THE ROLE OF NITRIC OXIDE IN STRESS-INDUCED GASTRIC DAMAGE IN CHOLESTATIC RATS

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Abstract - In this study the effect of nitric oxide synthesis inhibition on stress-induced gastric damage was evaluated in bile duct ligated, sham operated and unoperated rats. Animals were injected intraperitoneally with N^G-nitro-L-arginine methylester (L-NAME), 40 mg/kg, L-arginine, 200 mg/kg or saline, 30 min before water-immersion stress. One hour after water immersion, the animals were killed and their stomachs were removed for measurement of gastric mucosal damage. The results showed that L-NAME significantly enhances the development of gastric mucosal lesion in sham operated and unoperated rats, while in bile duct ligated animals, L-NAME decreases and L-arginine enhances the potentiation of stress-induced gastric mucosal damage. The results suggest that inhibition of nitric oxide synthase with L-NAME has different effects on stress-induced gastric damage in cholestatic rats compared with normal animals.

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Key words: Nitric oxide, cholestasis, stress gastric damage

INTRODUCTION

It is known that fatal upper gastrointestinal bleeding often occurs in critically ill or postoperative patients with obstructive jaundice (1), and the frequency of gastrointestinal ulcerations are higher in jaundiced patients compared with normal population (2). Several experimental studies have shown that gastric mucosa of cholestatic animals is more vulnerable to water-immersion stress (3) and gastroinvasive agents such as aspirin and taurocholate (4,5). Previous reports have also referred to decrease in gastric wall blood flow (6), decrease of mucosal noradrenaline and PGE2 (1), increased gastric acid output (3) and mucosal free radical formation (7) in rats with obstructive cholestasis. Recently it has been implicated that nitric oxide (NO) has an important role in regulation of gastric wall blood flow, gastric acid and mucus secretion (8). Tanaka and coworkers (9) have reported that preadministraion of a nitric oxide synthase (NOS) inhibitor, N^G-monomethyl L-arginine, enhances stress-induced gastric lesion development, and have suggested a protective function for NO against stress-induced gastric damage. The aim of the present study was to evaluate the role of NO on stress - induced gastric damage in cholestatic conditions.

MATERIALS AND METHODS

Animal manipulation

Male albino rats weighing 200-250 g were used in this experiment. All animals were given free access to food and water. Laparotomy was performed under general anesthesia induced by an intraperitoneal injection of Ketamine HCl (Gedoon Richter LTD, Hungary), 50 mg/kg and Xylazine HCl (Bayer AG, Leverkusen, Germany), 10 mg/kg. The bile duct was isolated and doubly ligated using the method of Cameron and Oakley (10). Sham operation consisted of laparotomy and bile duct identification and manipulation without ligation. Unoperated age-matched rats also served as controls. Two weeks after the operation, the rats were fasted for 24 hours, but permitted free

access to water. In this 24-hour period the rats were housed in individual cages with a wire-mesh floor to prevent coprophagy.

Drug administration and water immersion stress

Rats were injected intraperitoneally with N^G-nitro-L-arginine methylester (L-NAME), 40 mg/kg (Sigma, St. Louis, MO, USA) or L-arginine, 200 mg/kg (Merck, Germany), which was dissolved in isotonic saline, 30 min prior to water - immersion stress (11). In each group (bile duct ligated, sham operated and unoperated rats), a number of animals were chosen as control and were treated with an equivalent volume of saline. The rats were restrained in a wire cage and immersed up to the depth of the xiphoid process in a 21-23°C water bath for one hour to produce water-immersion stress-induced gastric mucosal damage (8). Then the rats were sacrificed under ether anesthesia and blood samples were collected for determination of alkaline phosphatase activity.

Measurement of gastric mucosal lesions

The stomachs were removed and inflated by injection of 10 ml formalin 2% to fix the inner layers of gastric wall. After 20 min, the stomachs were incised along the greater curvature and lesions in the glandular portion were evaluated (12). Ulcer index, using the J-score (13), was calculated by classifying the erosions in size order: 0 to 1 mm in diameter=1, 1 to 2 mm=2 and greater than 2 mm=3. The sum of these points in each animal was defined as the ulcer index.

Measurement of alkaline phosphatase activity in plasma

Plasma alkaline phosphatase activity was measured with a colorimetric method, in which alkaline phosphatase catalyzed substrate para-nitrophenyl phosphate to 4-nitrophenoxide, a chromogenic material which can be detected with spectrophotometer in wavelength of 405 nm (14).

Statistical analysis

All data are presented as the mean±SEM. Statistical analysis of data was evaluated by means of analysis of variance (ANOVA) followed by the Newman-Keuls test for multiple comparisons, and a P-value of less than 0.05 was considered to be statistically significant.

RESULTS

One day after laparotomy, bile duct ligated (BDL) rats revealed manifestations of cholestasis (jaundice, dark urine and steatorrhea). After killing the animals, plasma alkaline phosphatase activities were significantly higher in BDL rats compared with sham operated (SHAM) and unoperated (UNOP) animals (BDL=331±5 U/l; SHAM=92±4 U/l; UNOP=93±4 U/l, $P<0.05$).

Fig. 1 shows the ulcer indices in SHAM and UNOP rats. There is no significant difference between SHAM/saline and UNOP/saline animals after stress-induced ulcerations. As is shown in this figure, L-NAME can increase the potentiation of stress-induced gastric damage in SHAM and UNOP animals ($P<0.05$ in both groups), while preadministration of L-arginine reduces the gastric mucosal lesion development ($P<0.05$ in SHAM group).

