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Suppressive effect of irsogladine maleate on *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG)-initiated and glyoxal-promoted gastric carcinogenesis in rats

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Abstract

The modifying effect of irsogladine maleate (IRG) on *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG)-initiated and glyoxal-promoted gastric carcinogenesis was examined in male Wister rats. Six-week-old rats were divided into ten groups. Groups 1 through 6 were given MNNG (100 mg/l in drinking water) for 25 weeks from the start of the experiment, whereas groups 7 through 10 received distilled water in the initiation phase as the vehicle treatment. Groups 1 and 8 were kept on the basal diet and distilled water throughout the experiment (55 weeks). Groups 2–8 were given 0.5% glyoxal in the drinking water for 30 weeks from 26th week of the experiment. Group 3 was fed the diet mixed with 100 ppm IRG for 25 weeks from the start of experiment. Groups 4 and 8 were fed the diet mixed with 100 ppm IRG for 30 weeks from 26th week of experiment. Groups 5 and 9 or 6 were given 100 or 25 ppm IRG containing diet, respectively throughout the experiment. Group 10 was given the basal diet and distilled water as the vehicle treated control. Tumors of upper digestive tracts (stomach and duodenum) were developed in groups: 1 (12/17 rats, 71%), 2 (11/12 rats, 92%), 3 (9/16 rats, 56%), 4 (5/12 rats, 42%), 5 (6/15 rats, 40%) and 6 (7/12 rats, 58%). High dose of IRG in initiation and/or promotion phase significantly reduced the incidence of tumors of the upper digestive tracts. The average numbers of the digestive tracts neoplasms in groups 3, 5 and 6 given glyoxal and IRG were less than those in group 2 which received only glyoxal. These results suggest that IRG could be a preventive agent against the occurrence of neoplasms of the upper digestive tract. © 2001 Published by Elsevier Science Ireland Ltd.

Keywords: Irsogladine maleate; Stomach carcinogenesis; Chemoprevention; *N*-Methyl-*N*-nitro-*N*-nitrosoguanidine; Rat

1. Introduction

Chemoprevention entails the concept that non-carcinogenic synthetic chemicals or naturally occurring products can inhibit the process of

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carcinogenesis. A number of agents have proved effective against chemical carcinogenesis in different organs (Wattenberg, 1985; Tanaka et al., 1990), and classified into two major categories of compounds, i.e. blocking and suppressing agents (Wattenberg, 1985).

The mechanisms of neoplastic development are considered to involve multistep genetic alterations (Weisburger and Williams, 1995). Using animal models, multistep carcinogenesis including stages of initiation, promotion and progression has been well investigated. In particular, studies on hepatocarcinogenesis have provided a number of information on these aspects (Salt and Farber, 1976; Pitot et al., 1978; Ito et al., 1980; Dragan and Pitot, 1992). Gap junctional intercellular communication (GJIC) has been reported to exert important roles in the control of cell proliferation or differentiation (Loewenstein, 1979; Yamaski et al., 1993; Tsuda et al., 1995; Sakamoto et al., 1992), and the modulation of GJIC is suggested to be involved in hepatocarcinogenesis, especially on the phase of promotion (Williams, 1981; Trosko and Chang, 1983; Yamaski et al., 1993). Gap junctions have been documented to mediate the transfer of signal-transducing factors such as calcium, cAMP, and inositol triphosphate (Saez et al., 1989). They are formed from oligometric proteins composed of six subunits, designated as connexons. More than 11 members of connexins (Cxs), are currently known in mammals. Of them, Cx 32, Cx 26 and Cx 43 are commonly expressed in the stomach (Paul, 1986; Zhang and Nicholson, 1989). Furthermore, Cx 32 is known to be decreased in neoplastic as well as non-neoplastic injured mucosa of the stomach (Ohkusa et al., 1995). Previously, we have demonstrated that in vivo exposure of liver tumor promoters, such as phenobarbital (PB) or dichlorodiphenyltrichloroethane (DDT), decreased the size, and altered the distribution, of gap junctions in rat hepatocytes, suggesting inhibitory effects of these agents on GJIC (Sugie et al., 1987). Depending on the evidences from different studies (Yamaski et al., 1993), it is presumed that agents that enhance functions of gap junctions may have suppressing effects on carcinogenesis.

