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• BRIEF REPORTS •

Chronic gastritis rat model and role of inducing factors

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Abstract

AIM: To establish an experimental animal model of chronic gastritis in a short term and to investigate the effects of several potential inflammation-inducing factors on rat gastric mucosa.

METHODS: Twenty-four healthy, male SD rats were treated with intragastric administration of 600 mL/L alcohol, 20 mmol/L sodium deoxycholate and 0.5 g/L ammonia (factor A), forage containing low levels of vitamins (factor B), and/or indomethacin (factor C), according to an $L_8(2^7)$ orthogonal design. After 12 wk, gastric antral and body mucosae were pathologically examined.

RESULTS: Chronic gastritis model was successfully induced in rats treated with factor A for 12 wk. After the treatment of animals, the gastric mucosal inflammation was significantly different from that in controls, and the number of pyloric glands at antrum and parietal cells at body were obviously reduced ($P<0.01$). Indomethacin induced gastritis but without atrophy, and short-term vitamin deficiency failed to induce chronic gastritis and gastric atrophy. In addition, indomethacin and vitamin deficiency had no synergistic effect in inducing gastritis with the factor A. No atypical hyperplasia and intestinal metaplasia in the gastric antrum and body were observed in all rats studied.

CONCLUSION: Combined intragastric administration of 600 mL/L alcohol, 20 mmol/L sodium deoxycholate and 0.5 g/L ammonia induces chronic gastritis and gastric atrophy in rats. Indomethacin induces chronic gastritis only. The long-term roles of these factors in gastric inflammation and carcinogenesis need to be further elucidated.

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INTRODUCTION

Chronic gastritis including chronic atrophic gastritis (CAG) is common and CAG is a precancer lesion. It is very important to study the etiology of chronic gastritis, especially CAG. We established an experimental animal model of chronic gastritis and investigated the effects of inducing factors on gastric mucosa of rats.

MATERIALS AND METHODS

Animals

Twenty-four healthy, male SD rats weighing 270-290 g were involved in this study. Animals were housed in a controlled environment with a 12/12 h light /dark cycle. The care and handling of the animals were in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Chemicals and experimental design

Pure ammonia (Wujin Chemicals Factory,Jiangsu, China) was used to be diluted to a 0.5 g/L solution. Pure alcohol (Yixing Nanxin Chemicals Factory, Jiangsu, China) was diluted to a 60% solution and sodium deoxycholate (DOC-Na) (SERVA Company) was dissolved into sterilized water to make a 20 mmol/L solution. A mixture of carboxymethyl cellulose containing 0.5 mg/mL indomethacin was dispensed.

In this study, an $L_8(2^7)$ orthogonal test was used and comprised 8 testing members, 3 treatment factors (A,B and C) and 2 levels (with or without treatment). Triple tests were conducted and 24 rats were used. Factor A: 0.5 g/L ammonia solution was used as drinking water everyday, intragastric administration of 2 mL of 600 mL/L alcohol was given twice in fasting per week and intragastric administration of 2 mL of 20 mmol/L DOC-Na without fasting everyday but intragastric administration twice in fasting per week was also given. Factor B: forage containing less vitamin was given. Factor C: intragastric administration with 1mg indomethacin was given everyday. All above doses were used for each rat, the testing period was 12 wk. The control rats had free access to normal rat chow and water.

Histology study

All rats were sacrificed with luxation of cervical vertebra and their stomachs were removed after 12 wk. Gastric mucosa for histological examinations was cut along the lesser curvature from the lower esophagus to the upper duodenum. Samples were immersed in buffered 40 g/L formaldehyde and embedded in paraffin. Paraffin sections were sliced, mounted on glass slides and stained with hematoxylin and eosin (H&E) for histological study. Inflammation grades of gastric antrum and body were based on semi-quantity. Four inflammation grades were classified in accordance with pathological diagnosis of chronic gastritis set up on Huston symposium in 1994[1]. Four typical signs of inflammation grades were described: 0: no inflammation, the presence of few leukocytes infiltration in gastric mucosa; 1: mild inflammation, a few leukocytes infiltration in upper mucosa or at bottom of gastric glands; 2: moderate inflammation, a large number of leukocytes infiltration in total mucosa; 3: severe inflammation, leukocytes infiltration in heaps in total mucosa. Each inflammation grading result was based on an average of grades of 10 fields under microscope. Thickness of lamina propria mucosa of the stomach was measured at given points which was 150±10 μm away from the boundary of forestomach in body while 150±10 μm away from pyloric ring in antrum. Percentage ratio of pyloric gland area to total lamina propria area at gastric antrum was 100 μm to 200 μm away from pyloric ring. Also 10 intact oxyntic glands were observed at the above points in gastric body, parietal cell number in each gland and the median number were calculated.

