ELSEVIER

European Journal of Pharmacology 370 (1999) 283–286

ejp

N^G-nitro-L-arginine methylester is protective against ethanol-induced gastric damage in cholestatic rats

Arezo Nahavandi [a,*], Ahmad Reza Dehpour [b], Ali Reza Mani [a], Homayoun Homayounfar [a], Ali Abdoli [a]

[a] *Department of Physiology, Iran University of Medical Sciences, PO Box 14155-6183, Tehran, Iran*
[b] *Department of Pharmacology, Tehran University of Medical Sciences, Tehran, Iran*

Received 16 November 1998; received in revised form 4 March 1999; accepted 9 March 1999

Abstract

In this study the effect of nitric oxide (NO) synthesis inhibition on ethanol-induced gastric damage was evaluated in bile duct-ligated, sham-operated and unoperated rats. The animals were injected intraperitoneally with saline, L-arginine (200 mg/kg) or N^G-nitro-L-arginine methylester (L-NAME) in doses of 5, 15 and 30 mg/kg, 30 min before ethanol administration. The animals were killed 1 h after ethanol administration and their stomachs were removed for measurement of gastric mucosal damage. The results showed that L-NAME significantly enhanced the development of gastric mucosal lesions in sham-operated and unoperated rats, while in bile duct-ligated animals, L-NAME decreased and L-arginine enhanced the potentiation of ethanol-induced gastric mucosal damage. The plasma level of nitrite and nitrate was also measured and was significantly higher in bile duct-ligated rats than in control groups. The results suggest that inhibition of NO synthase with L-NAME has different effects on ethanol-induced gastric damage in cholestatic groups and in normal rats and that these effects can be explained by overproduction of NO in bile duct-ligated animals.

Keywords: Cholestasis; Gastric damage; Nitric oxide (NO)

1. Introduction

Fatal upper gastrointestinal bleeding often occurs in critically ill or postoperative patients with obstructive jaundice (Urakawa et al., 1987), and the frequency of gastrointestinal ulcerations is higher in jaundiced patients than in the normal population (Bastid et al., 1990). Several experimental studies have shown that the gastric mucosa of cholestatic rats is more vulnerable than that of normal animals to water-immersion stress (Sasaki et al., 1986) and to gastroinvasive agents such as aspirin and taurocholate (Matsuo et al., 1989; Dehpour et al., 1998). Previous reports have also referred to a decrease in gastric wall blood flow (Sasaki et al., 1987), a decrease of mucosal noradrenaline and prostaglandin E_2 (Urakawa et al., 1987), an increased gastric acid output (Sasaki et al., 1986) and mucosal free radical formation (Shian et al., 1994) in rats with obstructive cholestasis.

MacNaughton et al. (1989) have reported that ethanol-induced gastric damage can be significantly reduced by nitric oxide (NO). It has been suggested that NO has an important role in the regulation of gastric wall blood flow (Pique et al., 1989), and of gastric acid and mucus secretion (Martinez-Cuesta et al., 1992; Brown et al., 1993). NO is synthesized from L-arginine by either a Ca^{2+}-dependent constitutive NO synthase or a Ca^{2+}-independent inducible NO synthase. Both NO synthase have been detected in gastric mucosal cells isolated from rats (Nishida et al., 1997). In the digestive system, NO produced by constitutive NO synthase is assumed to be cytoprotective while excessive NO produced by inducible NO synthase is cytotoxic (Nishida et al., 1997). Several studies have also suggested that there is overproduction of NO in experimental models of bile duct obstruction (Ferraz and Wallace, 1996; Ghafourifar et al., 1997; Inan et al., 1997) and the aim of the present study was to evaluate the role of NO in ethanol-induced gastric damage during cholestasis.

* Corresponding author. Tel.: +98-21-204-7429

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00168-5

2. Materials and methods

2.1. Animal manipulations

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, Budapest, Hungary), 50 mg/kg and xylazine HCl (Bayer, Leverkusen, Germany), 10 mg/kg. The bile duct was isolated and double-ligated using the method of Cameron and Oakley (1932). 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 h but were permitted free access to water. In this 24-h period the rats were housed in individual cages with a wire-mesh floor to prevent coprophagy.