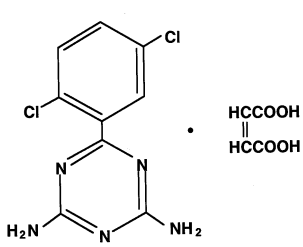

Fig. 1. Molecular structure of irsogladine maleate.

Recently, irsogladine maleate (IRG) (Fig. 1) being used as an antiulcer agent has been found to enhance GJIC (Ueda et al., 1991a, 1994, 1995) and the effectiveness of combinative chemotherapy of IRG and other agents is reported in metastatic tumors from gastric cancers (Hosokawa et al., 1994). We have examined the effect of IRG in gap junctions of the liver, and found that IRG inhibited decrease of Cx 32 induced by PB, and confirmed the suppressing effect of IRG on DEN-induced and PB-promoted rat hepatocarcinogenesis (Sugie et al., 1998).

Presently, possible modifying effect of IRG was examined in rats using *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG)-initiated and glyoxal-promoted gastric carcinogenesis. A promoting effect of glyoxal in two-stage carcinogenesis for glandular stomach cancers has been confirmed (Takahashi et al., 1989). Dose selection of IRG was based on maximum tolerated dose (MTD) values in a previous study (Sumi et al., 1986).

2. Materials and methods

2.1. Animals, diet, waters and carcinogen

Weanling male Wister rats, purchased from Shizuoka SLC, Co., Shizuoka, were used. Powdered CE-2 (CLEA Japan Inc., Tokyo) was used as a basal diet. DEN and PB were purchased from Nacalai Tesque Inc., Kyoto and Maruishi Pharm. Co., Osaka, respectively. IRG was synthesized by Nippon Shinyaku Co., Inc., Kyoto.

All animals were housed in wire cages (three rats/cage). They had free access to water and diets under controlled environmental conditions of humidity (50 ± 10%), lighting (12 h light/dark cycle)

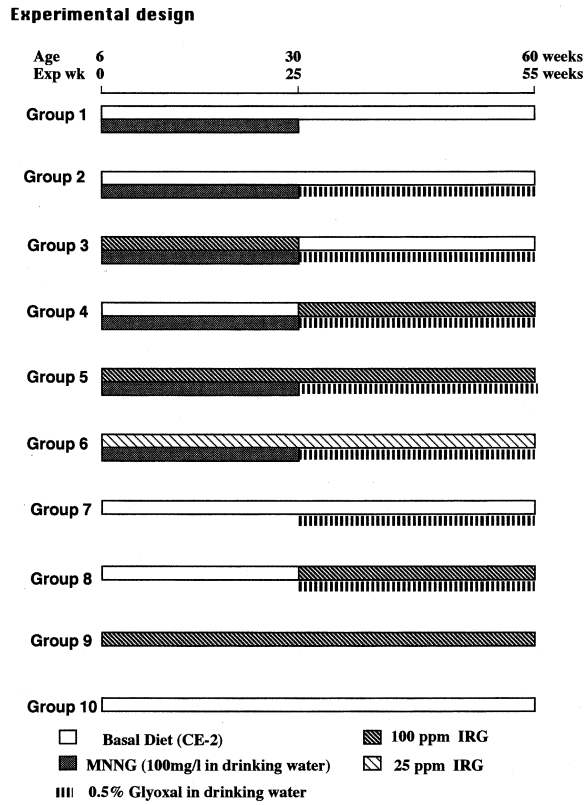

Fig. 2. Experimental design: □, basal diet (CE-2); ▨, 100 ppm IRG; ▧ 25 ppm IRG; ■, MNNG (100 mg/l in drinking water); ▥, 0.5% glyoxal in drinking water.

and temperature (23 ± 2 °C). The experimental diet mixed with IRG and drinking water containing PB were prepared weekly and stored in a cold room (4 °C).