Statistical analysis

All data were analyzed by using variance analysis of an $L_8(2^7)$ orthogonal test. $P<0.05$ was considered statistically significant.

RESULTS

Effect of inflammation grades on gastric mucosa

At antrum, inflammation grade of gastric mucosa induced by a single factor A was 1.50, 1.67 and 1.75 respectively on three repeated tests. It was significantly higher than that of the control. The grade induced by factor C also was higher than that of the control ($P<0.01$, Table1, Figure1). However, combined treatment of factors A+B+C, A+B, A+C had no effects on enhancing inflammation grades induced by a single factor A. In addition, a single factor B had no effect on inducing gastritis and inflammation grades induced by combined treatment of factors B+C had no difference from that induced by a single factor C (Table 1). The results were similar at gastric body (Table 1, Figure 2).

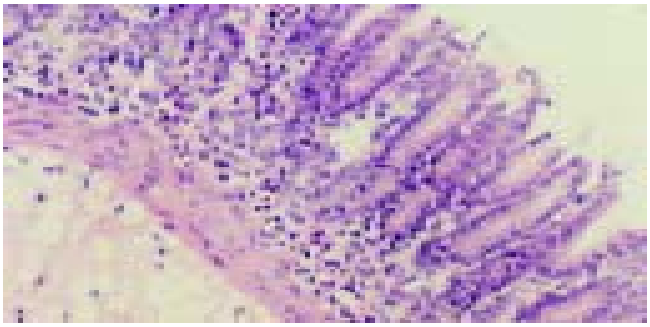

Figure 1 Severe infiltration of inflammatory cells, decreased thickness of lamina propria and lessened pyloric glands in gastric antrum of rats with gastritis induced by factor A. The results induced by factors A+B+C, A+C, A+B were similar. HE stain×100. Factor A: Combined intragastric treatments of 600 mL/L alcohol, 20 mmol/L sodium deoxycholate and 0.5 g/L ammonia;Factor B: Treatment of forage containing low levels of vitamins; Factor C: Treatment of indomethacin.

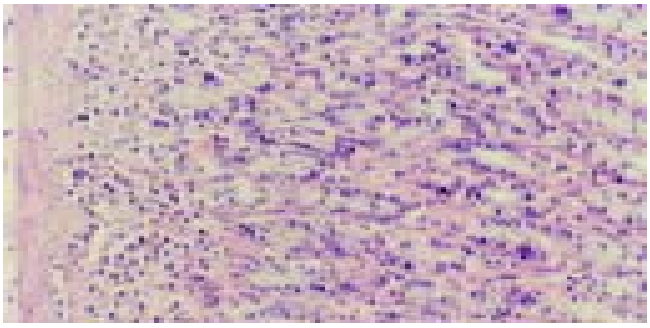

Figure 2 Severe infiltration of inflammatory cells, decreased parietal cells in oxyntic glands in gastric body of rats with gastritis induced by factor A with no changes in thickness of lamina propria. The results induced by factors A+B+C, A+C, A+B were similar. HE stain×100. Factor A: Combined intragastric treatments of 600 mL/L alcohol, 20 mmol/L sodium deoxycholate and 0.5 g/L ammonia; Factor B: Treatment of forage containing low levels of vitamins; Factor C: Treatment of indomethacin.

Effect of glandular atrophy on gastric mucosa

At antrum, the lamina propria mucosa of rats induced by single factor A was much thinner than that of the control ($P<0.01$, Table 2) while the lamina propria of rats induced by single factor B or C had no difference from that of the control and there was no statistical difference between that of rats induced by factors A+B+C, A+B, A+C and that of rats induced by single factor A (Table 2). By calculating the percentage ratio of pyloric gland area to total lamina propria area at antrum, we found the results were similar (Table 3, Figure 1). In addition, we found that although factors A, B or C had no effect on inducing the changes of lamina propria in gastric body, factor A induced a decrease of parietal cells in oxyntic glands. However, combined treatment of A+B+C, A+B, A+C had no synergistic effect with single factor A (Tables 2, 3 and Figure 2). Also no atypical hyperplasia and intestinal metaplasia in mucosa of gastric body and antrum in rats were observed in this study.

