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

Tijen UTKAN[1], Guner ULAK[1], N.Zafer UTKAN[2], H. Gokalp YILDIRAN[1], M. Nejat GACAR[1]

Depts. of [1] Pharmacology and Toxicology, [2] Surgery School of Medicine, Kocaeli University, Kocaeli, TURKEY

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 byN°-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 gastropratective role of endogenous NO. [Turk J Med Res 1996; 14(3):81-84]

Keywords: L-arginine, Nitric oxide, Ethanol, Stomach, Rats

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 glycryl 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 muco-sal hemodynamics, the contrubition 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.

Received: Nov. 20,1995 Accepted: Apr. 10,1996

Correspondence: Tijen UTKAN
School of Medicine Kocaeli University
Department of Pharmacology
and Toxicology
41900, KOCAELI

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 % eth-

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 pretreated 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 assesing the size of petechiae, five such lesions were considered to be equivelant to 1 mm of ulcer. The sum of the lesions lengths in each group was divided the te 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.

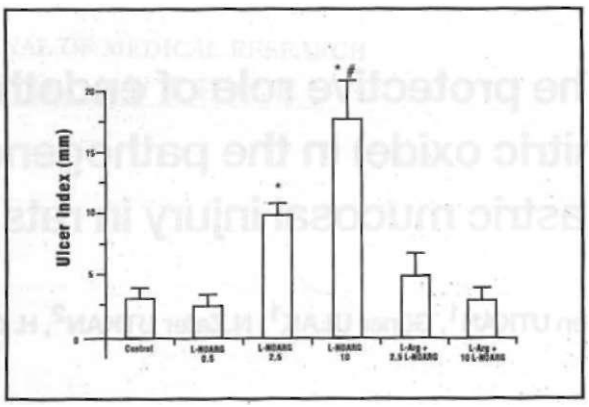

Figure **1,** Effect of N -nitro L-arginine (L-NOARG) on ethanol-induced gastric mucosal lesions in rats. Vehicle on L-NOARG (0.5,2.5 or 10 mg/kg i.v) was administrated 15 min before 30% ethanol was challenged. In some experiments, rats were pretraeted with L-arginine (500 mg/kg i.v) 10 min before L-NOARG (2.5 or 10 mg/kg i.v). 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.

RESULTS

Intragastric administration of 30 % ethanol caused 3.23±1.01 mm of macrscopic hemorrhagic lesions (Figure 1). Pretreatment with L-NOARG (2.5 and 10 mg/kg) significantly increased the hemorrhagic damage caused by 30 % ethanol in 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 ihibitors 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 adrenergil non choliherpiv relaxation in the mammalian gut has been shown (23).

Intragastric ethanol causes acute hemorrorhagic 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). These mediators in acute gastric mucosal injury also affect gastric microcirculation. The repulation of the of gastric mucosal microcirculation are mainly involved in the maintenance of gastric integrity and hence the local release of vasoactive mediators from endothelial cells of the microvasculature has a significant role. Intragastric ethanol stimulates ET-1 release which is

known as a potent ulcerogenic agent in rat gastric mucosa (17). The ulcerogenic action of ET-1 is due 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 inibitors 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 pathopyhsiology 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 againts 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 pat-hogenesis 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 ethanol most probably due to changes in gastric microcirculation. Moreover complete reversal of ethanol-induced gastric mucoal damage by L-arginine pretreatment indicates the gastroprotective role of endogenous NO.

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

Nitrik oksid sentaz enzimi ile L-arjininden sentez edilen NO'in gastrointestinal bütünlüğün sağlanmasında önemli bir modulator rolü vardır. Bu çalışmada, N°-nitro L-arjinin (L-NOARG) ile (2.5 veya 10 mg/kg i.v) endojen NO sentezinin inhibe edilmesi, sıçanlarda etanolun neden olduğu akut mide mukoza hasarını doza bağlı olarak arttırdı. L-NOARG'ın bu etkisi L-arjinin (500 mg/kg i.v) ile önlendi. Elde edilen sonuçlar endojen NO inhibisyonu etanolun neden olduğu akut mide mukoza! hasarını muhtemelen mikrosirkülasyonu etkileyerek artırdığını ve bu etkinin L-arjinin ile geri çevrilebilmesi etanolun oluşturduğu akut mide mukozal hasarında endojen NO'in koruyucu etkisi olduğunu göstermektedir. [Turk J Med Res 1996; 14 (3): 81-84]

