Asia, Central and South America, and Eastern Europe(1,2). Gastric cancer is still the most common cause of cancer mortality in Korea(3) and Japan(1). Data from epidemiologic and experimental studies suggest that the disease has an environmental etiology, with the diet as the most important factor(4-7). Excessive intake of highly salted food is, for example, closely associated with stomach cancer in man(8). It is important that chemopreventive agents active against development of gastric carcinomas be found for use in high risk populations.

Chelidonium majus L.(Papaveracea) is known to be a source of analgesic and antitumor agents with methanolic *Chelidonium majus L.* herb extracts(CH) being found to inhibit protein tyrosine kinase(PTK) activity, associated with cellular proliferation and neoplastic development, in human stomach cancer cell lines(SNU-1 or KATO-III)(9). The induction of gastric carcinomas with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine(MNNG) was first accomplished in rats in 1967(10). Since then, many strains of rats or mice have proved to be susceptible to the carcinogenic action of MNNG or *N*-methyl-*N*-nitrosourea(MNU), usually given in the drinking water over a period of weeks to months, and their use in experimental models has allowed a great deal of information to be obtained regarding the histogenesis and molecular characteristics of carcinogen-induced gastric cancers(11-18).

Medium-term rat gastric carcinogenesis models using pepsinogen isozyme 1 altered pyloric glands(PAPGs) as end-point lesions have proved suitable for the detection of chemopreventive and promoting effects of various chemicals(11,19,20). This approach is advantageous for medium-term screening of modifying agents

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rather than to carrying out the mere traditional two-year studies(19,21). Our protocol utilizes the potent enhancement by hypertonic saturated sodium chloride solution(S-NaCl), given simultaneously with 4-nitroquinoline-1-oxide(4NQO) or MNNG, of the yields of forestomach and glandular stomach tumors(6,7).

The present investigation was undertaken to examine the modifying effects of CH given during post-initiation stage on forestomach and glandular stomach carcinogenesis initiated by combined treatment with MNNG and S-NaCl in rats.

MATERIALS AND METHODS

Animals and chemicals

Six-week old, 64 male Wistar rats, were supplied by the National Institute of Safety Research, Korea. They were housed in polycarbonate cage with hard wood chips in an air-conditioned room(23±2°C, 55±10% R.H.) with a 12h light/dark cycle. Basal diets(Jeil Sugar Co., Seoul, Korea) and drinking water were available ad libitum. MNNG(CAS No. 70-24-7) was obtained from Fluka Chemie AG, Switzerland. The water extract of *Chelidonium majus L.*(Papaveracea) was prepared by immersing dried leaves and stems in hot water at 100°C for 60min and collecting the supernatant after high-speed centrifugation 3000rpm for 30min. Fresh preparations were made on a weekly basis and stored in a refrigerator. Concentration was equivalent to the final effective yields achieved with methanolic extraction (0.8g/100g herb), previously reported(9). Anti-rat-pepsinogen 1(Anti-rat-Pg) serum was a generous gift from Dr. Chie Furihata of the Department of Molecular Oncology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

Treatments

Group 1 rats were initially given MNNG(200mg/kg b.w.) by gavage at days 0 and 14 as well as S-NaCl (1ml per rat) every 3 days during weeks 0 to 3(6 times; on days 3, 7, 10, 14, 17, 21), and then placed on basal diet containing 0.1 or 0.2% CH for 16 weeks from week 4. Rats of Group 2 and 3 were treated with MNNG together with S-NaCl or saline(0.9% NaCl, 1ml per rat) respectively, timed as in Group 1 but without further treatment. All surviving rats were killed at week 20 and histopathologically investigated(Fig. 1). The body weights were measured weekly during the experiment. The organ weights were measured at the sacrifice.

Histological examination

Each forestomach was longitudinally cut into 6 to 9 strips, fixed in 10% neutral phosphate-buffered formalin, routinely processed for paraffin embedding, sectioned and stained with H&E(22). Diagnosis of preneoplastic and neoplastic lesions into forestomach (a) hyperplasia : mild, moderate or severe ; (b) papilloma(PAP) ; (c) squamous cell carcinoma(SCC) categories was made applying the criteria reported previously(23-26).