Table 1 $L_8(2^7)$ orthogonal test results of inflammation grades in stomachs of rats

Treatment factors	Triple orthogonal tests results					
	Antrum			Body		
A+B+C	2.10	1.83	2.00	1.67	1.60	1.64
A+B	1.67	1.50	1.63	1.30	1.40	1.38
A+C	2.10	2.10	1.83	1.67	1.64	1.67
A	1.50[b]	1.67[b]	1.75[b]	1.29[b]	1.25[b]	1.40[b]
B+C	1.17	1.25	1.00	1.00	1.08	0.90
B	0.75	0.75	0.83	0.60	0.67	0.63
C	1.25[d]	1.17[d]	1.00[d]	0.88[d]	1.00[d]	1.10[d]
Control	0.75	0.83	0.75	0.60	0.64	0.67

[b]$P<0.01$ *vs* the control, [d]$P<0.01$ *vs* the control. Factor A: Combined intragastric treatments of 60% alcohol, 20 mmol/L sodium deoxycholate and 0.5 g/L ammonia; Factor B: Treatment of forage containing low levels of vitamins; Factor C: Treatment of indomethacin.

Table 2 $L_8(2^7)$ orthogonal test results of thickness of lamina propria in stomachs of rats

Treatment factors	Triple orthogonal tests results (μm)					
	Antrum			Body		
A+B+C	40.0	38.5	36.5	310.0	290.0	305.0
A+B	43.5	41.5	45.0	383.5	283.5	338.5
A+C	46.5	36.5	36.5	311.5	313.5	325.0
A	43.5[b]	41.5[b]	45.0[b]	358.5	331.5	330.0
B+C	70.0	72.0	66.5	308.5	328.5	341.5
B	65.0	75.0	68.5	333.8	333.5	326.5
C	71.5	71.0	75.0	333.5	325.0	311.5
Control	66.5	71.5	75.0	300.0	310.0	313.5

[b]$P<0.01$ *vs* the control. Factor A: Combined intragastric treatments of 600 mL/L alcohol, 20 mmol/L sodium deoxycholate and 0.5 g/L ammonia; Factor B: Treatment of forage containing low levels of vitamins; Factor C: Treatment of indomethacin.

Table 3 $L_8(2^7)$ orthogonal test results of changes of glands in gastric lamina propria of rats

Treatment factors	Triple orthogonal tests results					
	Percentage ratio of pyloric gland area to total lamina propria at gastric antrum			Parietal cell number in observed oxyntic glands in gastric body		
A+B+C	0.2188	0.3750	0.3125	12	10	13
A+B	0.3750	0.3000	0.3750	14	12	14
A+C	0.3650	0.3125	0.3333	11	11	12
A	0.3438[b]	0.3750[b]	0.3230[b]	14[b]	12[b]	15[b]
B+C	0.5688	0.5150	0.5725	28	23	25
B	0.5385	0.5625	0.5313	25	26	23
C	0.5750	0.5840	0.6500	26	23	27
Control	0.5250	0.6050	0.5893	25	29	23

[b]$P<0.01$ *vs* the control. Factor A: Combined intragastric treatments of 600 mL/L alcohol, 20 mmol/L sodium deoxycholate and 0.5 g/L ammonia; Factor B: Treatment of forage containing low levels of vitamins; Factor C: Treatment of indomethacin.

DISCUSSION

CAG has been considered a precancerous disease[2], it is essential to establish a stable, economic and effective experimental animal model of chronic gastritis including CAG for further study on gastritis. Previous studies showed that three methods were practicable to establish experimental models. (1) Biologically induced animal models. Animals such as rats, cats were infected with *Helicobacter pylori* (*H pylori*) and an experimental model was induced. However, just some of these animals could be induced and models were unstable and it was time-consuming. (2) Animal models induced by physiochemical injury. Models of chronic gastritis were induced by single factors such as alcohol or sodium deoxycholate or ammonia or X-ray irradiation, but the models were unstable and atypical. (3) Immunologically induced animal models. Models of CAG were induced by hypodermic injection with homogeneic, xenogeneic or isogeneic stomach antigens,but the procedure was complicated and expensive, the effects were unstable[3-14].