REFERENCES

1. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology and pharmacology, Pharmacol. Rev. 1991; 43:109-41.
2. Rees DD, Palmer RMJ, Schulz R, et al. Characterisation of three inhibitors of endothelial nitric oxide synthases in vitro and in vivo. Br J Pharmacol 1990; 101: 746-52.
3. Moore PK, Alswayeh OA, Higgs EA. L-N°-nitro arginine (L-NOARG) a novel L-arginine reversible inhibitor of endothelium-dependent vasodiltion in vitro. Br J Pharmcol 1991; 99:408-12.
4. Li GG, Rand MJ. Nitric oxide and vasoactive intestinal polypeptide mediate nonadrenergic, noncholinergic inhibitory transmission to smooth muscle of the rat gastric fundus. Eur J Pharmacol 1990; 191:303-9.
5. Bult H, Boeckxtaens GE, Pelckmans PA, et al. Nitric oxide as an inhibitory nonadrenergic noncholinergic neurotransmitter. Nature 1990; 345:346-7.
6. Bult H, Boeckxtaens GE, Pelckmans PA, et al. Nitric oxide as an inhibitory nonadrenergic noncohiinergic neurotrans-

mitter. Nature 1990; 345: 346-7.

7. Desai KM, Sessa WC, Vane JR. Involvement of Nitric oxide in the reflex relaxation of the stomach to accommodate food or fluid. Nature 1991; 351:477-9.

8. trie K, Muraki T, Frukawa K, et al. L-N$^{\circ}$-nitroarginine inhibits nicotine-induced relaxation of isolated rat duodenum. Eur J Pharmacol 1991; 202: 285-8.

9. Martinez-Cuesta MA, Barrachina MD, Pique JM, et al. The role of nitric oxide and platelet-activating factor in the inhibition by endotoxin of pentagastrin-stimulated gastric acid secretion. Eur J Pharmacol 1992;218: 351-4.

10. Takeuchi K, Ohuchi T, Miyoke H, et al. Stimulation by nitric oxide synthase inhibits of gastric and duodenal HCO_3 secretion in rats. J Pharmacol Exp Ther 1993; 266:1512-19.

11. Whittle BJR, Lopez-Belmonte J, Moncada S. Regulation of gastric mucosal integrity by endogenous nitric oxide: Interactions with prostanoids and sensory neuropeptides in the rat. Br J Pharmacol 1991; 99: 607-11:

12. Kitagawa H, Taeda F, Kohei H. Effect of endothelium-derived relaxing factor on the gastric lesion induced by HCl in rats. J Pharmacol Exp Ther 1990; 253: 1133-37.

13. MacNaughton K, Cirin G, Wallace JL. Endothelium-derived relaxing factor (nitric oxide) has protective actions in the stomach. Ufe Sci 1989; 45:1869-76.

14. Yanagisawa M, Kurihara H, Kim et al. Novel pott vasoconstrictor peptid produced by vascular endothelial cells. Nature 1988;332:411-3.

15. Wallace JI, Ciino G, De Nucci G, et al. Endothelin has potent ulcerogenic and vasoconstrictor actions in the stomach. Am J Physiol 1989; 256: 661-5.

16. Whittle BJR, Esplugues JV. Induction of rat gastric damage by the endothelium-derived peptide endothelin. Br J Pharmacol 1988; 95:1011-4.

17. Mesud E, Kanawa S, Nogano K, et al. The role of endogenous endothelin in pathogenesis of ethanol induced gastric mucosal injury in rats Am J Physiol 1993; 265 (Gastrointest Liver Physiol 28): 474-81.

18. Lopez-Belmonte J, Whittle BJR, Moncada S. The actions of nitric oxide donors in the prevention or induction of injury to the rat gastric mucosa. Br J Pharmacol 1993; 108: 73-8.

19. Terano A, Hiraishi H, Ota S, et al. Role of superoxide and hydroxyl radicals in rat gastric injury induced by ethanol. Gastroenterol. Jpn 1989;24: 488-93.

20. Masuda E, Kawano S, Nagano K, et al. Endogenous nitric oxide modulates ethanol-induced gastric mucosal injury in rats. Gastroentrology 1995; 108: 58-64.

21. Pique JM, Whittle BJR, Espluges JV. The vasodilator role of endogenous nitric oxide in the rat gastric microcirculation. Eur J Pharmacol 1989; 174:293-6.

22. Tepperman BL, Whittle BJR. Endogenous nitric oxide and sensory neuropeptides interact in the modulation of the rat gastric microcirculation. Br J Pharmacol 1992; 105:171-5.

