PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

World J Gastroentero, 2001;7(3):376 - 380
World Journal of Gastroenterology
 ISSN 1007 - 9327

Carcinogenic potential of duodenal reflux juice from patients with long-standing postgastrectomy

Zhe Fu Ma[1], Zhong Yu Wang[1], Jun Ran Zhang[2], Peng Gong[1] and Hai Long Chen[1]

Subject headings gastrectomy; duodenal reflux; initiation; promotion; cell transformation; gastric stump cancer

Ma ZF, Wang ZY, Zhang JR, Gong P, Chen HL. Carcinogenic potential of duodenal reflux juice from patients with long-standing postgastrectomy. **World J Gastroenterol**, 2001;7(3):376-380

Abstract

AIM To determine whether study on the carcinogenic potential of reflux juice from patients with remote gastrectomy could clarify the inherent relationship between duodenal reflux and gastric stump cancer.

METHODS A total of 37 reflux juice samples (13 BillrothI, 24 BillrothII) were employed in the present study. A two-stage transformation assay using BALB/c 3T3 cells was carried out to test the initiating or promoting activity of these samples.

RESULTS Two of 18 (11.1%) reflux samples exerted initiating activities, whereas 9/19 (47. 4%) samples enhanced the MNNG-initiating cell transformation, suggesting the duodenal reflux juice might more frequently possess the tumor-promoter activity ($P = 0.029$). In addition, there was no difference in initiating activities of the samples irrespective of surgical procedures ($P = 0.488$), while Billroth II samples exhibited stronger tumor-promoter activity than Billroth I samples ($P = 0.027$). Furthermore, the promoter activities were well correlated with the histological changes of the stomas ($r_s = 0.625$, $P = 0.004$), but neither their cytotoxicities nor initiating activities had this correlation (Probabilities were 0.523 and 0.085, respectively).

CONCLUSION The duodenal reflux juice from patients with remote postgastrectomy did have carcinogenic potential, and suggested that tumor-promoting activity should principally account for the high incidence of gastric cancer in gastrectomy patients. In contrast, it is difficult to explain the high stump-cancer incidence with the "N-nitroso compounds" theory-a popular theory for the intact stomach carcinogenesis, and it seemed to be justified to focus chemoprevention of this cancer on the tumor-promoting potential of reflux juice.

[1]Department of General Surgery, First Hospital, Dalian Medical University, Dalian 116011, China
[2]Department of Radiation Oncology, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA 02129, USA
Dr. Zhe Fu Ma, graduated from Shanxi Medical College in 1993, now Ph.D., M.D. in Department of Surgery, Dalian Medical University, majoring gastroenteric cancer, having 4 papers published.
Correspondence to: Dr. Zhe Fu Ma. Department of General Surgery, First Hospital, Dalian Medical University, Dalian 116011, China
Tel. 0086-411-4720334
Email. address: mazhefu@usa.net
Received 2000-11-03 **Accepted** 2001-02-16

INTRODUCTION

Since gastric stump cancer was first described in 1922[1], it has been well established that the incidence of gastric carcinoma is increased in patients who have undergone a partial gastrectomy for peptic ulcer disease[2-6]. But the etiology and exact mechanism of gastric stump carcinogenesis are unclear. Decreased sensitivity of chief cells and parietal cells[7], alteration in gastrin level[8], hypoxia and hemodynamic changes[9], bacterial proliferation[10], and reflux[11,12] are the putative contributing factors. Among these, the excessive duodenal reflux induced by surgery seems to be the main risk factor, because the incidence of stump carcinomas is higher in Billroth II than in Billroth I[13,14], and most of the stump carcinomas are located near the stoma[15-17]. Nevertheless, what is the inherent association between the duodenal reflux and stump cancer To answer the question, a cell transformation assay was employed in our study to examine the carcinogenic potential, initiating and promoting activity, of reflux juices from patients with remote postgastrectomy, in terms of the common theory carcinogenesis is analytically considered to relate to the two stages, initiation and promotion[18-23].