Immunohistochemical staining of Pg 1

The glandular stomach was fixed in sublimated for-

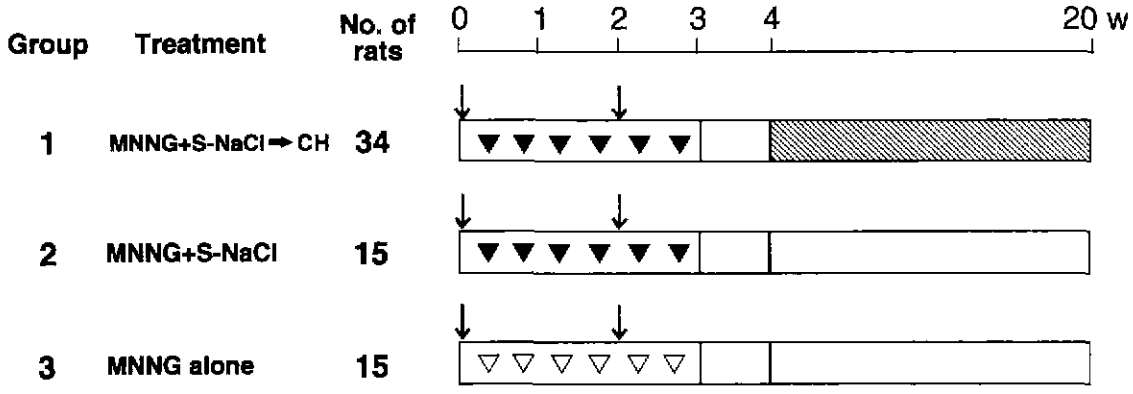

Fig. 1. Experimental protocol for the present medium-term rat stomach bioassay.
Group 1, MNNG+S-NaCl→CH; group 2, MNNG+2-NaCl; group 3, MNNG alone.
MNNG(↓), 200mg/kg body wt, i.g. twice for 2 weeks; S-NaCl(▼), Saturated sodium chloride solution(1ml/rat, i.g.); or Saline(▽), (1ml/rat, i.g.), 6 times during weeks 0 to 3; diagonal shading, *Chelidonium majus L.* herb herb extract (CH; 0.1 or 0.2% in the diet for 16 weeks); no shading, basal diet.

malin, cut into 6 strips and embedded in paraffin for subsequent immunohistochemical staining of Pg 1, as described earlier(11,19,20,27). The avidin-biotin-peroxidase(ABC) method was used to determine the localization of Pg 1 in the pyloric mucosa. Briefly endogenous peroxidase activity was blocked by treatment with methanolic hydrogen peroxide and sections were then sequentially treated with diluted normal goat serum, rabbit anti-rat Pg 1 IgG(1 : 20,000), biotin-labeled goat anti-rabbit IgG(1 : 400), and avidin-biotin-peroxidase complex(Vectastain Elite ABC kit, PK-6101, Vector Lab., Inc., CA, USA). Diaminobenzidine was used as a chromogen to demonstrate the sites of peroxidase binding and to aid orientation the sections were lightly counterstained with hematoxylin. As a negative control for the specificity of the anti-rat-Pg 1 antibody, it was replaced with normal rabbit serum.

Quantitative analysis

The numbers of pyloric glands demonstrating low or no of Pg 1 expression were quantitatively assessed by counting over 1,000 pyloric glands. Data were compared for statistical significance using the Dunnett's multiple comparison t-test for PAPGs, the Chi-square, and the cumulative Chi-square tests for tumor incidences in the forestomach.

RESULTS

Body and organ weights

The final body weight and food consumption rates were not significantly different between Groups 1(MNNG+S-NaCl→CH) and 2(MNNG+S-NaCl). The absolute and relative kidney weights in the animals receiving MNNG+S-NaCl→CH were significantly decreased as compared to the MNNG+S-NaCl(Group 2), respectively($p<0.01$, $p<0.05$)(Table 1).

Forestomach lesions

The incidences of hyperplasia and/or neoplastic lesions(PAP/SCC) of the forestomach did not show any significant intergroup differences. However, S-NaCl treatment was associated with a tendency for increase (Table 2).

PAPGs in pyloric mucosa

The average numbers of PAPGs in pyloric mucosa of the MNNG+S-NaCl→CH(0.1%) group were significantly decreased as compared to the MNNG+S-NaCl group case($p<0.02$)(Table 3). Values with MNNG +S-NaCl also were greater than for MNNG alone, but the difference was not significant(Table 3).

