PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

ELSEVIER

World J Gastroenterol 2005;11(32):5029-5031
World Journal of Gastroenterology ISSN 1007-9327

• *BRIEF REPORTS* •

Effect of *Helicobacter pylori* infection on gastric mucosal pathologic change and level of nitric oxide and nitric oxide synthase

Yong-Fu Wang, Chun-Lin Guo, Li-Zhen Zhao, Guo-An Yang, Peng Chen, Hong-Kun Wang

Yong-Fu Wang, Chun-Lin Guo, Li-Zhen Zhao, Guo -An Yang, Peng Chen, Hong-Kun Wang, First Hospital, Baotou Medical College, University of Science and Technology of Inner Mongolia, Baotou 014010, Inner Mongolia, China
Supported by the Science and Technology Committee of Baotou, China, No. 2000-26
Correspondence to: Dr. Yong-Fu Wang, Baotou Medical College, University of Science and Technology of Inner Mongolia, Baotou 014010, Inner Mongolia, China. wyf5168@hotmail.com
Telephone: +86-472-2178195 Fax: +86-472-2129235
Received: 2004-10-09 Accepted: 2004-12-21

Abstract

AIM: To investigate the level of nitric oxide (NO) and nitrous oxide synthase (NOS) enzyme and its effect on gastric mucosal pathologic change in patients infected with *Helicobacter pylori* (*H pylori*), and to study the pathogenic mechanism of *H pylori*.

METHODS: The mucosal tissues of gastric antrum were taken by endoscopy, then their pathology, *H pylori* and anti-CagA-IgG were determined. Fifty *H pylori* positive cases and 35 *H pylori* negative cases were randomly chosen. Serum level of NO and NOS was detected.

RESULTS: One hundred and seven cases (71.33%) were anti-CagA-IgG positive in 150 *H pylori* positive cases. The positive rate was higher especially in those with pre-neoplastic diseases, such as atrophy, intestinal metaplasia and dysplasia. The level of NO and NOS in positive group was higher than that in negative group, and apparently lower in active gastritis than in pre-neoplastic diseases such as atrophy, intestinal metaplasia and dysplasia.

CONCLUSION: *H pylori* is closely related with chronic gastric diseases, and type I *H pylori* may be the real factor for *H pylori*-related gastric diseases. Infection with *H pylori* can induce elevation of NOS, which produces NO.

Key words: *Helicobacter pylori*; Nitric oxide; Nitric oxide synthase; Gastric mucosa; Pathology

Wang YF, Guo CL, Zhao LZ, Yang GA, Chen P, Wang HK. Effect of *Helicobacter pylori* infection on gastric mucosal pathologic change and level of nitric oxide and nitric oxide synthase. *World J Gastroenterol* 2005; 11(32): 5029-5031
http://www.wjgnet.com/1007-9327/11/5029.asp

INTRODUCTION

There is evidence that *Helicobacter pylori* (*H pylori*) is closely related with gastric carcinoma, and is considered as the first grade oncogene of gastric carcinoma by World Health Organization (WHO). *H pylori* infection correlates closely with gastric mucous pathology[1-4].

NO is a medium produced in vessel endothelial cells or smooth muscle cells by NOS[5,6]. As an inflammatory medium, NO plays an important role in the physical function and pathological process. Changes of NO in serum and tissue are related with damage to gastric mucosa and *H pylori* infection[7-11].

This study aimed to investigate the changes of NO, NOS and the pathological transformation of gastric mucosa in patients infected with *H pylori*.

MATERIALS AND METHODS

Patients

Two hundred and eighty-two patients with chronic gastric disease were enrolled in this study. *H pylori* was detected by both rapid urease test and real-time fluorescent quantitative PCR in these patients. Anti-CagA-IgG was detected in the *H pylori* positive patients, the serum samples were collected from 50 *H pylori* positive patients and 35 *H pylori* negative patients for detection of NO and NOS.

Real-time fluorescent quantitative PCR

Real-time fluorescent quantitative PCR was performed with PCR kit (Da'an Gene Diagnosis Center, Guangzhou). Fluorescence was detected with a type DA620 fluorescent detector.

Cag A H pylori-IgG

CagA *H pylori* IgG was detected according to the manufacturer's instructions (Shanghai Jingying Biology Corporation).

Measurement of NO and NOS

Because NO could be converted into NO_2^- and NO_3^- *in vivo*, nitrate reductase was used to deoxidize NO_3^- into NO_2^-, and to determinate its concentration. NO and NOS were tested with the kits, (Nanjing Jiancheng Biology Corporation).