2.2. Drug administration and ethanol-induced gastric damage

The rats were injected intraperitoneally with L-arginine (Merck, Germany), 200 mg/kg (Gyires, 1994) or N^G-nitro-L-arginine methylester (L-NAME, Sigma, St. Louis, MO, USA) in doses of 5, 15 and 30 mg/kg dissolved in isotonic saline, 30 min prior to ethanol administration. In each group (bile duct-ligated, sham-operated and unoperated rats), a number of animals were chosen as controls and were treated with an equivalent volume of saline. The rats were given 1 ml of 96% ethanol by gavage and killed 1 h later (Gyires, 1994). The rats were killed under ether anesthesia and blood samples were collected for determination of alkaline phosphatase activity and plasma level of nitrite and nitrate.

2.3. Measurement of gastric mucosal lesions

The stomachs were removed and inflated by injection of 10 ml formalin 2% to fix the inner layers of the gastric wall. After 20 min the stomachs were incised along the greater curvature (Hara et al., 1991). They were examined for lesions of the glandular portion by an observer who was unaware of the treatment the rats have received. The area of the erosions (mm^2) was calculated by measuring the width and length of erosions under a stereoscopic microscope (Shibasaki et al., 1990).

2.4. Measurement of alkaline phosphatase activity in plasma

Plasma alkaline phosphatase activity was measured with a colorimetric method, in which alkaline phosphatase catalyzes the substrate *para*-nitrophenyl phosphate to 4-nitrophenoxide, a chromogenic material which can be detected with a spectrophotometer at a wavelength of 405 nm (Pincus et al., 1991).

2.5. Plasma nitrite and nitrate assay

Plasma nitrite and nitrate levels were measured based on Griess reaction as previously described (Moshage, 1997).

2.6. Statistical analysis

All data are presented as the means ± S.E.M. Statistical evaluation of the data was done with the analysis of variance (ANOVA) followed by the Newman–Keuls test for multiple comparisons, and a P-value less than 0.05 was considered statistically significant.

3. Results

One day after laparotomy, bile duct-ligated rats revealed manifestations of cholestasis (jaundice, dark urine and steatorrhea). Plasma alkaline phosphatase activity was significantly higher in bile duct-ligated rats than in sham-operated and unoperated animals, 352 ± 6, 91 ± 4 and 92 ± 4 U/l ($P < 0.05$), respectively. The plasma levels of nitrite and nitrates were significantly higher in the bile duct-ligated rats than in sham-operated and unoperated animals, 72 ± 6, 42 ± 2 and 39 ± 3 μM ($P < 0.01$), respectively and L-NAME (15 mg/kg) reduced plasma levels of nitrite and nitrate in all three groups (bile duct-ligated = 35 ± 5 μM; sham-operated = 22 ± 3 μM; unoperated = 24 ± 3 μM).

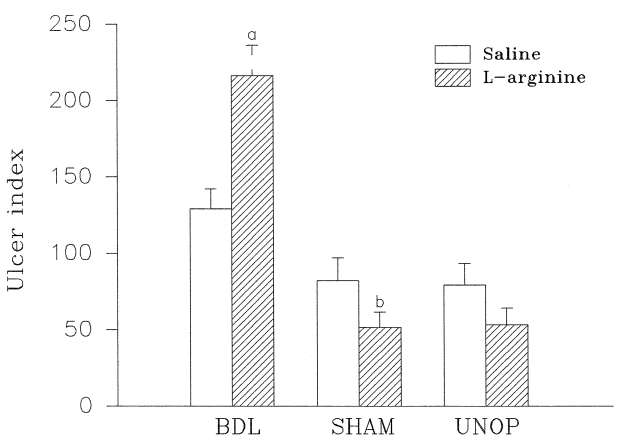

Fig. 1. Comparison of ulcer index (mm^2) between bile duct-ligated (BDL), sham-operated (SHAM) and unoperated (UNOP) rats, 1 h after ethanol administration. Hatched bars show the effect of L-arginine (200 mg/kg) administration on ethanol-induced gastric damage in the experimental groups. There were 8–10 rats were used in each group. Data are shown as means + S.E.M. There was no significant difference in mucosal damage between sham-operated and unoperated rats. Ulcer index was significantly higher in bile duct-ligated/saline rats than in sham-operated/saline and unoperated/saline animals ($P < 0.01$). (a) $P < 0.001$ in comparison with bile duct-ligated/saline. (b) $P < 0.05$ in comparison with sham-operated/saline.