MATERIALS AND METHODS

Sampling

Thirty-seven patients (10 women and 27 men; aged, 42-77 years) who underwent partial gastrectomy at least 10 years previously for benign ulcer disease received endoscopy. Each patient experienced either BillrothI or BillrothII procedures (Table 1). Endoscopy was carried out after 8 to 12 hours of fasting. The bile stained reflux fluid was aspirated in a sterile syringe, then sterilized by passing through a 0.22 μm Millipore filter and stored at -70℃ until analysis. The thirty seven patients were all eligible for this study (patients with histories of smoking were excluded from the study), and informed consent was obtained from each patient.

For the sake of enough reflux juice, samples from BillrothI or Billroth II patients were randomly extracted to make two almost equal sized groups to evaluate the initiating and promoting activity, respectively. At the end of the endoscopy, at least five biopsies in a circle around stoma were taken for histological assessment. Three grades were used which were, in ascending order of significance: chronic superficial gastritis (± minimal atrophic gastritis); atrophic gastritis/intestinal metaplasia; dysplasia[24].

Cytotoxicity assay

BALB/c 3T3 A31-1-1 cells 1×10^4 (one of three standardized cell lines generally recommended for the cell transformation assay)[25] were plated in each well of a 96-well plate covered with 100 μL DMEM (Dulbecco's modification of Eagle's medium, Gibco) supplemented with 10% FCS (fetal calf serum, Gibco) at 37℃ in a humidified incubator containing 5% CO_2 in air for 24 hours. The medium was then replaced by 100 μL medium containing reflux juice (15-20 doses designed serially per sample by a concentration gradient of 1.25% reflux juice), and further incubated for 24 hours. The culture was used as a negative control; 3-(4,5,dimethylthiazol-2yl)2,5-diphenyl tetrazolium bromide (MTT; Promega) was added (5 g/L) and the plates were incubated for a further 4 hours. The dye/medium in each well was carefully removed and 100 μL solubilization solution (Promega) was placed in each well for 1 hour. The plates were read at 570 nm in a microplate reader (Biorad 550). The mean absorbance was calculated and cell survival was expressed as the percentage absorbance of that in wells incubated with the negative control.

Transformation assay

Two-stage transformation was assayed by the protocol described by Hirakawa *et al*[26]. Only the volume of culture medium was changed from 5 mL to 4 mL. In the initial assay, actively growing cells (10^4 cells per 60 mm-diameter plastic dish) were plated. Cultures were incubated for 24 hours, reflux samples with graded concentrations (80% and 40% critical toxicity) were added for 72 hours (initiating phase), and 0.3 mg/L TPA (12-O-tetradecanoylphorbol 13-acetate) were present in the medium for 2 weeks 4 days after reflux juice was removed (promoting phase). The medium was then replaced with fresh, promoter-free medium and the culture was incubated for another 2 weeks. The culture medium was changed twice a week. Ten dishes were used for each sample in 2 independent tests. MNNG (N-methyl-N'-nitro-N-nitro-soguanidine) 0.5 mg/L and culture medium were used as positive and negative control, respectively. Finally, the culture cells were fixed and stained with Giemsa 5 weeks after plating. Type III transformed cell foci (deeply basophilic, criss-crossing, a dense layer formation and a random orientation of cells at the edges of foci[25] were counted. As for the transformation frequency (TF), the percentage of dishes with foci was calculated. In the promoting assay, 0.5 mg/L MNNG (dissolved in DMSO)was employed in the intiating phase, and reflux samples (25% toxicity) were added into the medium during the promoting phase. Twelve dishes were used for each sample in 2 independent tests. TPA 0.3 mg/L and culture medium were taken as positive and negative control, respectively. Other procedures of this assay were the same as described above.