Table 1. Body and relative organ weights

Group	Treatment	No. of rats	Body weight(g)		Relative organ weight(g%)	
			Initial(0wk)	Final(20wk)	Liver	Kidney
1	MNNG+S-NaCl→CH 0.1%	17	171.6±19.2	437.1±27.6	2.53±0.15	0.70±0.07**
	MNNG+S-NaCl→CH 0.2%	17	181.6±18.2	433.4±27.9	2.53±0.19	0.72±0.08*
2	MNNG+S-NaCl	15	176.0±21.7	456.1±36.6	2.58±0.22	0.77±0.08
3	MNNG alone	15	174.7±15.8	459.2±39.2	2.52±0.23	0.79±0.07

Data are mean±SD values
S-NaCl: Saturated sodium chloride by gavage(1ml per rat) during weeks 0 to 3
CH: *Chelidonium majus L.* herb extract at doses of 0.1 or 0.2% in the diet
*$p<0.05$, **$p<0.01$ compared to Group 2

Table 2. Incidences of forestomach lesions

Group	Treatment	No. of rats	Normal	Lesions			
				Hyperplasia	PAP	SCC	PAP/SCC
1	MNNG+S-NaCl→CH 0.1%	17	0	12(71)	4(23)	1(6)	5(30)
	MNNG+S-NaCl→CH 0.2%	17	0	12(71)	2(11)	3(18)	5(30)
2	MNNG+S-NaCl	15	1(7)	7(46)	6(40)	1(7)	7(47)
3	MNNG alone	15	2(13)	10(67)	3(20)	3(20)	3(20)

Numbers in parentheses are percentages of rats with the respective lesion type
PAP: papillomas; SCC: squamous cell carcinomas
S-NaCl: Saturated sodium chloride by gavage(1ml per rat) during weeks 0 to 3
CH: *Chelidonium majus L.* herb extract at doses of 0.1 or 0.2% in the diet

Table 3. Numbers of PAPG in Wistar rats

Group	Treatment	No. of rats	Incidence of PAPGs(%)	No. of PAPGs
1	MNNG+S-NaCl→CH 0.1%	15	100	2.39±0.85*
	MNNG+S-NaCl→CH 0.2%	16	100	3.25±1.49
2	MNNG+S-NaCl	13	100	4.13±2.21
3	MNNG alone	14	100	2.80±1.49

Data are mean±SD values
PAPGs: Pepsinogen 1 altered pyloric glands
No. of PAPGs: Numbers of PAPGs/100 pyloric glands
S-NaCl: Saturated sodium chloride by gavage(1ml per rat) during weeks 0 to 3
CH: *Chelidonium majus L.* herb extract at doses of 0.1 or 0.2% in the diet
*$p<0.02$ compared to Group 2

DISCUSSION

The results of the present study clearly demonstrated that treatment with 0.1% CH can exert a significant inhibitory effect on rat glandular stomach carcinogenesis after treatment with MNNG and S-NaCl, although this was not dose-dependent and in fed only a tendency for decrease was observed with the higher dose group.

With regard to the present initiation protocol it has been well demonstrated that NaCl promotes the development of epithelial tumors in the rat stomach(21, 28). Although the underlying mechanisms have not been clearly elucidated, several possibilities have been suggested. One is that a high concentration of NaCl causes injury to the mucous membrane, possibly due to a permeability imbalance, followed by reactive regenerative processes(6). Enhanced cellular proliferation as indicated by ODC activity is closely associated with tumor promotion(13,21,22,29,30). Another possibility concerns lipid peroxidation. Takahashi et al(28) demonstrated treatment with hypertonic NaCl to increase lipid peroxidation levels dose-dependently in both the gastric mucosa and urine of rats. This enhancing effect of hypertonic NaCl is very useful for medium-term assay systems designed to screen agents for their potential to modify glandular carcinogenesis, because of rapid induction of PAPGs(31). This has the two merits of allowing a shortening of the experimental duration and a lower requirement for test compounds.

Chelidonium majus L. is one of the Papaveraceae family of herbs which are found distributed throughout in East Asia(Northeastern China, Korea, and Southern Japan)(32,33). It contains species of alkaloids such as chelidonine(34), which have recently attracted attention for their pharmacologic actions as analgesics and also for antitumor properties in the patients with biliary and liver tumors in Austria, Russia, and Eastern Europe (34). The current results indicate that CH may help prevent stomach cancer in populations with a high salt intake.

The possession of potent anti-inflammatory infleunce effect(34) suggests that its preventive action could be due to effects on the immune system, oxygen radicals, and tissue turnover. It should be mentioned in this content that MNNG and hypertonic NaCl are associated with increased lipid peroxidation in the stomach mucosa (6,28,30). Further experimentation is required to determine the mechanisms of CH.

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