Fig. 2 shows the ulcer indices in BDL rats. Comparison of Fig. 1 and Fig. 2 reveals that gastric mucosal damage is significantly more severe in BDL/saline rats compared with SHAM/saline and UNOP/saline animals ($P<0.05$). As is shown in Fig. 2, preadministration of L-NAME in BDL rats prevents the gastric mucosal lesion development ($P<0.05$), while L-arginine increases the potentiation of stress-induced gastric damage ($P<0.05$).

DISCUSSION

The present study has shown that

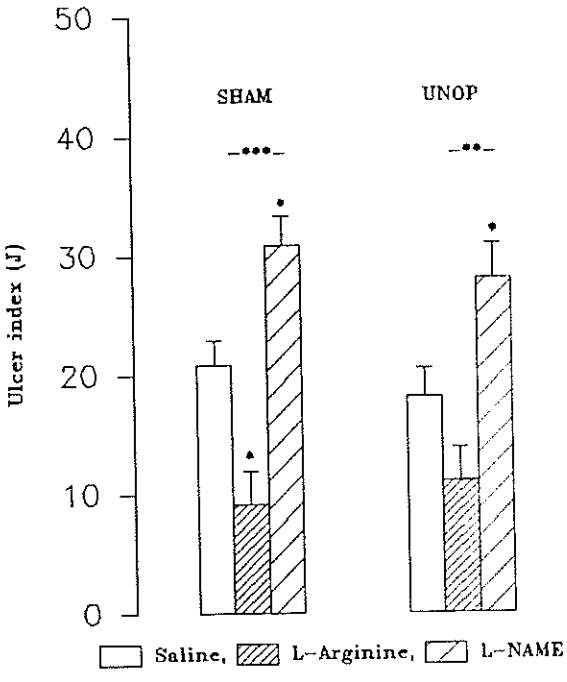

Fig. 1 Comparison of the ulcer index after L-NAME, L-arginine or saline administration in sham operated (SHAM) and unoperated (UNOP) rats. 6-8 rats were used in each group $^{*}P<0.05$ in comparison of saline group. $^{**}P<0.01$, $^{***}P<0.001$.

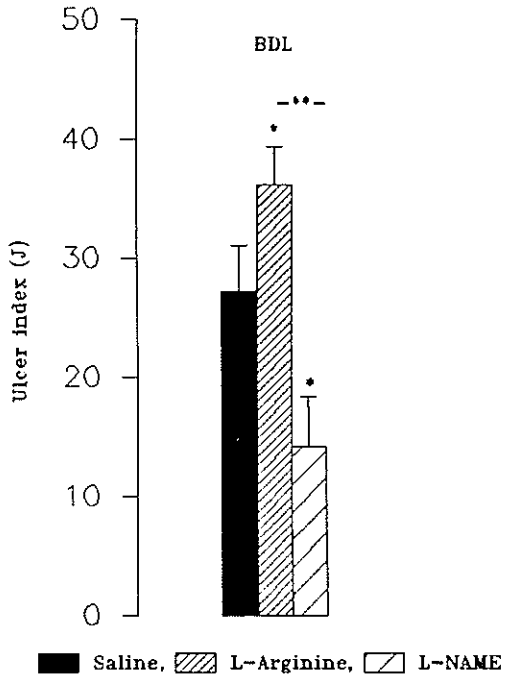

Fig. 2 Comparison of the ulcer index after L-NAME, L-arginine or saline administration in bile duct ligated (BDL) rats. 6-8 rats were used in each grouop. $^{*}P<0.05$ in comparison of saline group. $^{**}P<0.01$.

administration of L-NAME a nonselective NOS inhibitor prior to the water - immersion stress, significantly enhances the development of gastric mucosal lesion in normal rats (SHAM and UNOP), while in BDL groups, L-NAME decreases and L-arginine enhances the potentiation of stress-induced gastric mucosal damage. These results suggest that inhibition of NOS with L-NAME has different effects on stress-induced gastric damage in cholestatic condition compared with normal animals.

NO is synthesized from L-arginine by either a Ca^{+2}-dependent constitutive nitric oxide synthase (cNOS) or a Ca^{+2}-independent inducible nitric oxide synthase (iNOS). Both NOS have been detected in gastric mucosal cell isolated from rats (8). It has been widely accepted that in the digestive system, NO produced by cNOS is cytoprotective while excessive NO produced by iNOS is cytotoxic (8). Recently Zimmermann and coworkers (15) have reported that cNOS expression diminishes after bile duct ligation and

this change is reversed after biliary decompression surgery by choledoco-jejunostomy. Several studies have also suggested overproduction of NO in cirrhosis as well as experimental models of bile duct obstruction (16,17,18). According to Vallance and Moncada's hypothesis (18), NO overproduction may be due to elevated incidence of endotoxemia after bile duct ligation (19) that leads to overproduction of NO via a stimulatory effect on iNOS. However, some studies did not support this hypothesis (20,21). For example Fernandez and coworkers (20) could not show any significant increase of iNOS activity in bile duct ligated rats.

With regard to the fact that NO produced by cNOS is protective, while excessive NO produced by iNOS is cytotoxic in digestive system (8), the protective effect of L-NAME on stress-induced gastric damage in cholestatic rats may be due to overproduction of NO after bile duct ligation. The results of the present study suggest an important pathophysiologic role for arginine-nitric oxide pathway in the pathogenesis of the gastric ulcers in cholestatic subjects.

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