The shortcomings of the above methods have limited further studies on etiology of CAG. However, according to previous studies, we concluded that factors such as infection of *H pylori*, excessive drinking, reflux of duodenal juice, long-term intake of nonsteroid drugs, malnutrition that could induce continuous gastritis, were considered to be the etiology of CAG[15-25]. In our study we tried to administer 600 mL/L alcohol to injure gastric mucosa, 20 mmol/L sodium deoxycholate (DOC-Na) as a simulator to reflux of duodenal juice, 0.5 g/L indomethacin to interfere with synthesis of prostaglandin (PG) that was proved to be effective for preventing gastric mucosa from injury and 0.5 g/L ammonia as a simulator to *H pylori* to induce continuous gastritis in rats. Finally we found that combined administration of 600 mL/L alcohol, 20 mmol/L DOC-Na and 0.5 g/L ammonia for 12 wk could induce an animal model of chronic gastritis with some features of early CAG especially in antrum. Furthermore, our study showed that atrophy of gastric mucosa in rats also developed from gastric antrum to body. It proved once again that excessive drinking, reflux of bile and infection of *H pylori* played a vital role in inducing chronic gastric mucosal inflammation. It also suggests that the above factors may probably induce CAG. However, we found that indomethacin induced only chronic gastritis but not atrophy of glands in mucosa and had no effect on enhancing glandular atrophy induced by alcohol, ammonia and DOC-Na. In addition, single treatment of forage containing few vitamins in our study could not induce chronic gastritis and glandular atrophy, it is perhaps because the testing duration was too short. The long-term roles of these factors in gastric inflammation and atrophy need to be further elucidated.

In conclusion, an animal model of chronic gastritis can be established by combined intragastric administration with 60% alcohol, 20 mmol/L sodium deoxycholate and 0.5 g/L ammonia. These factors play a vital role in etiology of chronic atrophic gastritis.

REFERENCES

1 **Dixon MF**, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated sydney system. International workshop on the histopathology of gastritis, houstou 1994. *Am J Surg Pathol* 1996; **20**: 1161-1181

2 **Kapadia CR**. Gastric atrophy, metaplasia, and dysplasia: a clinical perspective. *J Clin Gastroenterol* 2003; **36**(5 Suppl): S29-36

3 **Hu PJ**, Zeng ZR, Lin HL, Chen MH, Chen W, Peng XZ. Effect of eradication of *Helicobacter pylori* on development and reversion of atrophic gastritis in animal study. *Zhonghua Xiaohua Zazhi* 2000; **20**: 155-158

4 **Oda T**, Marakami K, Nishizono A, Kodama M, Nasu M, Fujioka T. Long-term *Helicobacter pylori* infection in Japanese monkeys induces atrophic gastritis and accumulation of mutations in the p53 tumor suppressor gene. *Helicobacter* 2002; **7**: 143-151

5 **Kinoshita K**, Watanabe H, Ando Y, Katayama M, Yamamoto H, Hirano N, Yoshikuni S, Yamamoto T. Effects of subtotal resection of the fundus on development of intestinal metaplasia induced by X-ray irradiation in Donryu rats. *Pathol Int* 2000; **50**: 879-883

6 **Kawano S**, Tsujii M, Fusamoto H, Sato N, Kamada T. Chronic effect of intragastric ammonia on gastric mucosal structures in rats. *Dig Dis Sci* 1991; **36**: 33-38

7 **Watanabe H**, Hirose F, Takizawa S, Terada Y, Fujii I. Morphological and biochemical changes in the gastric mucosa of A/HEJ mice injected with a xenogeneic stomach antigen. *Acta Pathol Jpn* 1977; **27**: 869-876

8 **Watanabe H**, Uesaka T, Kido S, Ishimura Y, Shiraki K, Kuramoto K, Hirata S, Shoji S, Katoh O, Fujimoto N. Gastric tumor induction by 1, 2-dimethylhydrazine in Wistar rats with intestinal metaplasia caused by X-irradiation. *Jpn J Cancer Res* 1999; **90**: 1207-1211

9 **Eaton KA**, Radin MJ, Krakowka S. An animal model of gastric ulcer due to bacterial gastritis in mice. *Vet Pathol* 1995; **32:** 489-497