Statistical analysis

Data were presented as mean±SD and analyzed with SPSS software. Statistical analysis was performed using two-tailed Student's *t* test and χ^2 test. $P<0.05$ was considered statistically significant.

RESULTS

Relationship between H pylori infection and pathology

Among the 282 cases, *H pylori* was found in 150 cases, (53.19%), including 38.54% (37/96) in chronic superficial gastritis group, 51.26% (61/119) in atrophic gastritis group, 73.17% (30/41) in intestinal metaplasia group, and 84.62% (22/26) in dysplasia group. The *H pylori* positive rate in atrophic gastritis group was higher than that in chronic superficial gastritis group ($P<0.05$), and significantly higher in intestinal metaplasia group and dysplasia group than that in chronic superficial gastritis group ($P<0.01$, Table 1).

Table 1 *H pylori* positive rate in chronic gastric disease (%)

Group	*n*	*H pylori* positive	*H pylori* negative
CSG	96	37 (38.54)	59 (61.46)
CAG	119	61 (51.26)[a]	58 (48.74)
IM	41	30 (73.17)[b]	11 (26.83)
Dysplasia	26	22 (84.62)[b]	4 (15.38)
Total	282	150 (53.19)	132 (46.81)

[a]$P<0.05$, [b]$P<0.01$ *vs* CSG.

Relationship between anti-CagA-IgG and pathology

The anti-CagA-IgG positive rate was 71.33% (107/150) in 150 *H pylori* positive patients, including 40.54% (15/37) in chronic superficial gastritis group, 75.41% (46/61) in atrophic gastritis group, 86.67% (26/30) in intestinal metaplasia group and 90.91% (20/22) in dysplasia group. The anti-CagA-IgG positive rate in chronic superficial gastritis group was significantly lower than that in the other three groups (Table 2).

Table 2 Positive rate of anti-CagA in 150 *H pylori* positive patients (%)

Group	CSG	CAG	IM	Dysplasia
n	37	61	30	22
Anti-CagA Positive (%)	15 (40.54)[b]	46 (75.41)	26 (86.67)	20 (90.91)

[b]$P<0.01$ *vs* Cag, IM, dysplasia.

Relationship between NO, NOS, and H pylori infection

The serum concentration of NO and NOS was 87.6±16.1 μmol/L and 51.4±13.3 μmol/L respectively in *H pylori* positive group, and 69.8±19.4 μmol/L and 35.2±13.3 μmol/L respectively in *H pylori* negative group (Table 3).

Table 3 Serum concentration of No and NOS(mean±SD)

Group	*n*	NO(μmol/L)	NOS(μmol/L)
H pylori positive	50	87.6±16.1[b]	51.4±13.3[b]
H pylori negative	35	69.8±19.4	35.2±13.3

[b]$P<0.01$ *vs H pylori* negative group.

Relationship between NO, NOS, and pathology

The serum concentration of NO in chronic superficial gastritis group was significantly lower than that in atrophic gastritis group, intestinal metaplasia group and dysplasia group ($P<0.05$, Table 4).

Table 4 Serum concentration of NO in different pathological groups (μmol/L, mean±SD)

Group	*H pylori* positive		*H pylori* negative	
	n	Concentration	*n*	Concentration
CSG	16	80.0±14.6[a]	11	62.2±16.9[a]
CAG	25	95.4±8.4	21	74.6±19.2
IM	12	91.2±13.9	4	75.5±27.7
Dysplasia	9	95.3±10.3	3	71.5±19.6

[a]$P<0.05$ *vs* atrophic gastritis group, intestinal metaplasia group, and dysplasia group.

The serum concentration of NOS in chronic superficial gastritis group was significantly lower than that in atrophic gastritis group, intestinal metaplasia group and dysplasia group ($P<0.05$), but there was no significant difference among the four groups (Table 5).

Table 5 Serum concentration of NOS in different pathological groups (μmol/L, mean±SD)

Group	*H pylori* positive		*H pylori* negative	
	n	Concentration	*n*	Concentration
CSG	16	38.0±12.4[a]	11	31.7±9.4
CAG	25	57.6±8.3	21	35.4±13.0
IM	12	54.8±8.3	4	26.1±4.4
Dysplasia	9	59.6±9.4	3	46.1±22.9

[a]$P<0.05$ *vs* atrophic gastritis group, intestinal metaplasia group, and dysplasia group.

