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Dose-dependent Promotion Effects of Potassium Chloride on Glandular Stomach Carcinogenesis in Rats after Initiation with *N*-Methyl-*N'*-Nitro-*N*-Nitrosoguanidine and the Synergistic Influence with Sodium Chloride[1]

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ABSTRACT

The modifying effects of potassium chloride (KCl) ingestion on glandular stomach carcinogenesis were investigated in male Wistar rats induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and were compared with those of sodium chloride (NaCl). A total of 120 male 6-week-old Wistar rats were divided into six groups, each consisting of 20 animals. After initiation of treatment with a MNNG solution (100 parts/million) as their drinking water for 10 weeks, rats were fed a diet supplemented with 5% NaCl, 2.5% NaCl, 2.5% NaCl plus 2.5% KCl, 5% KCl, 2.5% KCl, or a basal diet alone for the following 62 weeks. Under this experimental condition, there were no statistical differences in the final body weights between groups. The incidences of adenocarcinomas in the glandular stomachs were significantly higher in the 5% NaCl and combined 2.5% NaCl-plus-2.5% KCl groups ($P < 0.05$ and 0.01) than in the MNNG alone (control) group. The incidences of atypical or precancerous hyperplasias in the glandular stomachs were increased significantly by the 5% NaCl, 2.5% NaCl-plus-2.5% KCl, and 5% KCl treatments ($P < 0.05$ or 0.01). The multiplicities of adenocarcinomas were significantly greater in the 5% NaCl, 2.5% NaCl, and combined NaCl-plus-KCl groups ($P < 0.05$ or 0.01) compared with the control value. The multiplicity data for atypical hyperplasias were most striking; namely, their multiplicities were increased significantly by the treatments of NaCl or KCl ($P < 0.01$) in a clear dose-dependent manner and enhanced synergistically by the combined treatment of NaCl and KCl. Because the concentrations of KCl used in this study were about 1.3 times lower than those of NaCl on a molar basis, although the doses of each chemical were exactly the same on a weight-percent basis, it is suggested that the enhancing effects of KCl might not be much different from those of NaCl.

The results in the present study thus indicate that, similarly to NaCl, KCl ingestion exerts dose-dependent promoting effects and a synergistic influence with NaCl when given during the postinitiation phase of two-stage glandular stomach carcinogenesis in rats.

INTRODUCTION

Epidemiologically, it has been reported that consumption of salty foods is associated closely with the relative risk for gastric cancer (1). In fact, it has been well documented in animal studies that NaCl has a gastric tumor-promoting activity as well as a coinitiating effect at high doses (2, 3). Recently, we have shown clear dose-dependent promotion effects of NaCl on glandular stomach carcinogenesis in rats initiated with MNNG[3] (4). Meanwhile, KCl has been used as a substitute food additive for NaCl in patients who need restriction of NaCl. Previously, KCl has been reported to exert an inhibitory effect on gastric tumor development in hypertensive rats caused by a combined treatment with deoxycorticosterone (DOCA) and NaCl (5). On the other hand, Furihata *et al.* (6) have suggested that high concentrations of KCl may exert a possible gastric tumor-promoting activity in a short-term bioassay using rats, as do high concentrations of NaCl. Thus, equivocal effects of KCl have been shown on glandular stomach carcinogenesis in rats. Nevertheless, the modifying effects of KCl on a simple two-stage gastric carcinogenesis model in normal rats have not been elucidated.

Because MNNG was shown to be an effective carcinogen, inducing gastric adenocarcinoma in rats (7), a two-stage, initiation-promotion carcinogenesis model for the rat glandular stomach has been established in our laboratory using administration of MNNG (8–12). In the present study, the modifying effects of KCl during the postinitiation stage were investigated with this two-stage rat glandular stomach carcinogenesis model.

MATERIALS AND METHODS

Chemicals and Animals. KCl (purity, >99.9%) and NaCl (purity, >99.5%) were purchased from Wako Pure Chemicals, Inc. (Osaka, Japan). MNNG was a commercially available preparation from Aldrich Chemical Co. (Milwaukee, WI). Male 6-week-old Wistar rats (Japan SLC, Inc., Shizuoka, Japan) were housed five animals per wire cage and maintained under standard laboratory conditions (room temperature, 23 ± 2°C; relative humidity, 60 ± 5%; and a 12-h/12-h light/dark cycle). They were fed a basal pellet diet, Oriental MF (Oriental Yeast Co., Ltd., Tokyo, Japan), supplemented with or without test chemicals.