10 **Lee A**, Chen M, Coltro N, O'Rourke J, Hazell S, Hu P, Li Y. Long term infection of the gastric mucosa with *Helicobacter species* does induce atrophic gastritis in an animal model of *Helicobacter pylori* infection. *Zentralbl Bakteriol* 1993; **280**: 38-50

11 **Wang X**, Willen R, Svensson M, Ljungh A, Wadstrom T. Two – year follow-up of *Helicobacter pylori* infection in C57BL/6 and Balb/cA mice. *APMIS* 2003; **111**: 514-522

12 **Ikeno T**, Ota H, Sugiyama A, Ishida K, Katsuyama T, Genta RN, Kawasaki S. *Helicobacter pylori*-induced chronic active gastritis, intestinal metaplasia, and gastric ulcer in Mongolian gerbils. *Am J Pathol* 1999; **154**: 951-960

13 **Kim DH**, Kim SW, Song YJ, Oh TY, Han SU, Kim YB, Joo HJ, Cho YK, Kim DY, Cho SW, Kim MW, Kim JH, Hahm KB. Long-term evaluation of mice model infected with *Helicobacter pylori*: focus on gastric pathology including gastric cancer. *Aliment Pharmacol Ther* 2003; **18**(Suppl 1): 14-23

14 **Bergin IL**, Sheppard BJ, Fox JG. *Helicobacter pylori* infection and high dietary salt independently induce atrophic gastritis and intestinal metaplasia in commercially available outbred Mongolian gerbils. *Dig Dis Sci* 2003; **48**: 475-485

15 **Kamada T**, Haruma K, Hata J, Kusunoki H, Sasaki A, Ito M, Tanaka S, Yoshihara M. The long-term effect of *Helicobacter pylori* eradication therapy on symptoms in dyspeptic patients with fundic atrophic gastritis. *Aliment Pharmacol Ther* 2003; **18**: 245-252

16 **Kearney DJ**, Ritchie K, Peacock JS. Gastric-juice ammonia assay for diagnosis of *Helicobacter pylori* infection and the relationship of ammonia concentration to gastritis severity. *Am J Gastroenterol* 2000; **95**: 3399-3403

17 **Huizenga JR**, Vissink A, Kuipers EJ, Gips CH. *Helicobacter pylori* and ammonia concentrations of whole, parotid and submandibular/sublingual saliva. *Clin Oral Investig* 1999; **3**: 84-87

18 **Bujanda L**. The effects of alcohol consumption upon the gastrointestinal tract. *Am J Gastroenterol* 2000; **95**: 3374-3382

19 **Thuluvath P**, Wojno KJ, Yardley JH, Mezey E. Effects of *Helicobacter pylori* infection and gastritis on gastric alcohol dehydrogenase activity. *Alcohol Clin Exp Res* 1994; **18**: 795-798

20 **Brown AS**, Fiatarone JR, Wood P, Bennett MK, Kelly PJ, Rawlins MD, Day CP, James OF. The effect of gastritis on human gastric alcohol dehydrogenase activity and ethanol metabolism. *Aliment Pharmacol Ther* 1995; **9**: 57-61

21 **Bielecki K**, Zawadzki JJ. Observations on gastric histology, endoscopy appearance and *Helicobacter pylori* after corrective surgery for bile reflux gastritis. *Mater Med Pol* 1994; **26**: 9-12

22 **Dixon MF**, Mapstone NP, Neville PM, Moayyedi P, Axon AT. Bile reflux gastritis and intestinal metaplasia at the cardia. *Gut* 2002; **51**: 351-355

23 **Ito Y**, Suzuki K, Ichino N, Imai H, Sakaguchi H, Hokama M, Nishii M, Nakano H. The risk of *Helicobacter pylori* infection and atrophic gastritis from food and drink intake: a cross-sectional study in Hokkaido, Japan. *Asian Pac J Cancer Prev* 2000; **1**: 147-156

24 **Kitahara F**, Shimazaki R, Sato T, Kojima Y, Morozumi A, Fujino MA. Severe atrophic gastritis with *Helicobacter pylori* infection and gastric cancer. *Gastric Cancer* 1998; **1**: 118-124

25 **Ohkuma K**, Okada M, Murayama H, Seo M, Maeda K, Kanda M, Okabe N. Association of *Helicobacter pylori* infection with atrophic gastritis and intestinal metaplasia. *J Gastroenterol Hepatol* 2000; **15**: 1105-1112

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