2.2. Experimental procedure

A total of 127 rats, 6 weeks of age, were divided into ten groups (Fig. 2). Groups 1 through 6 were given MNNG (100 mg/l in drinking water) for 25 weeks from the start of experiment, whereas groups 7 through 10 received distilled water in the initiation phase as the vehicle treatment. Groups 1 and 8 were kept on the basal diet and distilled water throughout the experiment (55 weeks). Groups 2–8 were given 0.5% glyoxal in the drinking water for 30 weeks from 26th week of experiment. Group 3 was fed the diet mixed with 100 ppm IRG for 25 weeks from the start of experiment. Groups 4 and 8 were fed the diet mixed with 100 ppm IRG for 30 weeks from 26th week of experiment. Groups 5 and 9 or 6 were given 100 or 25 ppm IRG containing diet throughout the experiment. Group 10 was given basal diet and distilled water as the vehicle treated control. At the termination of the experiment (55 weeks), complete autopsies on remaining animals were performed after sacrifice by ether anesthesia. At autopsy, the location, number and size of tumors were recorded. Tissues were fixed in 10% buffered formalin, embedded in paraffin blocks, and processed for routine histological observation with the use of hematoxylin and eosin stain. The other organs were also processed for routine histological preparations and diagnosed histopathologically.

2.3. Statistical analysis

Differences in incidences or severity of pathological lesions in the liver between groups were analyzed by the χ^2-test, Fisher's exact probability test or Student's t-test.

3. Results

3.1. General observations

Mean body weights at termination of the experiment at 55–60 weeks are shown in Table 1. Among the groups which did not receive carcinogen, those exposures administered IRG (groups 8 and 9), had smaller body weights than others (groups 7 and 10). Among the MNNG-exposed groups, the body weights of groups with treatment of IRG during promotion phase or whole period were also smaller than the groups without IRG (groups 1 and 2). No clear evidence for toxicity was observed in the animals exposed to IRG diet, microscopically.

3.2. Tumor incidences

Tumors developed only in the stomach, duodenum and liver of rats in groups 1–6, which were

given MNNG (Tables 2–4). Forestomach papillomas and adenocarcinomas of the glandular stomach and duodenum were present in these groups. Glandular stomach adenomas occurred in groups 1–3, and 6. A sarcoma of the glandular stomach was found in group 1. In the duodenum, an adenoma was present in group 4, sarcomas occurred in groups 1, 2, 4 and 5.

The incidences of total tumors of upper digestive tracts and those of neoplasms of glandular stomach of groups 3–5 were significantly lower than those of group 2 ($P < 0.05$, 0.02, 0.05 and 0.05, 0.02, 0.01, respectively) . The incidence of duodenal tumors of the group treated with lower dose of IRG (group 6) was also significantly lower than that of group 2 ($P < 0.05$). Average numbers of total tumors of the upper digestive tracts of groups 3, 5 and 6 were significantly smaller than of group 2 ($P < 0.05$, 0.02 and 0.002, respectively). Furthermore, the average numbers of tumors of the glandular stomach of groups 3 and 4 were significantly smaller than those of group 2 ($P < 0.05$ and 0.01, respectively). Those of groups 5 and 6 were also relatively smaller than those of group 2. Average numbers of duodenal tumors of groups 6 were significantly lower than those of group 2 ($P < 0.02$) (Tables 5–8).

Table 1
Body weights of rats in each group

Group	Treatment	No. of rats	Body weight (g)
1	MNNG alone	17	377 ± 40[a]
2	MNNG → Glyoxal	12	356 ± 33
3	MNNG + IRG → Glyoxal	16	344 ± 84
4	MNNG → Glyoxal + IRG	12	321 ± 26[b]
5	MNNG + IRG → Glyoxal + IRG	15	295 ± 27[c]
6	MNNG + irg → Glyoxal + irg	12	324 ± 34[d]
7	Glyoxal alone	10	440 ± 42
8	IRG + Glyoxal	10	387 ± 25[e]
9	IRG alone	10	417 ± 38[f]
10	No treatment	10	459 ± 31

IRG, 100 ppm irsogladine maleate; irg: 25 ppm irsogladine maleate.