Bioassay for Assessing Modifying Effects of Salts on Glandular Stomach Carcinogenesis. A total of 120 rats were divided into six groups, each consisting of 20 animals. Each group was given a 100-parts/million MNNG solution for 10 weeks as an initiation treatment. Rats were then fed a diet supplemented with 5% NaCl (group 1), 2.5% NaCl (group 2), 2.5% NaCl plus 2.5% KCl (group 3), 5% KCl (group 4), or 2.5% KCl (group 5), or a basal diet alone (group 6) for the following 62 weeks. The experimental animals were observed macroscopically for symptoms weekly and weighed once a month. Necropsy was performed on all animals that were found dead or killed on becoming moribund. At the end of the 72nd experimental week, all surviving animals were killed and necropsied. At necropsy, the stomach and other major organs were excised and subjected to careful macroscopic examination. The stomach was opened along the greater curvature, put on a filter paper, and then fixed in 10% buffered formalin. The fixed stomachs were cut into longitudinal strips, 3 mm wide, for examination of the entire gastric mucosa. After processing for histology by routine methods, sections were stained with H & E.

Statistical Analysis. The tumor incidences were analyzed by the Fisher's exact probability test or the χ^2 test. The lesion multiplicities and the body and organ weights were examined by the Student's t test.

RESULTS

Mortality and Body and Organ Weights. Only three rats in group 3 were found dead during the early stages of the experiment

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[3] The abbreviations used are: MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; DOCA, deoxycorticosterone; H & E, hematoxylin and eosin; ODC, ornithine decarboxylase; RDS, replicative DNA synthesis.

(one at week 16 and two at week 27). These three animals, in which no gastric lesions were noted at necropsy, were excluded from the effective number for assessing the glandular stomach carcinogenesis, because the first proliferative lesions in the glandular stomach were observed in a group 4 rat, which was sacrificed moribundly at week 62. Rats in group 1 showed a tendency to be lower in body weight gain than other groups during the experiment; however, no significant differences in the final body weights were noted between groups. Regarding organ weights, except for increases in heart and kidney weights in group 1 and kidney weights in group 3, no significant differences were found between groups.

Effects of KCl and NaCl on Gastric Tumor Development. Cancerous and precancerous lesions in the glandular stomach were diagnosed as adenocarcinomas and atypical hyperplasias as described previously (3, 12). Briefly, adenocarcinomas were judged as invasive growths of atypical tubules (Fig. 1, *A* and *B*). Atypical hyperplasias were defined as noninvasive growths of atypical tubules histologically similar to adenocarcinomas but lacking complete expansion to the whole layer of the lamina propria or expansive growths of atypical tubules with moderate to severe dysplasia but involving the whole layer of the lamina propria (Fig. 2, *A* and *B*). Simple and nonatypical hyperplastic lesions such as pyloric metaplasia were discriminated strictly from atypical hyperplasias. As shown in Table 1, the incidences of adenocarcinomas in rat glandular stomachs of groups 1 (45%) and 3 (65%) were significantly higher ($P < 0.05$ and 0.01) than the group 6 value (15%). Especially in pyloric adenocarcinoma development, 2.5% NaCl plus 2.5% KCl, but neither compound alone, exerted a promoting effect. Thus, the combined treatment of NaCl and KCl enhanced the carcinogenicity synergistically. The incidences of atypical hyperplasias in rat stomachs of groups 1 (90%), 3 (94%), and 4 (100%) were significantly higher ($P < 0.05$ or 0.01) than the group 6 value (60%). The incidences of atypical hyperplasias, thus, were significantly increased by the high dose of KCl as they were by the same concentration of NaCl. Although atypical hyperplasias were located preferably in the fundic mucosa regardless of the treatments of NaCl or KCl, the incidences of atypical hyperplasias in the pyloric mucosa of groups 3 (71%) and 4 (80%) were also increased significantly ($P < 0.05$ and 0.01) compared with the group 6 value (35%). Similarly, as shown in Table 2, the mean numbers per animal, *i.e.*, multiplicities of adenocarcinomas, in the glandular stomachs of groups 1 (0.55), 2 (0.55), and 3 (0.82) were significantly greater ($P < 0.05$ or 0.01) than in group 6 (0.15). The multiplicities of atypical hyperplasias in the glandular stomachs of groups 1 (4.05), 2 (2.70), 3 (4.12), 4 (4.50), and 5 (2.90) were significantly higher ($P < 0.01$) than the group 6 value (1.05). The multiplicity data, especially for fundic atypical hyperplasias, demonstrated clearly the dose-dependent promotion effects of KCl as well as NaCl, and their synergistic influence was most striking in the multiplicity data for pyloric adenocarcinomas.

