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Gastric Lesions in Rats Fed Salted Food Materials Commonly Eaten by Japanese

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Abstract

A high intake of salted food is thought to be related to the high incidence of stomach cancer in Japan. In the present study, female F344 rats were divided into four groups. They were fed a nutritionally deficient purified diet (Group 1) and standard purified diet (Group 3) for 113 weeks and the same diets supplemented with salted cuttlefish guts, broiled, salted, dried sardines, pickled radish, and soy sauce (Groups 2 and 4). The incidence of papillomas and ulcers of the forestomach was highest in Group 4, which was given the standard diet supplemented with the salty food materials ($p < 0.05$). These results suggest the importance of salted food as a suspicious causal factor in human stomach cancer in Japan.

(Nutr Cancer 14, 127-132, 1990)

Introduction

The mortality and incidence of stomach cancer in Japan are still the highest in the world, although a declining trend has been shown for the last 25 years (1). From many epidemiological studies, salty foods, especially broiled, salted, dried fish and salted, pickled vegetables, have been reported to be high risk factors for stomach cancer in Japan (1-5). It has already been reported that broiled sardines contain mutagenic and carcinogenic heterocyclic amines that induce tumors of the liver, forestomach, and intestine in mice and rats (5-9). A cohort study of Japanese people also indicated that frequent consumption of broiled fish was significantly associated with mortality from cancer of all types, including stomach cancer (10). The highest mortality of stomach cancer in Japan has been observed in Toyama, Niigata, Yamagata, and Akita prefecture, which face the Japan Sea and are snowy during the winter. People in these areas ingest a great deal of rice and most frequently ingest broiled, salted sardines, pickled radish, and various kinds of salty foods cooked with soy sauce, especially in prewar times. Furthermore, their diet was nutritionally deficient,

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consisting of insufficient dairy products and also a low intake of protein, fruits, and vegetables.

In the present study, rats were fed a nutritionally deficient purified diet and standard purified diet. The same diets were supplemented with the foods thought to be risk factors to investigate their importance as causative factors in stomach cancer and to determine the influence of nutritional conditions on the development of stomach cancer.

Materials and Methods

Animals and Housing Conditions

Six-week-old female F344 rats obtained from Charles River (Kanagawa, Japan) were used for the experiment. They were housed one to a hanging plastic cage, with sterilized soft wood chips as bedding, in a barrier-sustained animal room at 23 ± 1°C, 55 ± 5% humidity, with a 12:12-hour light-dark cycle.

Diet and Experimental Procedure

A total of 69 rats were randomly divided into four groups and treated as follows. Group 1 (18 rats) was given a nutritionally deficient (low-protein, low-lipid, low-vitamin, low-mineral) purified, powdered diet (Table 1). Group 2 (17 rats) was given the same diet as Group 1, but the diet was supplemented with powdered, salted, cuttlefish guts, broiled, salted, and dried sardines, pickled radish, and soy sauce (Table 1). Group 3 (18 rats) was given a standard nutritional, purified, powdered diet (Table 2). Group 4 (16 rats) was given the same standard diet as Group 3, but the diet was supplemented with the same foods as for Group 2 (Table 2). The total amount of food contained in the diet of Groups 2 and 4 was 4.6%.

The salt content in the diet was kept at less than 3%. Casein, cellulose powder, sucrose, α-cornstarch, and Harper mineral mixture were purchased from Oriental Yeast (Tokyo, Japan). Choline chloride and most chemicals used for the preparation of the vitamin mixture were obtained from Wako Pure Chemical Industries (Osaka, Japan). Salted fish guts were usually kept at −80°C in a freezer. When necessary, the frozen guts were sliced, made into a viscous liquid state using a mixer, freeze-dried, and powdered. Pickled radish was also sliced, squashed with a mixer, freeze-dried, and powdered. Broiled, salted, dried sardines were also powdered. Soy sauce was directly mixed with the diet. The diet used in each group

Table 1. Nutritionally Deficient Purified Diet Given to Group 1

	Grams	Percent
Casein	204.0[a]	8.0
Corn oil	76.5	3.0
AIN-76 vitamin mixture (Ref.11)	12.7	0.5
Harper mineral mixture (Ref. 12)	76.5[a]	3.0
Cellulose powder (fiber)	51.0	2.0
50% Choline chloride solution	10.2	0.4
Sucrose: α-cornstarch (1:2)	2,550.0	83.1[b]

a: Casein and mineral mixture were reduced to 127 g (5%) and 51 g (2%) 8 wks after feeding was begun.

b: Diet given to Group 2 was the same as Group 1 with addition of powdered, salted, cuttlefish guts (15.3 g), broiled, salted, and dried sardines (40.8 g), pickled radish (28.0 g), and soy sauce (33.0 ml). Amount of sucrose: α-cornstarch (1:2) was 78.5% in Group 2 diet in beginning of experiment, whereas it was 83.1% in Group 1 diet. Ratio of additional foods, such as salted cuttlefish guts, in Groups 2 or 4 was 4.6% throughout experiment.