[a] Mean ± SD.

[b] Significantly different from group 2 by Student's t-test ($P < 0.005$).

[c] Significantly different from group 2 by Student's t-test ($P < 0.0001$).

[d] Significantly different from group 2 by Student's t-test ($P < 0.05$).

[e] Significantly different from group 10 by Student's t-test ($P < 0.0001$).

[f] Significantly different from group 10 by Student's t-test ($P < 0.05$).

4. Discussion

The results of the present study clearly indicate the inhibitory effect of IRG on MNNG-induced carcinogenesis in the upper digestive tract. In this study, a suppressive effect of IRG was particularly apparent in stomach carcinogenesis when given in the promotion phase together with glyoxal. We have reported the chemopreventive effect of IRG in DEN-induced and PB-promoted hepatocarcinogenesis in rats (Sugie et al., 1998). In that study, IRG was given during the promotion phase and the suppressive effect of IRG on hepatocarcinogenesis was prominent in the tumor induction promoted by PB. Results of the present study are basically in agreement with such data and appear to confirm that IRG principally acts as an antipromotor. IRG has been used as a mucosal protective agent, whose action is partially explained as enhancement of mucosal blood flow, increase of cellular cyclic AMP and facilitation of gap-junctional intercellular communication (Ueda et al., 1991b; Tatsumi et al., 1998). Cx 32 is reported to decrease in injured or neoplastic mucosa of the stomach (Ohkusa et al., 1995). Such protective effect of IRG for the gastric mucosa may be importantly concerned with the inhibition of MNNG-induced initiation.

IRG has been known to enhance GJIC (Ueda et al., 1991a, 1994, 1995). An inverse correlation between the expression of Cx 32 and BrdU labeling index is reported in partial hepatectomized liver (Yamaski et al., 1993; Tsuda et al., 1995). Reduction of GJIC is suggested to be associated with the process of mitosis (Stein et al., 1992). Thus, modulation of carcinogenesis by GJIC is considered to be concerned with its biological effects, especially in the promotion phase (Yamaski et al., 1993). Tsuda et al. (1995) reported a

Table 2
Incidence and multiplicity of gastric tumors

Group	Treatment	No. of rats	Forestomach	Glandular stomach			
			Papilloma	Adenoma	Adenocarcinoma	Sarcoma	Total
1	MNNG	17	4 (24%) 0.24 ± 0.42[a]	1 (6%) 0.06 ± 0.24	2 (12%) 0.12 ± 0.32	1 (6%) 0.06 ± 0.24	4 (24%) 0.24 ± 0.42
2	MNNG → Glyoxal	12	3 (25%) 0.25 ± 0.43	2 (17%) 0.17 ± 0.37	7 (58%)[b] 0.58 ± 0.49[d]	0	9 (75%)[c] 0.75 ± 0.43[d]
3	MNNG + IRG → Glyoxal	16	1 (6%) 0.06 ± 0.24	1 (6%) 0.06 ± 0.24	5 (31%) 0.31 ± 0.46	0	6 (38%)[e] 0.38 ± 0.48[f]
4	MNNG → Glyoxal + IRG	12	3 (25%) 0.25 ± 0.43	0	3 (25%) 0.25 ± 0.43	0	3 (25%)[g] 0.25 ± 0.43[h]
5	MNNG + IRG → Glyoxal + IRG	15	1 (7%) 0.07 ± 0.25	0	5 (33%) 0.40 ± 0.61	0	5 (33%)[g] 0.40 ± 0.61
6	MNNG + irg → Glyoxal + irg	12	1 (8%) 0.08 ± 0.28	1 (8%) 0.08 ± 0.28	4 (33%) 0.33 ± 0.47	0	5 (42%) 0.42 ± 0.49

