The protective role of endothelium-derived relaxing factor (nitric oxide) in the pathogenesis of ethanol-induced gastric mucosal injury in rats

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Nitric oxide (NO) synthesized from L-arginine by constitutive NO synthase, has an important modulatory role in the regulation of gastrointestinal integrity. Inhibition of endogenous NO formation by N°-nitro L-arginine (L-NOARG) (2.5 or 10 mg/kg i.v.) dose-dependently increased ethanol-induced gastric mucosal injury in rats. The effects of L-NOARG was abolished by pretreatment with L-arginine (500 mg/kg i.v.). These results indicate that inhibition of endogenous NO formation released from gastric mucosal vasculature may cause the gastric mucosa more susceptible to injury by ethanol most probably due to changes in gastric microcirculation. Moreover complete reversal of this increased ethanol-induced gastric mucosal damage by L-arginine pretreatment indicates the gastroprotective role of endogenous NO. [Turk J Med Res 1996; 14(3):81-84]

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NO is synthesized from L-arginine by constitutive NO synthase in endothelial cells and in neuronal tissue (1). Based on the use of NO synthase inhibitors such as L-NMMA and L-NOARG, NO has been postulated as a mediator of the nonadrenergic, noncholinergic (NANC) relaxation of the intestinal smooth muscle, including that of rat or guinea pig stomach and duodenum as well as canine duodenum (2-8).

Recently, NO was shown to play an important role in the regulation of gastric mucosal blood flow (9), gastric mucus, acid and alkaline secretion and thus in the maintenance of gastric mucosal integrity (9-11). NO donors such as glyceryl trinitrate, isoamyl nitrate or nitroprusside can protect against acute hemorrhagic mucosal injury provoked by alcohol and other topical irritants (12,13).

Endothelium-1 (ET-1) is a 21 amino acid peptide with potent vasoconstrictor actions (14). Recently, it has been shown to induce gastric mucosal injury most likely due to vasoconstriction in the stomach (15,16). It is reported that endogenous ET-1 plays an essential role in the pathogenesis of ethanol-induced gastric mucosal injury by causing microcirculatory disturbances (17). Lopz-Belmonte et al, have recently suggested that exogenous NO can protect the rat gastric mucosal damage induced by the vasoconstrictor peptide ET-1 (18). Although it is postulated that NO released locally by ethanol may influence gastric mucosal hemodynamics, the contribution of the endogenous NO in the protection of gastric mucosa from damage induced by ethanol is not yet fully clear (19). Since endogenous NO formation can be specifically inhibited by L-NOARG, we have now used this L-arginine analogue to investigate the role of endogenous NO produced by constitutive nitric oxide the synthase in the pathogenesis of gastric mucosal damage provoked by ethanol.

MATERIALS AND METHODS

Wistar albino rats of either sex, weighing 180-250 g, were deprived food but allowed free access to water 24 h before the experiment. Ethical approval was granted by the Ethic Committee of Kocaeli University. The animals were divided into following six groups; vehicle (phosphate buffered saline, PBS) plus 30 % ethanol; 0.5, 2.5 or 10 mg/kg L-NOARG plus 30 % ethan-
anol; 500 mg/kg L-arginine plus 2.5 or 10 mg/kg L-NOARG plus 30 % ethanol (5-7 rats in each group).

Fifteen minutes after intravenous administration of the vehicle (PBS: phosphate- buffered saline) or L-NOARG, dissolved in PBS, 30% ethanol (0.5 ml/100 g b. wt.) was given intragastrically by an orogastric tube (No 2. Rusch). In some experiments, rats were pre-treated with L-arginine, dissolved in distilled water, 10 minutes before the injection of L-NOARG. The rats were killed by an overdose of ether 30 minutes after the challenge of ethanol and the stomachs were removed and opened along the greater curvature. Macroscopic gastric mucosal lesions were measured as the sum of the length of hemorrhagic erosion. When assessing the size of petechiae, five such lesions were considered to be equivalent to 1 mm of ulcer. The sum of the lengths of each group was divided to the number of rats in that group and expressed as the mean ulcer index (21).

All drugs were prepared daily and purchased from Sigma (Sigma, St. Louis USA).

The data were compared by one-way analysis of variance (ANOVA) followed by Least Significant Differences (LSD) test. An associated probability (p value) of <0.05 was considered to be statistically significant.

RESULTS

Intragastric administration of 30 % ethanol caused 3.23±1.01 mm of macroscopic hemorrhagic lesions (Figure 1). Pretreatment with L-NOARG (2.5 and 10 mg/kg) significantly increased the hemorrhagic damage caused by 30 % ethanol to a concentration-dependent manner (10.12±0.89 mm and 17.8±2.04 mm, respectively) (p<0.05). This increase in mucosal damage caused by L-NOARG was significantly reduced by pretreatment with L-arginine (50 mg/kg) (5.05±1.76 mm and 3.08±1.88 mm respectively) (p<0.05) 0.5 mg/kg L-NOARG had no significant effect on hemorrhagic damage induced by 30 % ethanol (2.62±1.03 mm) (Figure 1).

DISCUSSION

In the present study, inhibition of NO released from gastric mucosal vasculature by pretreatment with L-NOARG, significantly increased the acute hemorrhagic gastric injury induced by 30 % ethanol. Pretreatment with L-arginine, a precursor of NO significantly reduced the mucosal injury. These results confirm the previous report by Mesuda et al that inhibition of endogenous NO by L-NNA induces extensive hemorrhagic changes in the gastrointestinal mucosa of rats (20).