Statistical analysis

One-way ANOVA was used to search for differences in the average TF values between BillrothI or Billroth II groups. In other sections, two-tailed Fisher's exact test was performed. A probability of $P<0.05$ was considered statistically significant and we used Bonferroni's method to get the nominal level for each comparison of TF between a sample and the culture control.

RESULTS

In the 2-stage transformation assay, without a known promoter TPA, a 3-day initiating treatment with a potent carcinogen MNNG at 0.5 mg/L had not significantly yielded an increasing number of transformed foci. While MNNG caused a very remarkable transformation with subsequent TPA (0.3 mg/L) promoting treatment (data not shown). The results suggested that the present assay system work well enough to examine whether an agent could exert an initiating or promoting activity. In addition, the doses of bile samples used in the cell transformation assay were selected based on the results of MTT (toxicity) assay. A small concentration gradient (1.25% reflux juice, v:v) was used in MTT assay to obtain the critical cytotoxicity more accurately. Because the toxicity of the samples were different (ranging from 75 to 250 μL aspirate/mL medium), a unified criterion (the same percent of each sample's own toxicity) was used to compare TF values among various samples effectively. In the initiating assay, the aspirate concentration was extended to a high dose, 80% critical toxicity, to ensure that our conclusions are free from false negative results. A low dose, 25% toxicity, was used in the promoting assay for the sake of the culture cells having to survive a long-term exposure to reflux juice (2 weeks). In addition, this dose-design method makes it feasible to study on the carcinogenic potential independent of the cytotoxicity of reflux juice.

Carcinogenic potential in the transformation assay

Six Billroth I and I2 Billroth II samples were randomly distributed into the initiating group. Two of 18 samples exerted significantly initiating activity compared with negative control group in the TPA-promoting cell transformation assay (Figure 1a). As

analysed by subsite, 1/6 Billroth I and 1/I1 BillrothII samples were positive. There was no marked difference in the average TF values between the two groups (Figure 1b) either at the high or low doses (data not shown for the low dose, 40% toxicity).

The residual samples, 7 Billroth I and 12 Billroth II, made up the promoting group. As shown in Figure 2a, there was an increased number of transformed foci in the MNNG-initiated target cells followed by applications of 9 of 19 samples. The nine positive samples included 2 BillrothI and 7 BillrothII aspirate. A significant difference in the average TF values was observed between the two groups ($P<0.05$, Figure 2b).

In comparison of the results between the initiating and promoting assay, the promoting positive rate (9/19, 47.4%) was statistically higher than initiating positive rate (2/18, 11.1%) ($P = 0.029$). Therefore, the reflux juice might usually exhibit the promoting activity and sparsely possess the promoting potential.

Histological grades with carcinogenic potential and cytotoxicity of reflux juice

A stronger correlation (Spearman's rank correlation coefficient = 0.625, $P = 0.004$) was found between the histological grades of stoma and the promoting activity, but not between histological grades and the initiating activity (Table 2). In addition, with the upward shift of histological grades, a small decrease in the toxic concentrations of reflux juice was observed in Table 1, which did not reach statistical significance.

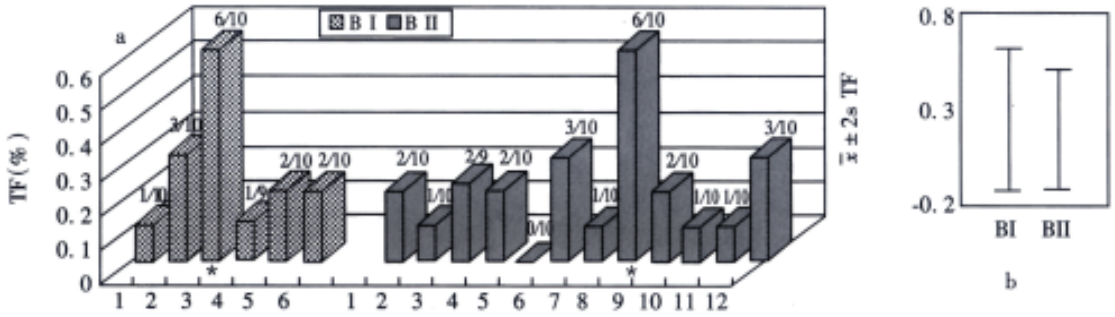

Figure 1 The intiating activity of reflux juice in tranformation assay. *Positive cases. TF of culture control was 7% (4/57). The average TF of BillrothI (BI) and Billroth II (BII) groups shown in Figure 1b.