[a] Mean ± SD.
[b] Significantly different from group 1 by Fisher's exact probability test ($P<0.02$).
[c] Significantly different from group 1 by Fisher's exact probability test ($P<0.01$).
[d] Significantly different from group 1 by Student's t-test ($P<0.005$).
[e] Significantly different from group 2 by Fisher's exact probability test ($P<0.05$).
[f] Significantly different from group 2 by Student's t-test ($P<0.05$).
[g] Significantly different from group 2 by Fisher's exact probability test ($P<0.02$).
[h] Significantly different from group 2 by Student's t-test ($P<0.01$).

Table 3
Incidence and multiplicity of duodenal tumors

Group	Treatment	No. of rats	Duodenum			
			Adenoma	Adenocarcinoma	Sarcoma	Total
1	MNNG	17	0	8 (47%) 0.53 ± 0.61[a]	1 (6%) 0.06 ± 0.24	9 (53%) 0.59 ± 0.60
2	MNNG → Glyoxal	12	0	3 (25%) 0.25 ± 0.43	3 (25%) 0.25 ± 0.43	6 (50%) 0.50 ± 0.50
3	MNNG + IRG → Glyoxal	16	0	4 (25%) 0.25 ± 0.43	0	4 (25%) 0.25 ± 0.43
4	MNNG → Glyoxal + IRG	12	1 (8%) 0.08 ± 0.28	1 (8%) 0.08 ± 0.28	2 (17%) 0.17 ± 0.37	4 (33%) 0.33 ± 0.47
5	MNNG + IRG → Glyoxal + IRG	15	0	2 (13%) 0.13 ± 0.34	1 (7%) 0.07 ± 0.25	3 (20%) 0.20 ± 0.40
6	MNNG + irg → Glyoxal + irg	12	0	1 (8%) 0.08 ± 0.28	0	1 (7%)[b] 0.08 ± 0.28[c]

[a] Mean ± SD.
[b] Significantly different from group 2 by Fisher's exact probability test ($P<0.05$).
[c] Significantly different from group 2 by Student's t-test ($P<0.02$).

progressive decrease of Cx 32 expression in liver carcinogenesis together with an inverse correlation of the expression with hepatocellular proliferation, suggesting that Cx 32 expression has an important role in the hepatocarcinogenesis. The role of gap junction Cx 32 in the carcinogenesis is

supported by the observation that tumors derived from Cx 32-gene-transfected human hepatoma cells injected into nude mice were smaller size than those derived from non-transfected cells (Eghbali et al., 1991). The negative growth control in in vivo and in vitro is also observed in the other tumorigenic cell lines transfected by gap junction genes (Mehta et al., 1991; Zhu et al., 1991; Naus et al., 1992; Rose et al., 1993; Mesnil et al., 1995). It is speculated that connexin gene

Table 4
Incidence of neoplasms

Group	Treatment	No. of rats	Total tumors of upper digestive tracts			Cholangioma
			Benign tumors	Malignant tumors	Total	
1	MNNG	17	5 (29%) 0.29 ± 0.46[a]	10 (59%) 0.76 ± 0.73	12 (71%) 1.06 ± 0.80	3
2	MNNG → Glyoxal	12	5 (42%) 0.42 ± 0.49	10 (83%) 1.08 ± 0.64	11 (92%) 1.50 ± 0.76	1
3	MNNG + IRG → Glyoxal	16	2 (13%) 0.13 ± 0.33	7 (44%) 0.56 ± 0.70	9 (56%)[b] 0.69 ± 0.68[c]	2
4	MNNG → Glyoxal + IRG	12	4 (33%) 0.33 ± 0.47	4 (33%) 0.50 ± 0.76	5 (42%)[d] 0.83 ± 1.07	0
5	MNNG + IRG → Glyoxal + IRG	15	1 (7%) 0.07 ± 0.25[f]	6 (40%) 0.60 ± 0.80	6 (40%)[e] 0.67 ± 0.84[g]	0
6	MNNG + irg → Glyoxal + irg	12	2 (17%) 0.17 ± 0.37	5 (42%) 0.42 ± 0.49[f]	7 (58%) 0.58 ± 0.49[h]	0