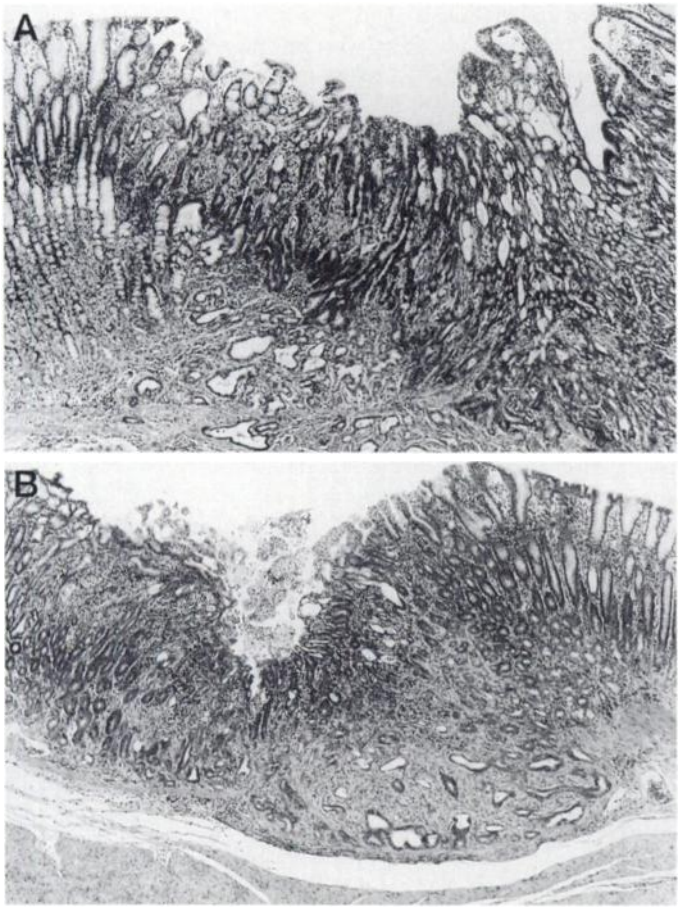

Fig. 1. *A*, photomicrograph showing a fundic adenocarcinoma in a rat of group 1 (H & E; ×38). *B*, photomicrograph showing a pyloric adenocarcinoma in a rat of group 3 (H & E; ×38).

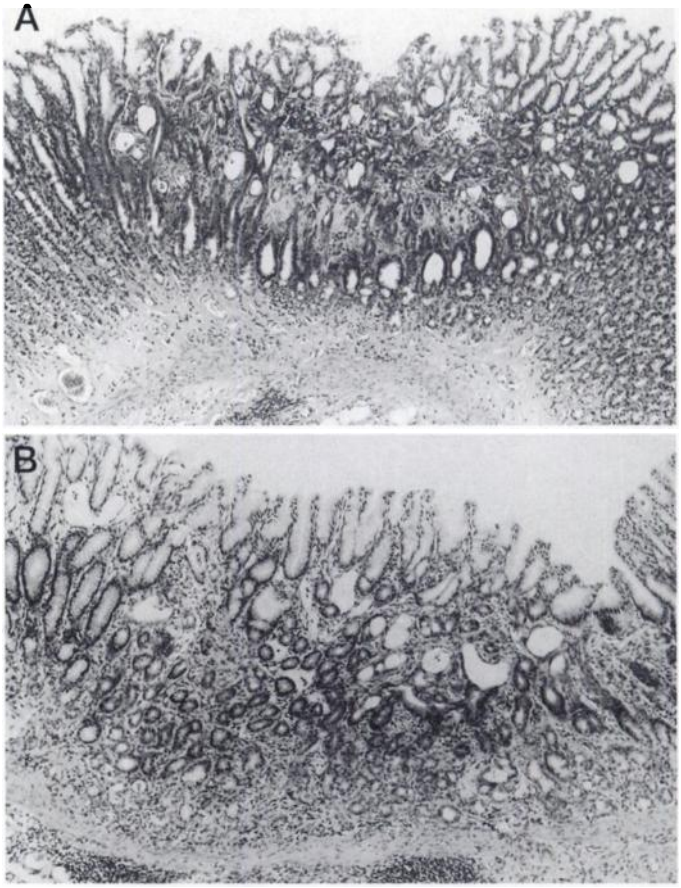

Fig. 2. *A*, photomicrograph showing a fundic atypical hyperplasia in a rat of group 1 (H & E; ×59). *B*, photomicrograph showing a pyloric atypical hyperplasia in a rat of group 3 (H & E; ×59).