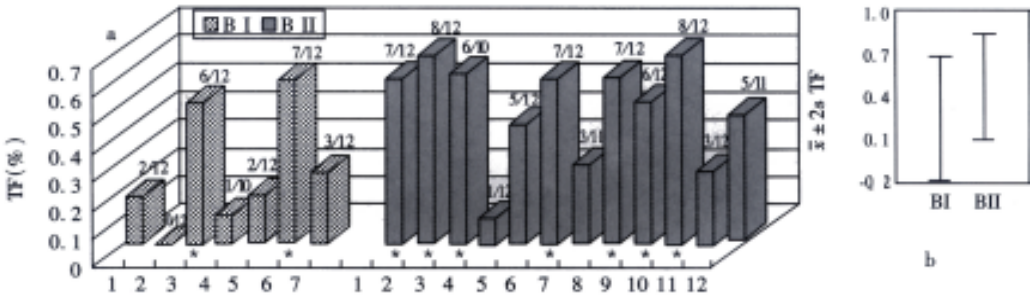

Figure 2 The promoting activity of reflux juice in tranformation assay. *Positive cases. TF of culture control was 7% (4/59). The average TF of BillrothI (BI) and Billroth II (BII) groups shown in Figure 2b.

Table 1 Operation types and critical toxicity of reflux juice in each histological grade

Histological grades	Billroth I	Billroth II	Critical toxic concentrations (%, v:v)	Correlation between histological grades and toxicity[a]
1 Chronic superficial gastritis	7	10	16.4±4.0	
2 Atrophic gastritis/intestinal metaplasia	4	7	15.8±5.0	
3 Dysplasia	2	7	12.9±4.6	r_s = -0.287, P = 0.085

[a]Two tailed spearman's rank correlation.

Table 2 Histological grades of stoma and carcinogenic potential of reflux juice in the cell transformation assay

Histological grades	TF (%)		Correlation coefficient[a]	
	Initiating	Promoting	Initiating	Promoting
1 Chronic superficial gastritis	20.1±18.4 (8)	25.3±18.7 (9)		
2 Atrophic gastritis/intestinal metaplasia	26.0±20.7 (5)	48.1±20.1 (6)	r_s = 0.161	r_s = 0.625
3 Dysplasia	20.4±7.10 (5)	56.7±10.6 (4)	P = 0.523	P = 0.004

[a]Two tailed Spearman's rank correlation. The number of the patients for each histological grade is inside the parentheses.

DISCUSSION

In present study, the duodenal reflux juice from patients with remote postgastrectomy exerted more frequently tumor-promoter activity compared to the initiating (mutagenic) activity. In addition, there was no difference in the initiating activity of reflux samples irrespective of surgical procedures. While the BillrothII aspirates exhibited a stronger tumor-promoter activity than BillrothI, in accordance with many epidemical findings, stump cancer preferred to the BillrothII procedure. All these results strongly suggested that partial gastrectomy - perse- should be responsible for the reflux promoting activity, implying an etiological role for the promoting activity of duodenal reflux juice in the pathogenesis of gastric stump cancer. It is just the tumor-promoter activity, a characteristic beyond what an intact stomach usually possesses, that may elucidate the high incidence of gastric cancer in postgastrectomy patients than in general population.