23. Li CG, Rand MJ. Nitric oxide and vasoactive intestinal polypeptide mediate nonadrenergic noncholinergic inhibitory tranmission to smooth muscle of the rat gastric fundus. Eur J Pharmacl 1990; 191:303-9.

24. Laine L, Weinstein VM. Histology of alcoholic hemorrhagic "gastritis" a prospective evaluation. Gastroenterology 1988; 94:1254-62.

25. Oates PJ, Hakkinen Jp. Studies on the mechanism of ethanol-induced gastric damage in rats. Gastroenterology 1988; 94:10-21.

26. Yonei Y, Guth PH. Lipoxygenase metabolites in the rat gastric microvascular response to intragastric ethanol. Gastroenterology 1989; 97: 304-12.

27. Yonei Y, Wayland H, Guth PH. Role of arachidonic acid metabolites in ethanol vasoaction in rat gastric submucosa. Am J Physiol 1988; 225 (Gastro Intest Liver Physiol 18): 731-7.

28. Kvietys PR, Twohing B, Danzell J, et al. Ethanol-induced injury to the rat gastric mucosa. Gastroenterology 1990; 98: 909-20.

29. Ogle J, Cho C, Tong M, et al. The influence of verapamil on the gastric effects of stress in rats. Eur J Pharmcol 1985; 112:399-404.

30. Konturek SJ, Brzozowski T, Drozdowicz D, et al. Role of leukotrienes and platelet activating factor in acute gastric mucosal lesions in rats. Eur J Pharmacol 1989; 164: 285-92.

31. Masuda E, Kawano S, Nagano K, et al. Effect of intravascular ethanol on modulation of gastric mucosal integrity possible role of endothelin-1. Am J Physiol 1992; 262(Gastrointest Liver Physiol 25): 785-90.

32. Wallace JL, Keenan CM, MacNaughton KW, et al. Comparison of the effects of endothelin-1 and endothelin-3 on the rat stomach. Eur J Pharmacol 1989; 167: 41-7.

33. Masuda E, Kawano S, Nagano K, et al. Ethanol-induces endogenous nitric oxide from gastric vasculature in vitro (Abstr). Gastroenterology 1993; 104:141-5.

34. Lamarque D, Whitle BRJ. Involvement of superoxide and xanthine oxidase in neutrophil-independent rat gastric damage induced by NO donors. Br J Pharmacol 1995; 116: 1843-8.

35. Gryglewski RJ, Palmer RMJ, Moncada S. Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. Nature 1986; 320: 454-6.

36. Pihan G, Regillo C, Szabo S. Free radicals and lipid peroxidation in ethanol- or aspirin-induced gastric mucsal injury. Digest Dis Sci 1987; 32:1395-401.

mitter. Nature 1990; 345: 346-7.

7. Desai KM, Sessa WC, Vane JR. Involvement of Nitric oxide in the reflex relaxation of the stomach to accommodate food or fluid. Nature 1991; 351:477-9.

8. Irie K, Muraki T, Frukawa K, et al. L-N$^{\omega}$-nitroarginine inhibits nicotine-induced relaxation of isolated rat duodenum. Eur J Pharmacol 1991; 202: 285-8.

9. Martinez-Cuesta MA, Barrachina MD, Pique JM, et al. The role of nitric oxide and platelet-activating factor in the inhibition by endotoxin of pentagastrin-stimulated gastric acid secretion. Eur J Pharmacol 1992;218: 351-4.

10. Takeuchi K, Ohuchi T, Miyoke H, et al. Stimulation by nitric oxide synthase inhibits of gastric and duodenal HCO_3 secretion in rats. J Pharmacol Exp Ther 1993; 266:1512-19.

11. Whittle BJR, Lopez-Belmonte J, Moneada S. Regulation of gastric mucosal integrity by endogenous nitric oxide: Interactions with prostanoids and sensory neuropeptides in the rat. Br J Pharmacol 1991; 99: 607-11!

12. Kitagawa H, Taeda F, Kohei H. Effect of endothelium-derived relaxing factor on the gastric lesion induced by HCl in rats. J Pharmacol Exp Ther 1990; 253: 1133-37.

13. MacNaughton K, Cirin G, Wallace JL. Endothelium-derived relaxing factor (nitric oxide) has protective actions in the stomach. Life Sci 1989; 45:1869-76.