DISCUSSION

H pylori infection plays a leading role in the pathogenesis of chronic gastritis. Furthermore, *H pylori* infection is also a high risk factor for the development of gastric cancer [12]. *H pylori* can destroy gastric mucosa, leading to inflammation of gastric mucosa and digestive symptoms.

Our study showed that the *H pylori* positive rate in chronic superficial gastritis group was 38.54%, suggesting that *H pylori* is related to inflammation of gastric mucosa. Other factors may be involved in inflammation of gastric mucosa, such as pH value, mucus, glycoprotein. But in atrophic gastritis group, intestinal metaplasia group, and dysplasia group, the *H pylori* positive rate was 51.26%, 73.17% and 84.62%, respectively, indicating that *H pylori* infection has a close relationship with gastric pre-neoplastic diseases, such as atrophy, intestinal metaplasia, and dysplasia.

It was reported that *H pylori* has two types. Type I *H pylori* possesses high virulence energy producing cytotoxin-associated protein A and vacuole toxin, which are responsible for inflammatory response of gastric epithelial cells, and promotes cell proliferation and apoptosis[13,14]. Therefore, type I *H pylori* has a close relationship with development of gastric pre-neoplastic diseases[15-18]. Our study showed that

the pathological change of gastric mucosa was parallel with the anti-CagA-IgG positive rate. These observations support the hypothesis that type I *H pylori* infection is a high risk factor for the development of gastric pre-neoplastic diseases.

It has been proved that there are lots of NOS in smooth muscle cells and myenteric nerve plexus of stomach[19], which are induced to produce endogenic NO by cytotoxins of *H pylori*. Moreover, a high pH value is beneficial for anaerobes to colonize in the stomach, and can degrade nitrate of food into nitrite. NO is regarded as an important inflammatory medium, related with acute and chronic inflammatory responses[20-22]. But NO seems to have both beneficial and harmful effects on different stages of inflammation. In earlier period, NO can relieve mucosal inflammation and prevents cellular damage. However, it can prevent cellular apoptosis, induce mutation and contribute to the development of gastric pre-neoplastic diseases in later period[23].

In this study, the levels of NO and NOS in chronic superficial gastritis group were significantly lower than those in pre-neoplastic diseases groups, such as atrophic gastritis group, intestinal metaplasia group, and dysplasia group in *H pylori* positive patients, but the condition existed not only in *H pylori* positive group, but also in *H pylori* negative group, suggesting that the serum level of NO induced by *H pylori* may be related with pre-neoplastic diseases. In *H pylori* negative patients, the levels of NOS had no difference in every pathological group, but the levels of NO were significantly higher in gastric pre-neoplastic disease groups, showing that other ways may stimulate the producing of NO besides *H pylori* in pre-neoplastic diseases. However, we believe that NO plays an important role in the development of pre-neoplastic diseases.

REFERENCES

1 **Sepulveda A,** Peterson LE, Shelton J, Gutierrez O, Graham DY. Histological patterns of gastritis in *H pylori*-infected individuals with a family history of gastric cancer. *Am J Gastroenterol* 2002; **97**: 1365-1370

2 **Nogueira C,** Figueiredo C, Carneiro F, Gomes AT, Barreira R, Figueira P, Salgado C, Belo L, Peixoto A, Bravo JC, Bravo LE, Realpe JL, Plaisier AP, Quint WG, Ruiz B, Correa P, van Doorn LJ. *Helicobacter pylori* genotypes may determine gastric histopathology. *Am J Pathol* 2001; **158**: 647-654

3 **Parsonnet J,** Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK. Helicobacter pylori infection and the risk of gastric carcinoma. *N Engl J Med* 1991; **325**: 1127-1131

4 **Takeuchi K,** Ohno Y, Tsuzuki Y, Ando T, Sekihara M, Hara T, Kuwano H. *Helicobacter pylori* infection and early gastric cancer. *J Clin Gastroenterol* 2003; **36**: 321-324

5 **Antos D,** Enders G, Rieder G, Stolte M, Bayerdorffer E, Hatz RA. Inducible nitric oxide synthase expression before and after eradication of Helicobacter pylori in different forms of gastritis. *Immunol Med Microbiol* 2001; **30**: 127-131

6 **Yanaka A,** Muto H, Fukutomi H, Ito S, Silen W. Role of nitric oxide in restitution of injured guinea pig gastric mucosa *in vitro*. *Am J Physiol* 1995; **268** (6 Pt1): G933-942