As for the etiology of gastric cancer, dietary factor has been emphasized principally since some procarcinogens, such as nitrates, can often enter the diet by vegetables, preservatives of food, even drinking water. Nitrite may derive from nitrate by the flora of the mouth or stomach (human saliva typically contains 6-10 mg/L nitrite and 15-35 mg/L nitrate[27], and further react with secondary amines to give rise to N-nitroso compounds which are strong carcinogens suspected of playing a role in upper gastrointestinal carcinogenesis[28] because of their spontaneous synthesis from dietary components and their ability to alkylate nucleic acids. This "N-nitroso compounds" theory has provided a potential explanation for some geographic regions at a very high risk for gastric cancer[29]. But whether this theory can work as well to interpret the high incidence of gastric cancer in the postgastrectomy patients is still a matter of dispute[30-34]. Although we did not measure the concentrations of N-nitroso compounds directly, the initiating assay can effectively detect the mutagenicity of the whole human reflux juice that may contain various mutagenic or carcinogenic substances inclusive of the N-nitroso compounds. When analysing our data from another angle, the initiating or mutagenic activity of reflux samples did not correlate to the histological grades of anastomotic area (Table 2), whereas the promoting activity significantly augmented with the progression of histological abnormalities. Thus, a causal role for the promoting activity of reflux juice in the pathogenesis of stump cancer was further suggested, implying that the tumor-promoter activity might principally account for the high incidence of gastric cancer in the long-standing postgastrectomy patients relative to the initiating activity. While this did not, of course, exclude that the initiating activity or mutagenic activity was indispensable to the stump carcinogenesis. But a perfect explanation for the high stump-cancer incidence seemed to be not available by the "N-nitroso compounds" theory, as it is different from the setting of intact stomach carcinoma. Rising gastric pH in the presence of bacteria after gastrectomy might not favor the formation of the mutagenic or carcinogenic compounds (e.g., N-nitroso compounds)[14,35], and those with a high concentration enough to take an initiating or mutagenic effect were seldom present in the reflux juice of postgastrectomy patients. In addition, as shown in Table 1, with the histological manifestations of the stoma, a slight increase in the non-specific cytotoxicity of reflux juice was observed. Although it did not reach the statistical significance, whether the result alluded to a synergic effect for the reflux toxicity in the gastric stump carcinogenesis deserved further studies.

What is the exact nature of the tumor-promoting species in the reflux juice Some substances exerting a persistent action (e.g., cell differentiating or proliferating[36-39]) must exist in the reflux juice. Unconjugated and secondary bile acids might be first candidates, which have been suggested to take a part in the colon cancer[40-46]. These bacteria-degraded bile acids also present in the gastric aspirate from remote postgastrectomy patients though consisting of a small portion of the whole reflux bile acids. Secondly, lysophosphatidylcholine (lysoPC), the product of phosphatidylcholine hydrolysis by phospholipase A2, has been suggested to play a role in the pathogenesis of gallbaldder cancer in the APBDJ (Anomalous Pancreaticobiliary Ductal Junction) patients[47]. LysoPC might also be produced in the stump stomach due to the reflux juice containing aberrant pancreatic juice and bile. It has been reported that lysoPC at much lower concentrations significantly enhanced the activation of protein kinase C (PKC)[48-50] and regulated cell differentiation[51] if diacylglycerol (DAG) was available. And the unconjugated bile acids happened to remarkably generate DAG[52]. Therefore, whether the above two factors could cooperate to make the reflux juice exhibit tumor-promoter activity This issue also warrants further investigations.

For the first time, we confirmed that the duodenal reflux juice from the long-standing postgastrectomy patients did exert the tumor-promoting activity and initiating activity, and further suggested that tumor-promoting potential should be mainly responsible for the high stump-cancer incidence. Simultaneously, our results demonstrated that a close relationship between the histological changes of anastomotic site and the reflux tumor-promoting activity, thus directly supporting to conduct endoscopic surveillance for postgastrectomy patients with precancerous lesions

(e.g., moderate and severe dysplasia) in stoma. In addition, to decrease the incidence of stump cancer effectively, it seems reasonable not only to perform reconstruction procedures (e.g., Roux en Yanastomosis) for those with severe duodenal reflux, but also to focus the chemoprevention of this cancer on tumor-promoting potential of the reflux juice.