Table 2. Standard Nutritional Purified Diet Given to Group 3		
	Grams	Percent
Casein	510.0	20.0
Corn oil	127.5	5.0
AIN-76 vitamin mixture	25.5	1.0
Harper mineral mixture	127.5	5.0
Cellulose powder (fiber)	51.0	2.0
50% Choline chloride solution	10.2	0.4
Sucrose: α-cornstarch (1:2)	2,550.0	66.6[a]

a: Diet given to Group 4 was prepared in same manner with addition of same food materials used for Group 2 diet. Thus, sucrose: α-cornstarch (1:2) was 62.0% in Group 4 diet.

was prepared at intervals of about two weeks and kept at −20°C in a freezer. Food and water were given ad libitum. Body weight was measured once a week until the end of the experiment. The experiment was terminated 113 weeks after the start of feeding. All animals were autopsied, whether killed because of their moribund condition or at the termination of the experiment. Tissues were fixed in 10% neutralized formalin, sectioned, and stained with hematoxylin and eosin.

Statistical Analysis

The difference in the incidence of main stomach lesions in rats between the corresponding two groups (i.e., Group 1 vs. 2 or 3 and Group 2 or 3 vs. 4) was statistically tested using the chi-square test. If the number of animals was less than five, Yates' revise was adopted. The comparison of the mean body weight of the rats between the corresponding two groups was performed using Welch's *t* test.

Results

Body Weight and Survival

The changes in body weight of the rats in the four groups are shown in Figure 1. The mean body weight of the rats in Group 1 that received a nutritionally deficient diet was always less than that of the rats in Groups 2 and 3 ($p < 0.01$).

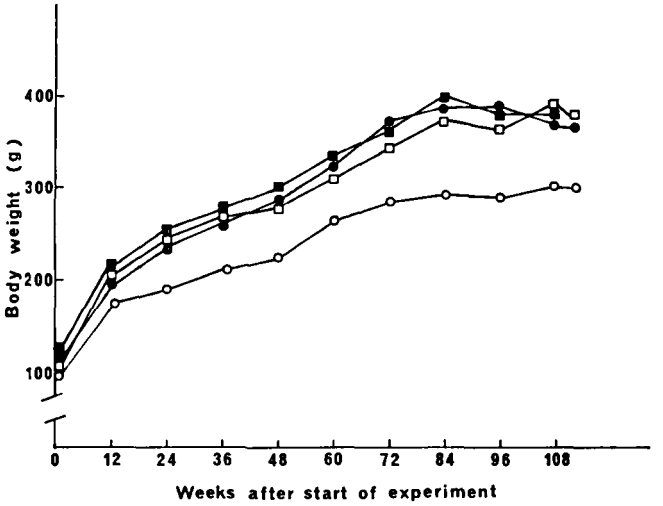

Figure 1. Changes in average body weight of rats in each group. *Open circles,* Group 1; *closed circles,* Group 2; *open squares,* Group 3; *closed squares,* Group 4.

More than 90% of the rats in each group survived beyond 74 weeks after the start of experiment (Figure 2). Although all the rats in Group 4 survived until 83 weeks, they subsequently began to die rapidly, and only one rat survived until the termination of the experiment. The survival rate of rats at the termination of the experiment was highest (65%) in Group 2 and lowest (6%) in Group 4. A significant difference was observed between these two groups ($p < 0.01$). In Groups 1 and 3, it was 56% and 33%, respectively.

Stomach

Hyperplasia of the forestomach was found in all of the groups. The incidence of the hyperplasia varied, depending on the group, from about 40% (Groups 2 and 3) to 60% (Groups 1 and 4) (Table 3). However, there was no significant difference between corresponding groups (Group 1 vs. 2 or 3) (Group 2 or 3 vs. 4). The incidence of papillomas of the forestomach was 17%, 24%, 5.6%, and 50% in Groups 1, 2, 3, and 4, respectively (Table 3). There was a significant difference in the incidence of papillomas between Group 3 (1/18) and Group 4 (8/16) ($p < 0.05$). Thus, it was shown that the addition of food materials significantly increased the incidence of papillomas of the forestomach in rats fed a standard purified diet.

However, this was not observed in the case of rats fed a nutritionally deficient diet (Group 1 vs. 2). A high incidence of ulcerations in the forestomach was observed, especially in Group 4 (7/16, 44%), and ulcus penetrans was also observed in two out of seven rats. All these stomach ulcers invariably developed near the limiting ridge in the anterior wall of the forestomach, and the adhesion of the omentum to the ulcerated stomach wall was observed in the case of ulcus penetrans. No stomach ulcers were observed in Group 1, whereas one rat was affected in Groups 2 and 3. A statistical analysis concerning the development of stomach ulcers proved a significant difference between the corresponding two groups, Group 4 and 2 and Group 4 and 3 ($p < 0.05$); that is, the incidence of the stomach ulcer was remarkably high in Group 4. On the other hand, ulcerations were not observed in the glandular stomach, although erosions were infrequently encountered.