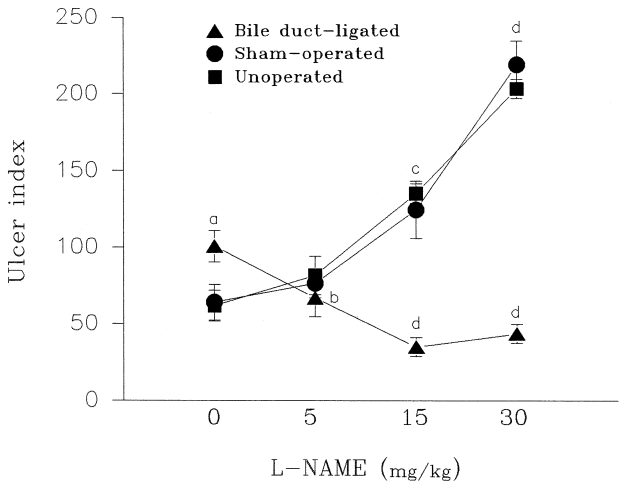

Fig. 2. Comparison of ethanol-induced gastric damage between bile duct-ligated, sham-operated and unoperated rats given L-NAME (dose–response experiments). There were 8–10 rats in each group. Data are shown as means ± S.E.M. (a) $P < 0.01$ in comparison with sham-operated and unoperated. (b) $P < 0.05$ in comparison with bile duct-ligated (dose = 0). (c) $P < 0.01$, (d) $P < 0.001$ (compared with dose = 0 of related groups).

Fig. 1 shows the ulcer indices in bile duct-ligated, sham-operated and unoperated rats. There was no significant difference between sham-operated/saline and unoperated/saline animals after ethanol-induced ulcerations but the gastric mucosal damage was significantly more severe in bile duct-ligated/saline rats than in sham-operated/saline and unoperated/saline animals ($P < 0.01$). As is shown in this figure, L-arginine increased the potentiation of ethanol-induced gastric damage in bile duct-ligated rats ($P < 0.001$), while preadministration of L-arginine reduced the gastric mucosal lesion development in sham-operated and unoperated animals ($P < 0.05$ in sham-operated group).

Fig. 2 shows the dose-dependent effect of L-NAME on ethanol-induced gastric damage in the experimental groups. As is shown in this figure, L-NAME had a different effect on gastric damage in bile duct-ligated rats in comparison with that in sham-operated and unoperated animals. L-NAME was protective against ethanol-induced gastric damage in bile duct-ligated rats and the most protective dose of L-NAME was 15 mg/kg, while in the control groups (sham-operated and unoperated) L-NAME induced a dose-dependent increase in mucosal lesion development.

4. Discussion

The present results have shown that administration of L-NAME a non-selective NO synthase inhibitor, prior to ethanol administration significantly enhanced the development of gastric mucosal lesions in normal rats (sham-operated and unoperated animals), while in bile duct-ligated groups, L-NAME decreased and L-arginine enhanced the potentiation of ethanol-induced gastric mucosal damage. These results suggest that inhibition of NO synthase with L-NAME has different effects on ethanol-induced gastric damage in rats with cholestasis and in the control groups.

Several studies have suggested overproduction of NO in cholestasis as well as in animal models of cirrhosis (Heinemann and Stauber, 1995; Ferraz and Wallace, 1996; Ghafourifar et al., 1997; Inan et al., 1997). In addition, the gastric wall blood flow response to exogenous NO/cGMP-dependent vasodilators is impaired in bile duct-ligated rats (Ferraz and Wallace, 1996). According to the hypothesis of Vallance and Moncada (1991), NO overproduction may be due to an elevated incidence of endotoxemia after bile duct-ligation (Raynolds et al., 1995; Inan et al., 1997) and endotoxemia may induce NO overproduction directly or indirectly through cytokines. Since Wardle and Wright (1970) first suggested an association between endotoxemia and cholestasis in 1970, there has been increasing evidence that gut-derived endotoxins are implicated in the pathophysiology of cholestasis (Inan et al., 1997). Chen et al. (1997) have reported that inducible NO synthase mRNA expression is increased in digestive tissue in response to endotoxin, while constitutive NO synthase mRNA showed simultaneous reduction in endotoxin-treated rats. However, the results of some studies did not support Vallance and Moncada's hypothesis (Fernandez et al., 1995; Kanwar et al., 1996). For example, Fernandez et al. (1995) could not show any significant increase of inducible NO synthase activity in bile duct-ligated rats.