7 **Bredt DS,** Hwang PM, Glatt CE, Lowenstein C, Reed RR, Snyder SH. Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. *Nature* 1991; **351**: 714-718

8 **Lin HC**, Yang MC, Hou MC, Lee FY, Huang YT, Lin LF, Li SM, Hwang SJ, Wang SS, Tsai YT, Lee SD. Role of endotoxaemia in hyperdynamic circulation in rats with extrahepatic or intrahepatic portal hypertension. *J Gastroenterol Hepatol* 1996; **11**: 422-428

9 **Lin RS,** Lee FY, Lee SD, Tsai YT, Lin HC, Lu RH, Hsu WC, Huang CC, Wang SS, Lo KJ. Endotoxemia in patients with chronic liver diseases: relationship to severity of liver diseases, presence of esophageal varices, and hyperdynamic circulation. *J Hepatol* 1995; **22**: 165-172

10 **Albillos A,** de la Hera A, Gonzalez M, Moya JL, Calleja JL, Monserrat J, Ruiz-del-Arbol L, Alvarez-Mon M. Increased lipopolysaccharide binding protein in cirrhotic patients with marked immune and hemodynamic derangement. *Hepatology* 2003; **37**: 208-217

11 **Vallance P,** Moncada S. Hyperdynamic circulation in cirrhosis: a role for nitric oxide? *Lancet* 1991; **337**: 776-778

12 **Yao YL,** Zhang WD. The develop in research of nosogenetic factors of *H pylori*. *Shijie Huaren Xiaohua Zazhi* 2002; **10**: 455-458

13 **Telford JL,** Covacci A, Ghiara P, Montecucco C, Rappuoli R. Unravelling the pathogenic role of *Helicobacter pylori* in peptic ulcer:potential new therapies and vaccines. *Trends Biotecchnol* 1994; **12**: 420-426

14 **Marchetti M,** Arico B, Burroni D, Figura N, Rappuoli R, Ghiara P. Development of a mouse model of *Helicobacter pylori* infection that mimics human disease. *Science* 1995; **267**: 1655-1658

15 **Xiao S,** Liu W. More attention to the diversity of clinical outcome in *Helicobacter pylori*. *Zhonghua Neike Zazhi* 1999; **38**: 437-438

16 **Hu FL,** Zhou DY, Jia PQ. The basic and the clinical of *Helicobacter pylori* infection. 1thed. *Beijing Technol Pub China* 2002: 172-176

17 **Zhang L,** Zhang LX, Zhang NX, Liu YG, Yan XJ, Han FC, Huo Y. The case control study of the relationship of *Helicobacter pylori* CagA and gastric carcinoma. *Shijie Huaren Xiaohua Zazhi* 2002; **10**: 593-594

18 **Yang WH,** Lin SR, Jin Z. The relationship of *Helicobacter pylori* nosogenetic factors and gastric mucosa pathology. *Zhonghua Xiaohua Zazhi* 2000; **1**: 56-57

19 **Stuehr DJ,** Marletta MA. Mammalian nitrate biosynathesis: mourse mactrophages prodece nitrate and nitrate in response to Escherichis coli lipopolysscaride. *Proc Natl Acad Sci USA* 1985; **82**: 7738-7742

20 **Mannick EE,** Bravo LE, Zarama G, Realpe JL, Zhang XJ, Ruiz B, Fontham ET, Mera R, Miller MJ, Correa P. Inducible nitric oxide synthase, nitrosine and apoptosis in *helicobacter pylori* gastritis. effect of a antibiotics and antioxidants. *Cancer Res* 1996; **56**: 3238-3243

21 **Akimoto M,** Hashimoto H, Shigemoto M, Yamashita K, Yokoyama I. Changes of nitric oxide and growth factors during gastric ulcer healing. *J Cardiovasc Pharmacol* 2000; **36**(5 Suppl 1): S282-S285

22 **Zhang X,** Ruiz B, Correa P, Miller MJ. Cellular dissociation of NF-kappaB and inducible nitric oxide synthase in *Helicobacter pylori* infecion. *Free Radic Biol Med* 2000; **29**: 730-735

23 **Natanson C**, Hoffman WD, Suffredini AF, Eichacker PQ, Danner RL. A therapeutic target in sepsis. *Am Intern Med* 1994; **120**: 778-783

Science Editor Wang XL and Guo SY **Language Editor** Elsevier HK