REFERENCES

1 Balfour DC. Factors influencing the life expectancy of patients operated on for gastric ulcer. *Ann Surg,* 1922;6:405-408
2 Carter DC. Cancer after peptic ulcer surgery. *Gut,* 1987;28:921-923
3 Offerhaus GJA, Tersmette AC, Huibregtse K, Vandestadt J, Tersmette KWF, Stijnen T, Hoedemaeker PJ, Vandenbroucke JP, Tytgat GNJ. Mortality caused by stomach cancer after remote partial gastrectomy for benign condition: 40 years of follow up of an Amsterdam cohort of 2633 postgastrectomy patients. *Gut,* 1988;29:1588-1590
4 Stalsberg H, Taksdal S. Stomach cancer following gastric surgery for benign conditions. *Lancet,* 1971;ii:1175-1177
5 Kaminishi M, Shimizu N, Shiomoyama S, Yamaguchi H, Ogawa T, Sakai S, Kuramoto S, Oohara T. Etiology of gastric remnant cancer with special reference to the effects of denervation of the gastric mucosa. *Cancer,* 1995; 75:1490-1496
6 Tersmette AC, Goodman SN, Offerhaus JA, Tersmette KWF, Giardiello FM, Vandenbroucke JP, Tytgat GNJ. Multivariate Analysis of the risk of stomach cancer after ulcer surgery in an Amsterdam Cohort of postgastrectomy patients. *Am J Epidemiol,*1991;134:14-21
7 Ruddell WSJ, Bone ES, Hill MJ, Blendis LM, Walters CL. Gastric juice nitrite: a risk factor for cancer in the hypochlorhydria stomach. *Lancet,* 1976;ii:1037-1039
8 Sumiyoshi H, Yasui W, Ochiai A. Effects of gastrin on tumor growth and cyclic nucleotide metabolism in xenotransplantable human gastric and colonic adenocarcinomas in nude mice. *Cancer Res,* 1984;44:4276-4280
9 Kamada T, Kawano S, Sato N. Gastric mucosal blood distribution and its change in healing process of gastric ulcer. *Gastroenterology,* 1983;84:1541-1546
10 Muscroft TJ, Deane SA, Youngs Dl. The microflora of the postoperative stomach. *Br J Surg,* 1981;68:560-564
11 Domell-f L, Eriksson S, Mori H, Weisburger JH, Williams GM. Effect of bile acid gavage or vagotomy and pyloroplasty on gastrointestinal carcinogenesis. *Am J Surg,* 1981;142:551-554
12 Dewer P, Dixon MF, Jonston D. Bile reflex and degree of gastritis in patients with gastric ulcer: before and after operation. *J Surg Res,* 1984;37: 277-284
13 Caygill CPJ, Hill MJ, Kirkham JS, Northfield TC. Mortality from gastric cancer following gastric surgery for peptic ulcer. *Lancet,* 1986;i:929-931
14 Richard-Boland C, Scheiman JM. Tumors of the stomach. In: Yamada T, Alpers DH, Owyang C, Powell DW, Silverstein FE., eds. Textbook of Gastroenterology. 2nd ed. J. B. Lippincott: *Philadelphia,* 1995:2254-2265
15 Langhans P, Heger RA. Operation-sequel carcinoma of the stomach. *World J Surg,* 1981;5:595-605
16 Pointner R, Schwab G, Konigstrainer A. Gastric stump cancer: etiopathological and clinical aspects. *Endoscopy,* 1989;21:115-119\
17 Fukuzawa K, Noguchi Y, Matsumoto A. Alterations on DNA proliferation in gastric stump mucosa with special reference to topography. *Surgery,* 1996;119:191-197
18 Weinstein IB, Troll W. National cancer institute workshop on tumor promotion and cofactors in carcinogenesis. *Cancer Res,* 1977;37:3461-3463