[a] Mean ± SD.
[b] Significantly different from group 2 by Fisher's exact probability test ($P<0.05$).
[c] Significantly different from group 2 by Student's t-test ($P<0.01$).
[d] Significantly different from group 2 by Fisher's exact probability test ($P<0.02$).
[e] Significantly different from group 2 by Fisher's exact probability test ($P<0.01$).
[f] Significantly different from group 2 by Student's t-test ($P<0.05$).
[g] Significantly different from group 2 by Student's t-test ($P<0.02$).
[h] Significantly different from group 2 by Student's t-test ($P<0.002$).

Table 5

Group	Treatment	No. of rats	Forestomach	Glandular stomach				Duodenum
			Papilloma	Adenoma	Adenocarcinoma	Sarcoma	Total	Adenoma
1	MNNG	17	0.24 ± 0.42[a]	0.06 ± 0.24	0.12 ± 0.32	0.06 ± 0.24	0.24 ± 0.42	0
2	MNNG → Glyoxal	12	0.25 ± 0.43	0.17 ± 0.37	0.58 ± 0.49[b]	0	0.75 ± 0.43[b]	0
3	MNNG + IRG → Glyoxal	16	0.06 ± 0.24	0.06 ± 0.24	0.31 ± 0.46	0	0.38 ± 0.48[c]	0
4	MNNG → Glyoxal + IRG	12	0.25 ± 0.43	0	0.25 ± 0.43	0	0.25 ± 043[d]	0.08
5	MNNG + IRG → Glyoxal + IRG	15	0.07 ± 0.25	0	0.40 ± 0.61	0	0.40 ± 0.61	0
6	MNNG + irg → Glyoxal + irg	12	0.08 ± 0.28	0.08 ± 0.28	0.33 ± 0.47	0	0.42 ± 0.49	0

[a] Mean ± SD.
[b] Significantly different from group 1 by Student's t-test ($P<0.005$).
[c] Significantly different from group 2 by Student's t-test ($P<0.05$).
[d] Significantly different from group 2 by Student's t-test ($P<0.01$).

Table 6
Multiplicity of gastric tumors

Group	Treatment	No.of rats	Forestomach	Glandular stomach			
			Papilloma	Adenoma	Adenocarcinoma	Sarcoma	Total
1	MNNG	17	0.24 ± 0.42[a]	0.06 ± 0.24	0.12 ± 0.32	0.06 ± 0.24	0.24 ± 0.42
2	MNNG → Glyoxal	12	0.25 ± 0.43	0.17 ± 0.37	0.58 ± 0.49[b]	0	0.75 ± 0.43[b]
3	MNNG + IRG → Glyoxal	16	0.06 ± 0.24	0.06 ± 0.24	0.31 ± 0.46	0	0.38 ± 0.48[c]
4	MNNG → Glyoxal + IRG	12	0.25 ± 0.43	0	0.25 ± 0.43	0	0.25 ± 0.43[d]
5	MNNG + IRG → Glyoxal + IRG	15	0.07 ± 0.25	0	0.40 ± 0.61	0	0.40 ± 0.61
6	MNNG + irg → Glyoxal + irg	12	0.08 ± 0.28	0.08 ± 0.28	0.33 ± 0.47	0	0.42 ± 0.49

[a] Mean ± SD.
[b] Significantly different from group 1 by Student's t-test ($P<0.005$).
[c] Significantly different from group 2 by Student's t-test ($P<0.05$).
[d] Significantly different from group 2 by Student's t-test ($P<0.01$).