NO is important in the gastric defence mechanism by regulating the mucosal blood flow and gastric mucus secretion and some NO synthase inhibitors are shown to decrease the gastric mucosal blood flow (9,21,22). Lack of the gastroprotective effect and decrease in gastric mucosal blood flow by NO synthase inhibitors suggest the role of endogenous NO in gastric defense mechanisms. The participation of NO in electrically-evoked, nerve mediated NANC (non adrenergic non cholinergic) relaxation in the mammalian gut has been shown (23).

Intragastric ethanol causes acute hemorrhagic erosion of the gastric mucosa in humans and animals (24,25). In addition, intragastric ethanol causes submucosal venous constriction resulting in gastric mucosal microcirculatory disturbances and possibly damage to the vascular endothelium (25-27). Various mediators of ethanol-induced gastric mucosal injury have been suggested, including neutrophils, free radicals, leukotriens, prostaglandins, PAF and ET-1 (17,26-31).

Intragastric ethanol stimulates ET-1 release which is

Figure 1, Effect of N-nitro L-arginine (L-NOARG) on ethanol-induced gastric mucosal lesions in rats. Vehicle on L-NOARG (0.5,2.5 or 10 mg/kg iv) was administrated 15 min before 30% ethanol was challenged. In some experiments, rats were pre-treated with L-arginine (500 mg/kg iv) 10 min before L-NOARG (2.5 or 10 mg/kg iv). The stomach was excised 30 min of the challenge with ethanol. Each column represent the mean±S.E.M of 5-7 experiments. *: p<0.05 significantly different from the control and L-arginine group and #: p<0.05 significantly different from the 2.5 mg/kg L-NOARG group.
known as a potent ulcerogenic agent in rat gastric mucosa (17). The ulcerogenic action of ET-1 is due to its vasoconstrictor properties in the stomach (15,16). Vasoconstriction can promote ulceration because an adequate supply of blood to the mucosa is essential for the rapid removal of back-diffusing acid and for the supply of oxygen and nutrients to the cell lining the lumen (32). The balance between endothelium-derived vasoconstrictor mediators and endothelium derived vasodilator is important in ethanol induced gastric mucosal hemodynamic changes. Since endothelin mediated vasoconstriction in the gastric mucosa may be predominant, NO induced vasodilatation may be masked (17, 33). By the use of inhibitors of NO biosynthesis such as L-NAME, the role of endogenous NO in the regulation of the rat gastric microcirculation under both resting and stimulated conditions has been established (21,22).

It has been demonstrated that increment of the intravascular ethanol concentration leads to local endogenous NO release from the gastric vasculature, and this is postulated to modulate ethanol-induced gastric mucosal vascular tone too (31). Inhibition of NO formation by L-NNA caused mucosal tissue hypoxia due to the decrement in the resting mucosal blood flow and concurrent administration of L-arginine reduced these changes. This suggest that endogenous NO may be released and/or produced from the gastric vasculature and plays an important role in gastric mucosal hemodynamics (20). However the relative contrubition by endogenous NO to the pathophysiology of the ethanol-induced gastric mucosal microcirculatory disturbances has not been clarified. It is reported that exogenous NO can protect the rat gastric mucosa from damage induced by topical irritants. However, NO derived from exogenous sources can likewise exert a dual action on the integrity of the gastric mucosa. Intragastric application of NO donors can protect the gastric mucosa. Intragastric application of NO donors can protect the gastric mucosa against acute hemorrhagic mucosal injury by topical irritants (12,13). By contrast higher doses of NO donors can themselves provoke extensive hemorrhagic mucosal damage (18). This damage may involve the production of the cytotoxic peroxynitrite from NO and superoxide anion (34). However, since superoxide dismutase reduces the inactivation of EDRF superoxide anion may also be involved in the pathogenesis of ethanol-induced gastric mucosal injury (35, 37).

In conclusion, the results obtained in this study suggest that inhibition of endogenous NO formation released from gastric mucosal vasculature may cause the gastric mucosa more susceptible to injury by ethan-

Endotel kaynaklı gevşetici faktörün (nitrik oksid) şişanlarda etanolun neden olduğu mide hasan üzerine koruyucu etkileri

Nitrik oksid sentaz enzimi ile L-argininden sentez edilen NO'in gastrointestinal bütünlüğün sağlanmasında önemli bir modulator rolü vardır. Bu çalışmada, N°-nitro L-arginin (L-NOARG) ile (2,5 veya 10 mg/kg i.v) endojen NO sentezinin inhibe edilmesi, şişanlarda etanolun neden olduğu akut mide mukoza hasarına doza bağlı olarak artırıldı. L-NOARG’ın bu etkisi L-arginin (500 mg/kg i.v) ile önledi. Elde edilen sonuçlar endojen NO inhibisyonu etanolun neden olduğu akut mide mukoza hasarı muhtemelen mikrosirkülasyonu etkileyerek artığı ve bu etkini L-arginin ile geri çevirebilemesi etanolun oluşturduğu akut mide mukoza hasarında endojen NO'nun koruyucu etkisi olduğunu göstermektedir. [Turk J Med Res 1996; 14 (3): 81-84]

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