19 Foulds L. The natural history of cancer. *J Chronic Dis,* 1958;8:12-37
20 Nowell P. The clonal evolution of tumor cell populations. *Science,* 1976; 194:23-28
21 Farber E, Cameron R. The sequential analysis of cancer development. *Adv Cancer Res,* 1980;31:125-226
22 Heeker E, Fusenig NE, Kunz W, Marks F, Thielmann HW (eds.). Carcinogenesis a Comprehensive Survey, Vol. 7. New York: *Raven Press,* 1982
23 Melnick RL, Kohn MC, Portier CJ. Implications for risk assessment of suggested nongenotoxic mechanisms of chemical carcinogenesis. *Environ Health Perspect,* 1996;104(Suppl 1):123-134
24 Morson BC, Sobin CH, Grundmann E, Johansen A, Nagayo T, Serck-Hanssen A. Precancerous conditions and epithelial dysplasea in the stomach. *J Clin Pathol,* 1980;33:711-721
25 Dunkel VC, Rogers C, Swierenga SHH, Brillinger RL, Gilman JPW, Nestmann ER. Recommended protocols based on a survey of current practice in genotoxicity testing laboratories: III. Cell transformation in C3H/10T1/2 mouse embryo cell, BALB/c 3T3 mouse fibroblast and Syrian hamster embryo cell cultures. *Mutation Res,* 1991;246:285-300
26 Hirakawa T, Kakunaga T, Fujiki H, Sugimura T. A new tumor-promoting agent, dihydroteleocidin B, markedly enhances chemically induced malignant cell transformation. *Science,* 1982;216:527-529
27 Tannenbaum SR, Sinskey AJ, Weisman M, Bishop W. Nitrite in human saliva: its possible relationship to nitrosamine formation. *JNCI,* 1974;53:79-84
28 Deng DJ. Progress of gastric cancer etiology: N-nitrosamides in the 1990s. *World J Gastroenterol,* 2000;6:613-618
29 Hartman PE. Review: putative mutagens and carcinogens in foods.I. Nitrate/nitrite ingestion and gastric cancer mortality. *Environmental Mutagenesis,* 1983;5:111-117
30 Schlag P, Ulrich H, Merkle P, B-ckler R, Peter M, Herfarth CH. Are nitrite and N-nitroso compounds in gastric juice risk factors for carcinoma in the operated stomach? *Lancet,* 1980;i:727-729
31 Hall CN, Darkin D, Brimblecombe R. Evaluation of the nitrosamine hypothesis of gastric carcinogenesis in precancerous conditions. *Gut,* 1986;27: 491-498
32 Watt PCH, Sloan JM, Donaldson JD. Relation between gastric histology and gastric juice pH and nitrite and N-nitroso compound concentrations in the stomach after surgery for duodenal ulcer. *J Clin Pathol,* 1984;37:511-519
33 Keighley MRB, Youngs D, Poxon V, Morris D, Muscroft TJ, Burdon DW, Barnard J, Bavin PMG, Brimblecombe RW, Darkin DW, Moore PJ, Viney N. Intragastric N-nitrosation is unlikely to be responsible for gastric carcinoma developing after operations for duodenal ulcer. *Gut,* 1984;25:238-245
34 Reed PI, Smith PLR, Haines K. Gastric juice N-nitrosamines in health and gastroduodenal disease. *Lancet,* 1980;i:550-552
35 Tersmette AC, Giardiello FM, Tytgat GNJ. Carcinogenesis after remote peptic ulcer surgery: the long-term prognosis of partial gastrectomy. *Scand J Gastroenterol,* 1995;30(Suppl 212):96-99
36 Offerhaus GJA, Stadt JVD, Samson G, Tytgat GNJ. Cell peoliferatoon kinetics in the gastric remnant. *Eur J Cancer Clin Oncol,* 1985;21:73-79