Histopathology of Organs Other Than the Stomach. Tubule regeneration and degeneration were evident in the kidneys of groups 1 and 3, whereas these findings were mild or absent in the other groups. No particular findings were observed in the lung, liver, and heart in each group, although the heart weight in group 1 was higher than in group 6. Thus, renal toxicity was suggested only in rats treated with 5% NaCl or 2.5% NaCl plus 2.5% KCl.

Table 1 *Incidence of gastric proliferative lesions in rats treated with MNNG*

Group[a]	Effective no. of rats	No. of rats with gastric lesions (%)					
		Fundus		Pylorus		Total	
		ADC[b]	AH	ADC	AH	ADC	AH
1. 5% NaCl	20	4 (20.0)[c]	17 (85.0)[d]	7 (35.0)	11 (55.0)	9 (45.0)[c]	18 (90.0)[c]
2. 2.5% NaCl	20	5 (25.0)[c]	16 (80.0)[c]	5 (25.0)	11 (55.0)	8 (40.0)	17 (85.0)
3. 2.5% NaCl + 2.5% KCl	17	4 (23.5)[c]	15 (88.2)[d]	9 (52.9)[c]	12 (70.6)[c]	11 (64.7)[d]	16 (94.1)[c]
4. 5% KCl	20	0	19 (95.0)[d]	5 (25.0)	16 (80.0)[d]	5 (25.0)	20 (100)[d]
5. 2.5% KCl	20	0	15 (75.0)	4 (20.0)	9 (45.0)	4 (20.0)	17 (85.0)
6. Basal diet	20	0	9 (45.0)	3 (15.0)	7 (35.0)	3 (15.0)	12 (60.0)

[a] After initiation with MNNG.
[b] ADC, adenocarcinoma; AH, atypical hyperplasia.
[c] Significantly different from group 6 ($P < 0.05$).
[d] Significantly different from group 6 ($P < 0.01$).

DISCUSSION

The results in the present study indicate clearly that KCl exerts dose-dependent promotion effects and that the effects are synergistic with NaCl ingestion when given during the postinitiation phase of two-stage glandular stomach carcinogenesis in rats. In clear contrast to our results, the opposite, *i.e.*, inhibitory effects of KCl on glandular stomach carcinogenesis, have been reported in hypertensive rats induced by a combination treatment of DOCA and NaCl (5). In the previous study by Tatsuta *et al.* (5), administration of DOCA and NaCl increased the norepinephrine concentration in the gastric wall and the cell proliferation of the gastric mucosa; however, KCl supplementation decreased both levels in DOCA- and NaCl-hypertensive rats. Based on these results, they have suggested that the sympathetic nervous system plays an important role in glandular stomach carcinogenesis, probably associated with cell proliferation of mucosal epithelia in the pylorus (5).

Meanwhile, Furihata *et al.* (6) have shown that some potassium salts may exert tumor-promoting activities in the glandular stomach mucosa of rats, as do sodium salts such as NaCl and Na_2CO_3. For example, KCl, K_2SO_3, and $K_2S_2O_5$ increased levels of ODC activity as well as RDS in the pyloric mucosa of the stomach, both of which have been proposed as parameters for assessing tumor-promoting activity (13). An increase in RDS provides direct evidence of enhanced cell replication (13). The availability of ODC, a rate-limiting enzyme in the biosynthesis of polyamines, is suggested to be essential for growing or regenerating tissues (14), because high levels of ODC have been detected in proliferating tissues, including malignant tumors (15–17). It has also been indicated that exposure of organ-specific carcinogens to rodents gives rise to a significant increase of ODC activity in target organs (18). Thus, the gastric carcinogens such as MNNG and the gastric tumor promoters such as NaCl proved to accelerate the cell-proliferative activity in the stomach mucosa of rats, judging from ODC as well as RDS levels (13). We have also demonstrated that, similarly to NaCl, $K_2S_2O_5$ enhances two-stage gastric carcinogenesis in rat stomach mucosa initiated with MNNG (8). Therefore, the results in the present study are in good agreement with these data. The mechanism on increased cell proliferation in the gastric mucosa by KCl remains to be elucidated; however, similar effects of NaCl, such as direct irritation to the gastric mucosa, destruction of the mucous barrier, and indirect influence through lipid peroxidation, might be involved (4, 19).