Table 7
Multiplicity of duodenal tumors

Group	Treatment	No. of rats	Duodenum			
			Adenoma	Adenocarcinoma	Sarcoma	Total
1	MNNG	17	0	0.53 ± 0.61[a]	0.06 ± 0.24	0.59 ± 0.60
2	MNNG → Glyoxal	12	0	0.25 ± 0.43	0.25 ± 0.43	0.50 ± 0.50
3	MNNG + IRG → Glyoxal	16	0	0.25 ± 0.43	0	0.25 ± 0.43
4	MNNG → Glyoxal + IRG	12	0.08 ± 0.28	0.08 ± 0.28	0.17 ± 0.37	0.33 ± 0.47
5	MNNG + IRG → Glyoxal + IRG	15	0	0.13 ± 0.34	0.07 ± 0.25	0.20 ± 0.40
6	MNNG + irg → Glyoxal + irg	12	0	0.08 ± 0.28	0	0.08 ± 0.28[b]

[a] Mean ± SD.
[b] Significantly different from group 2 by Student's t-test ($P<0.02$).

Table 8
Multiplicity of total tumors

Group	Treatment	No. of rats	Total tumors of upper digestive tracts		
			Benign tumors	Malignant tumors	Total
1	MNNG	17	0.29 ± 0.46[a]	0.76 ± 0.73	1.06 ± 0.80
2	MNNG → Glyoxal	12	0.42 ± 0.49	1.08 ± 0.64	1.50 ± 0.76
3	MNNG + IRG → Glyoxal	16	0.13 ± 0.33	0.56 ± 0.70	0.69 ± 0.68[b]
4	MNNG → Glyoxal + IRG	12	0.33 ± 0.47	0.50 ± 0.76	0.83 ± 1.07
5	MNNG + IRG → Glyoxal + IRG	15	0.07 ± 0.25[c]	0.60 ± 0.80	0.67 ± 0.84[d]
6	MNNG + irg → Glyoxal + irg	12	0.17 ± 0.37	0.42 ± 0.49[b]	0.58 ± 0.49[e]

[a] Mean ± SD.
[b] Significantly different from group 2 by Student's t-test ($P<0.01$).
[c] Significantly different from group 2 by Student's t-test ($P<0.05$).
[d] Significantly different from group 2 by Student's t-test ($P<0.02$).
[e] Significantly different from group 2 by Student's t-test ($P<0.002$).

expression basically increases gap junctional protein, enhances the intercellular communication and relates to the control of cell proliferation. This may be one of possible mechanisms for the suppressing effect of IRG on the carcinogenesis.

Recently, IRG has been reported to inhibit the induction of tissue-type plasminogen activator synthesis in the endothelial cells and suppress the angiogenesis induced by epidermal growth factor (Sato et al., 1993a). The induction of tissue-type plasminogen activator is considered to be indispensable for growth factor-dependent angiogenesis (Sato et al., 1993b). Larson and Haudenschild (1988) reported that junctional coupling is slightly reduced during repairing of wounded aortic endothelial cells. On the other hand, Pepper and Meda (1992) indicated that migrating endothelial cells express plasminogen activator activity, while GJIC itself increases during the migration of endothelial cells. These findings suggest that IRG may suppress angiogenesis (Sipos et al., 1994) through an abrogation of the induction of plasminogen activator by modulating GJIC of endothelial cells. This may be another possibility for the mechanism of the modifying effect of IRG on the hepatocarcinogenesis.

Meanwhile, IRG has been shown to activate GJIC through M1 muscarinic acetylcholine receptor (Ueda et al., 1995). Activation of M1 receptor inhibits Raf activation induced by growth factors (Russel et al., 1994). Accordingly, chemopreventive effect of IRG on the carcinogenesis may be related to the signaling through M1 muscarinic acetylcholine receptor.

Although more studies are necessary to confirm detailed mode of actions of IRG and their underlying mechanisms during carcinogenesis, the results of the present investigation strongly suggest that IRG could be a promising chemopreventive agent for human cancers of the digestive tract, including stomach.

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