We have shown that the plasma level of nitrite and nitrate is higher in cholestatic rats than in normal animals and this finding confirms the assumption of increased production of NO in cholestasis. The source of this overproduction however remains to be determined. Physiologically, endogenous NO produced by constitutive NO synthase regulates mucosal perfusion and has been suggested to protect the gastrointestinal mucosa from a variety of stimuli (Chen et al., 1997). Pathophysiologically, overproduction of NO may be involved in gastrointestinal injury (Nishida et al., 1997) and cell death through oxidative stress (Scarlett et al., 1996), DNA damage (Salgo et al., 1995), activation of poly (ADP-ribose) polymerase (Szabo, 1997) or dysregulation of cytosolic calcium (Richter et al., 1997). The protective effect of L-NAME on ethanol-induced gastric damage in bile duct-ligated rats can be explained by NO overproduction in cholestasis and suggests an important pathophysiological role for the arginine–NO pathway in the pathogenesis of gastric ulcers in cholestatic subjects.

Acknowledgements

Authors wish to express their thanks to Mr. Roozegar Seifi for his cooperation in the study.

References

Bastid, C., Tellechea, J., Sahel, J., 1990. Does jaundice increase the frequency of gastroduodenal ulcerations?. Hepatol. Gastroenterol. 37, 612–614.

Brown, J.F., Keates, A.C., Hanson, P.G., Whittle, B.J.R., 1993. Nitric oxide generators and cGMP stimulate mucus secretion by rat gastric mucosal cells. Am. J. Physiol. 265, G418–G422.

Cameron, G.R., Oakley, C.L., 1932. Ligation of common bile duct. J. Pathol. Bacteriol. 35, 769–798.

Chen, K., Inoue, M., Wasa, M., Fukuzawa, M., Kamata, S., Okada, A., 1997. Expression of endothelial constitutive nitric oxide synthase mRNA in gastrointestinal mucosa and its down regulation by endotoxin. Life Sci. 61, 1323–1329.

Dehpour, A.R., Mani, A.R., Alikhani, Z., Zeinoddini, M., Toor Savadkoohi, S., Ghaffari, K., Sharif, A., Sabbagh, B., Nowroozi, A., Sadr, S., 1998. Enhancement of aspirin-induced gastric damage by cholestasis in rats. Fundam. Clin. Pharmacol. 12, 442–445.

Fernandez, M., Garcia-Pagan, J.C., Casadevall, M., Bernadich, C., Peria, C., Whittle, B.J.R., Pique, J.M., Bosch, J., Rodes, J., 1995. Evidence against a role for inducible nitric oxide synthase in the hyperdynamic circulation of portal-hypertensive rats. Gastroenterology 108, 1487–1495.

Ferraz, J.G.P., Wallace, J.L., 1996. Prostaglandins modulate the responsiveness of the gastric microcirculation to sodium nitroprusside in cirrhotic rats. Hepatology 23, 123–129.

Ghafourifar, P., Dehpour, A.R., Akbarloo, N., 1997. Inhibition by L-NA, a nitric oxide synthase inhibitor, of naloxone-precipitated withdrawal signs in a mouse model cholestasis. Life Sci. 60, 265–270.

Gyires, K., 1994. The role of endogenous nitric oxide in the gastroprotective action of morphine. Eur. J. Pharmacol. 255, 33–37.

Hara, N., Hara, Y., Natsume, Y., 1991. Gastric hyperacidity and mucosal damage caused by hyperthermia correlates with increase in GABA concentrations of the rat brain. Eur. J. Pharmacol. 194, 77–81.

Heinemann, A., Stauber, R.E., 1995. The role of inducible nitric oxide synthase in vascular hyperactivity of endotoxin-treated and portal hypertensive rats. Eur. J. Pharmacol. 278, 87–90.

Inan, M., Sayek, I., Tel, B.C., Sahin-Erdemil, I., 1997. Role of endotoxin and nitric oxide in the pathogenesis of renal failure in obstructive jaundice. Br. J. Surg. 84, 943–947.

Kanwar, S., Kubes, P., Tepperman, B.L., Lee, S.S., 1996. Nitric oxide synthase activity in portal hypertensive and cirrhotic rats. J. Hepatol. 25, 85–89.