37 Fukuzawa K, Noguchi Y, Matsumoto A. Alterations on DNA proliferation in gastric stump mucosa with special referance to topography. *Surgery,* 1996;119:191-197
38 Deinlin E, Schmidt H, Reinmann JF, Pietrusky RG, Hornstein OP. DNA flow cytometric measurements in inflammatory and malignant human gastric lesions. *Virchows Arch,* 1983;402:185-193
39 Teodori L, Capurso L, Cordelli E. Cytometrically determined relative DNA content as an indicater of neoplasia in gastric lesions. *Cytometry,* 1984;5:63-70
40 Suglyama Y, Sohma H, Ozawa M, Hada R, Mikami Y, Konn M, Ono K. Regurgitant bile acids and mucosal injury of the gastric remnant after partial gastrectomy. *Am J Surg,* 1987;153:399-403
41 Huang XP, Fan XT. Bile acids, non-phorbol-ester-type tumor promoters, stimulate the phosphorylation of protein kinase C substrates in human platelets and colon cell line HT29. *Int J Cancer,* 1992;52:444-450
42 Armstrong D, Rytina ER, Murphy GM. Gastric mucosal toxicity of duodenal juice constituents in the rat. Acute studies using ex vivo rat gastric chamber model. *Dig Dis Sci,* 1994;39:327-339
43 Jung B, Vogt T, Mathieu-Daude F, Welsh J, Mcclelland M, Trenkle T, Weitzel C, Kullmann F. Estrogen-reponsive RING finger mRHA inducton in gastrointestinal carcinoma cells following bile acid treatment. *Carcinogenesis,* 1998;19:1901-1906
44 Lapre JA, Meer RVD. Diet-induced increase of colonic bile aceds stimulates lytic activity of fecal water and proliferation of colonic cells. *Carcinogenesis,* 1992;13:41-44
45 Rose RC, Nahrwold DL. Bile acids: effects on absorption of 1,2-Dimethylhydrazine and 7, 12-Dimethylbenz(a) anthracene in the colon of the rat and guinea pig. *JNCI,* 1982;68:619-622
46 Mower HF, Ray RM, Shoff R, Stemmermann GN, Nomura A, Glober GA, Kamiyama S, Shimada A, Yamakawa H. Fecal bile acids in two Japannese populations with different colon cancer risks. *Cancer Res,* 1979;39:328-331
47 Sugiyama Y, Kobori H, Hakamada K, Seito D, Sasaki M. Altered bile composition in the gallbladder and common bile duct of patients with anomalous pancreaticobiliary ductal junction. *World J Surg,* 2000;24:17-21
48 Sasaki Y, Asaoka Y, Nishizuka Y. Potentiation of diacylglycerol-induced activation of protein kinase C by lysophospholipids. *FEBS Lett,* 1993;320:47-51
49 Oishi K, Raynor RL, Charp PA, Kuo JF. Regulation of protein kinase C by lysophospholipids. *J Biol Chem,* 1988;263:6865-6871
50 Asaoka Y, Oka M, Yoshida K, Sasaki Y, Nishizuka Y. Role of lysophosphatidylcholine in T-lymphocyte activation: Involvement of phospholipase A2 in signal transduction through protein kinase C. *Proc Natl Acad Sci USA,* 1992;89:6447-6451
51 Asaoka Y, Yoshida K, Sasaki Y, Nishizuka Y. Potential role of phospholipase A2 in HL-60 cell differentiation to macrophages induced by protein kinase C activation. *Proc Natl Acad Sci USA,* 1993;90:4917-4921
52 Rao YP. Activation of protein kinase C á and ä by bile acids: correlation with bile acid structure and diacylglycerol formation. *J Lipid Res,* 1997;38:1395-1411

Edited by Ma JY