14. Yanagisawa M, Kurihara H, Kim et al. Novel pott vasoconstrictor peptid produced by vascular endothelial cells. Nature 1988;332:411-3.

15. Wallace JI, Cuno G, De Nucci G, et al. Endothelin has potent ulcerogenic and vasoconstrictor actions in the stomach. Am J Physiol 1989; 256: 661-5.

16. Whittle BJR, Esplugues JV. Induction of rat gastric damage by the endothelium-derived peptide endothelin. Br J Pharmacol 1988; 95:1011-4.

17. Mesud E, Kanawa S, Nogano K, et el. The role of endogenous endothelin in pathogenesis of ethanol induced gastric mucosal injury in rats Am J Physiol 1993; 265 (Gastrointest Liver Physiol 28): 474-81.

18. Lopez-Belmonte J, Whittle BJR, Moneada S. The actions of nitric oxide donors in the prevention or induction of injury to the rat gastric mucosa. Br J Pharmacol 1993; 108: 73-8.

19. Terano A, Hiraishi H, Ota S, et al. Role of superoxide and hydroxy! radicals in rat gastric injury induced by ethanol. Gastroenterol. Jpn 1989;24: 488-93.

20. Masuda E, Kawano S, Nagano K, et al. Endogenous nitric oxide modulates ethanol-induced gastric mucosal injury in rats. Gastroentrology 1995; 108: 58-64.

21. Pique JM, Whittle BJR, Espluges JV. The vasodilator role of endogenous nitric oxide in the rat gastric microcirculation. Eur J Pharmacol 1989; 174: 293-6.

22. Tepperman BL, Whittle BJR. Endogenous nitric oxide and sensory neuropeptides interact in the modulation of the rat gastric microcirculation. Br J Pharmacol 1992; 105:171-5.

23. Li CG, Rand MJ. Nitric oxide and vasoactive intestinal polypeptide mediate nonadrenergic noncholinergic inhibitory tranmission to smooth muscle of the rat gastric fundus. Eur J Pharmacl 1990; 191: 303-9.

24. Laine L, Weinstein VM. Histology of alcoholic hemorrhagic "gastritis" a prospective evaluation. Gastroenterology 1988; 94:1254-62.

25. Oates PJ, Hakkinen Jp. Studies on the mechanism of ethanol-induced gastric damage in rats. Gastroenterology 1988; 94:10-21.

26. Yonei Y, Guth PH. Lipoxygenase metabolites in the rat gastric microvascular response to intragastric ethanol. Gastroenterology 1989; 97: 304-12.

27. Yonei Y, Wayland H, Guth PH. Role of arachidonic acid metabolites in ethanol vasoaction in rat gastric submucosa. Am J Physiol 1988; 225 (Gastro Intest Liver Physiol 18): 731-7.

28. Kvietys PR, Twohing B, Danzell J, et al. Ethanol-induced injury to the rat gastric mucosa. Gastroenterology 1990; 98: 909-20.

29. Ogle J, Cho C, Tong M, et al. The influence of verapamil on the gastric effects of stress in rats. Eur J Pharmcol 1985; 112: 399-404.

30. Konturek SJ, Brzozowski T, Drozdowicz D, et al. Role of leukotrienes and platelet activating factor in acute gastric mucosal lesions in rats. Eur J Pharmacol 1989; 164: 285-92.

31. Masuda E, Kawano S, Nagano K, et al. Effect of intravascular ethanol on modulation of gastric mucosal integrity possible role of endothelin-1. Am J Physiol 1992; 262(Gastrointest Liver Physiol 25): 785-90.

32. Wallace JL, Keenan CM, MacNaughton KW, et al. Comparison of the effects of endothelin-1 and endothelin-3 on the rat stomach. Eur J Pharmacol 1989; 167:41-7.

33. Masuda E, Kawano S, Nagano K, et al. Ethanol-induces endogenous nitric oxide from gastric vasculature in vitro (Abstr). Gastroenterology 1993; 104:141-5.

34. Lamarque D, Whitle BRJ. Involvement of superoxide and xanthine oxidase in neutrophil-independent rat gastric damage induced by NO donors. Br J Pharmacol 1995; 116: 1843-8.

35. Gryglewski RJ, Palmer RMJ, Moncada S. Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. Nature 1986; 320: 454-6.

36. Pihan G, Regillo C, Szabo S. Free radicals and lipid peroxidation in ethanol- or aspirin-induced gastric mucsal injury. Digest Dis Sci 1987; 32: 1395-401.