The different modulating effects of KCl on glandular stomach carcinogenesis in rats between the present results and the previous data by Tatsuta *et al.* (5) remain unknown. However, except for induction of hypertension by DOCA, the dose levels of KCl might be at least partly attributable to these conflicting effects, because a similar but opposite phenomenon has been demonstrated in the effects of $CaCl_2$ (6, 20). Namely, it has been elucidated in short-term bioassays that high doses (around 700 mM) of $CaCl_2$ inhibit effectively the stimulation of replicative DNA synthesis in rat stomach mucosa caused by NaCl administration, whereas low doses (100–400 mM) of this calcium salt exert ODC induction and RDS stimulation in the stomach mucosa of rats (6, 20). In fact, we have shown that $CaCl_2$ at relatively high doses (1.0–0.2% in drinking water) inhibit two-stage glandular stomach carcinogenesis in rats significantly when given during the postinitiation stage (10). In addition to the doses used, the approaches of administration may be related to the different influence, because KCl was fed at doses of 5.0 and 2.5% in the present study, whereas it was given in drinking water at a dose of 1.0% in the experiment by Tatsuta *et al.* (5).

The neutral salt KCl has also been demonstrated to exert a weak promoting effect on urinary bladder carcinogenesis in rats, although the enhancing activity of the alkalizing salt $KHCO_3$ was much stronger (21). Lina *et al.* (21) have shown that KCl does not affect the pH of the urine, although the increase in urinary K^+ concentrations was comparable to those obtained with an equimolar amount of $KHCO_3$. Thus, it has been indicated that the simultaneous occurrence of increased urinary potassium ion concentration and elevated urinary pH

Table 2 *Multiplicity of gastric proliferative lesions*

Group[a]	No. of gastric lesions per rat (mean ± SD)					
	Fundus		Pylorus		Total	
	ADC[b]	AH	ADC	AH	ADC	AH
1. 5% NaCl	0.20 ± 0.41	3.30 ± 2.92[c]	0.35 ± 0.49	0.75 ± 0.79	0.55 ± 0.69[d]	4.05 ± 3.12[c]
2. 2.5% NaCl	0.25 ± 0.44	1.90 ± 1.45[c]	0.30 ± 0.57	0.80 ± 0.83	0.55 ± 0.76[d]	2.70 ± 1.75[c]
3. 2.5% NaCl + 2.5% KCl	0.24 ± 0.44	2.65 ± 1.77[c]	0.59 ± 0.62[d]	1.47 ± 1.42[c]	0.82 ± 0.73[c]	4.12 ± 2.67[c]
4. 5% KCl	0	3.05 ± 1.67[c]	0.25 ± 0.44	1.45 ± 1.32[c]	0.25 ± 0.44	4.50 ± 1.76[c]
5. 2.5% KCl	0	2.25 ± 2.15[c]	0.20 ± 0.41	0.65 ± 0.88	0.20 ± 0.41	2.90 ± 2.45[c]
6. Basal diet	0	0.70 ± 0.86	0.15 ± 0.37	0.35 ± 0.49	0.15 ± 0.37	1.05 ± 1.05

[a] After initiation with MNNG.
[b] ADC, adenocarcinoma; AH, atypical hyperplasia.
[c] Significantly different from group 6 ($P < 0.01$).
[d] Significantly different from group 6 ($P < 0.05$).

is crucial for urinary bladder tumor development (22, 23). In this context, the increased concentration of K^+ itself in the stomach might not be essential for promoting gastric tumor development.

In the comparison of gastric tumor-promoting activity between NaCl and KCl, KCl seemed less effective than NaCl, because the doses of KCl applied in the present study resulted consistently in less-remarkable enhancing effects compared with the same doses of NaCl on a weight-percent basis. However, the fact that the molecular weight of KCl (M_r 74,560) is approximately 1.3 times greater than that of NaCl (M_r 58,440) suggests that the promoting effects of KCl might be almost similar to those of NaCl on a molar basis, judging from the incidence and multiplicity data for gastric adenocarcinomas and atypical hyperplasias. This suggestion may be in line with the different severity of renal toxicity by these salts. It should be kept in mind that, besides NaCl, various salts of food additives, including KCl, could exert gastric tumor-promoting activity synergistically.

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