MacNaughton, W.K., Cirino, G., Wallace, J.L., 1989. Endothelium-derived relaxing factor (nitric oxide) has protective action in the stomach. Life Sci. 45, 1869–1876.

Martinez-Cuesta, M.A., Barrachina, M.D., Pique, J.M., Whittle, B.J.R., Esplugues, J.V., 1992. The role of nitric oxide and platelet-activating factor in the inhibition by endotoxin of pentagastrin-stimulated gastric acid secretion. Eur. J. Pharmacol. 218, 351–354.

Matsuo, T., Sasaki, T., Kamiyama, Y., Nitoh, H., Funayama, Y., Takahashi, M., Fukushima, K., Matsuno, S., 1989. Taurocholate-induced gastric damage in rats with obstructive jaundice. Scand. J. Gastroenterol. Suppl. 162, 83–86.

Moshage, H., 1997. Nitric oxide determination: much ado about NO-thing?. Clin. Chem. 43, 553–556.

Nishida, K., Ohta, Y., Ishiguro, I., 1997. Role of gastric mucosal constitutive and inducible nitric oxide synthesis in the development of stress-induced gastric mucosal lesions in rats. Biochem. Biophys. Res. Commun. 236, 275–279.

Pincus, M.R., Zimmermann, H.J., Henry, J.B., 1991. Clinical enzymology. In: Henry, J.B. (Ed.), Clinical Diagnosis and Management by Laboratory Methods. Saunders, Philadelphia, PA, pp. 250–285.

Pique, J.M., Whittle, B.J.R., Esplugues, J.V., 1989. The vasodilator role of endogenous nitric oxide in the rat gastric microcirculation. Eur. J. Pharmacol. 174, 293–296.

Raynolds, J.V., Murchan, P., Redmond, H.P., Warson, P.W., Leonard, N., Hill, A., Clarke, P., Marks, P., Keane, F.B., Tanner, W.A., 1995. Failure of macrophage activation in experimental obstructive jaundice. Br. J. Surg. 82, 534–538.

Richter, C., Ghafourifar, P., Schweizer, M., Laffranchi, R., 1997. Nitric oxide and mitochondrial calcium. Biochem. Soc. Trans. 25, 914–918.

Salgo, M.G., Stone, K., Squadrito, G.L., Battista, J.R., Pryor, W.A., 1995. Peroxynitrite causes DNA nicks in plasmid pBR322. Biochem. Biophys. Res. Commun. 210, 1025–1030.

Sasaki, I., Miyakawa, H., Kameyama, J., Kamiyama, Y., Sato, T., 1986. Influence of obstructive jaundice on acute gastric ulcer, intragastric PH and potential difference in rats. Tohoku J. Exp. Med. 150, 161–168.

Sasaki, I., Konno, Y., Kamiyama, Y., Sato, T., 1987. Acute gastric ulceration in rats with obstructive jaundice with special reference to gastric mucosal blood flow. Tohoku J. Exp. Med. 151, 351–358.

Scarlett, J.L., Packer, M.A., Porteous, C.M., Murphy, M.P., 1996. Alterations to glutathione and nicotinamide nucleotides during the mitochondrial permeability transition induces by peroxynitrite. Biochem. Pharmacol. 52, 1047–1055.

Shian, W.M., Sasaki, I., Kamiyama, Y., Natio, H., Funayama, Y., Takahashi, M., Iwatuki, A., Matsuno, S., 1994. Mucosal TBA reactants and glutathione levels in acute gastric mucosal injury in rats with obstructive jaundice. Ther. Res. 1, 77–83.

Shibasaki, T., Yamauchi, N., Hatta, M., Mauda, A., Oono, H., Wakabayashi, I., Ling, N., Demura, H., 1990. Brain corticotrophin-releasing factor acts as inhibitor of stress-induced gastric erosion in rats. Life Sci. 47, 925–932.

Szabo, C., 1997. Role of poly (ADP-ribose) synthetase activation in the suppression of cellular energetics in response to nitric oxide and peroxynitrite. Biochem. Soc. Trans. 25, 919–924.

Urakawa, T., Nagahata, Y., Nacamoto, M., Kumagai, K., Saitoh, Y., 1987. An approach to the mechanism of acute ulceration in obstructive jaundice. Scand. J. Gastroenterol. 22, 634–640.

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

Wardle, E.N., Wright, N.A., 1970. Endotoxin and acute renal failure associated with obstructive jaundice. Br. Med. J. 4, 472–474.