Discussion

The mortality from stomach cancer in Japan is the highest in the world. In the present study, rats were fed a nutritionally deficient purified diet and standard nutritional purified diet supplemented or not supplemented with special foods that have been most frequently used in high mortality areas of stomach cancer in Japan and regarded as suspicious causal agents in stomach cancer (1–5). The incidence of papillomas of the forestomach in the

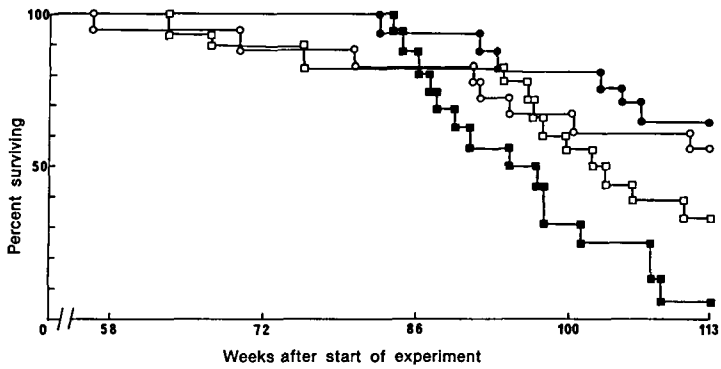

Figure 2. Percent survival of rats in each group. *Symbols* are as for Fig. 1.

Table 3. Gastric Lesions in Rats of Each Group

Group	No. of Rats Used	No. of Rats With Lesions[a]		
		Forestomach hyperplasia	Forestomach papilloma	Forestomach ulcer
1	18	11 (61)	3 (17.0)	0
2	17	7 (41)	4 (24.0)	1 (5.9)
3	18	7 (39)	1 (5.6)	1 (5.6)
4	16	10 (63)	8 (50.0)	7 (44.0)

a: Nos. in parentheses indicate percent.

present study was 5.6–50.0%. In particular, rats in Group 4 showed the highest incidence, and a significant difference was observed compared with Group 3 ($p < 0.05$).

Ulcerations in the forestomach were also observed most frequently in Group 4, and there was a significant difference compared with Groups 2 and 3 ($p < 0.05$). The significant high incidence of both papillomas and ulcerations in Group 4 suggested that these lesions were induced by salty foods supplemented to the standard diet. Salty foods used in the present study were salted cuttlefish guts, broiled, salted, dried sardines, pickled radish, and soy sauce. The mutagenicity of salted cuttlefish guts was reported by Kamiyama and Michioka (13). It has also been reported that carcinogenic heterocyclic amines are contained in broiled sardines (5–9). Furthermore, a higher intake of sodium chloride and salted pickles has been shown to be related to the higher incidence of stomach cancer by epidemiological and experimental studies (1–5,14). The presence of a precursor of mutagenic nitroso compounds in soy sauce and the mutagenicity of soy sauce treated with nitrite were also reported (15,16). These mutagenic and carcinogenic substances and sodium chloride contained in salty foods are inferred to be causative factors that induced ulcers from erosions in mucous membrane and papillomas from hyperplasia.

It is also probable that sodium chloride acted as a promoter to mutagenic and carcinogenic substances in induction of papillomas, although the concentration in the diet was relatively low. The salt content in the diet in the present experiment was kept at less than 3% because the main purpose of this study was to test the influence of the mutagenic or related food substance. It may be probable that stomach cancer was not induced because the salt content was relatively low in this experiment.

However, it is also conceivable that hyperplastic lesions or papillomas progress to cancer under the different regimen with the same diet as in Group 4. Furthermore, it can be also assumed that cancer may be induced from the regenerative epithelium of ulceration in the forestomach under a certain condition. Sugimura and co-workers (17) reported that *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) administration at a lower concentration and for a shorter period was more favorable for the production of adenocarcinomas, specifically in the glandular stomach, than at a higher concentration or for a longer period. The authors also reported that papillomas of the forestomach were much frequently observed than were tumors of the glandular stomach when rats received MNNG in a higher concentration continuously for a longer period. The majority of the animals bearing ulcers in Group 4 were emaciated and killed because of moribund condition. Thus, it can be assumed that carcinoma may be induced in the forestomach or glandular stomach by administration of the standard nutritional diet supplemented with lower concentration of suspicious food materials and for a shorter period.

There are two possible reasons for the lower incidence of ulcer and papilloma in nutritionally deficient rats fed salty food. 1. The content of sodium chloride in diet of each group was Group 1, 0.55%; Group 2, 1.35%; Group 3, 1.23%; and Group 4, 2.25%. That

is, the content in Group 2 was lower compared with Group 4. 2. It was reported that dietary protein deficiency decreases the microsomal protein and cytochrome P_{450} content of the liver; parallel with the degree of protein deprivation, activation of dimethylnitrosamine to a mutagen is decreased and inactivation of MNNG mutagenicity is reduced (18). These observations may provide another basis for explaining the changes in carcinogenic effects resulting from protein and other dietary deficiencies.

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