ELSEVIER

European Journal of Pharmacology 378 (1999) 123–127

ejp

www.elsevier.nl/locate/ejphar

Short communication

Effects of nitric oxide donors GEA 3162 and SIN-1 on ethanol-induced gastric ulceration in rats

M. Zaini Asmawi [a,b], Eeva Moilanen [a,c], Kari Annala [a], Petri Rahkonen [a], Hannu Kankaanranta [a,d,*]

[a] *Medical School, University of Tampere, P.O. Box 607, Tampere, FIN-33101, Finland*
[b] *School of Pharmaceutical Sciences, University of Science Malaysia, 11800 Penang, Malaysia*
[c] *Department of Clinical Chemistry, Tampere University Hospital, Tampere, Finland*
[d] *Department of Respiratory Medicine, Tampere University Hospital, Tampere, Finland*

Received 25 May 1999; received in revised form 18 June 1999; accepted 22 June 1999

Abstract

Low doses of the intragastrically (i.g.) administered nitric oxide (NO) donors, 1,2,3,4-oxatriazolium,5-amino-3-(3,4-dichlorophenyl)-chloride (GEA 3162; 0.3 mg/kg) and 3-morpholino-sydnonimine (SIN-1; 1 mg/kg), inhibited gastric ulceration induced by ethanol (94%) in anesthetized rats. In contrast, higher doses of these NO donors administered i.g. exacerbated the damage. When administered intravenously, the NO donors had no effect on ethanol-induced gastric lesions although a clear blood pressure-lowering effect was seen. Neither the inhibition nor the exacerbation of ulceration was correlated with changes in blood pressure or prostaglandin E_2 release from the mucosal tissue. The relatively small difference between the gastroprotective and damaging doses suggests that orally administered NO donors, especially in the case of GEA 3162, may have a narrow gastric safety margin.

Keywords: GEA 3162; SIN-1 (3-morpholino-sydnonimine); Gastric mucosa; Nitric oxide (NO); Prostaglandin E_2

1. Introduction

The nitric oxide (NO) donor *S*-nitroso-*N*-acetylpenicillamine has been shown to have a gastroprotective effect in a model of gastric injury (Lopez-Belmonte et al., 1993). NO regulates gastric mucosal blood flow (Lippe and Holzer, 1992), acid and mucus secretion (Brown et al., 1993), and enteric nerve function and contributes to cellular integrity and mucosal protection (Brown et al., 1993; Konturek et al., 1993). A NO-releasing moiety (nitrobutyl) has been coupled to an anti-inflammatory drug and found to reduce the ulcerogenic side effects of the drug without losing its anti-inflammatory activity (Wallace and Chin, 1997).

The integrity of gastrointestinal epithelial cells can be disrupted by higher concentrations of NO (Tepperman et al., 1993). The NO released by a NO donor may induce cytotoxicity, through its action with the reactive oxygen metabolite superoxide anion ($O_2^{\cdot -}$), to produce peroxynitrite ($ONOO^-$). This potent oxidizing agent can initiate lipid peroxidation and produce extreme cellular damage (Crow and Beckman, 1995). These data suggest that NO may have a bifunctional concentration-dependent effect on gastric mucosa.

Recently, a structurally different NO donor, 1,2,3,4-oxatriazolium,5-amino-3-(3,4-dichlorophenyl)-chloride (GEA 3162), was developed (Corell et al., 1994; Karup et al., 1994; Kankaanranta et al., 1996). GEA 3162 quickly releases NO spontaneously in aqueous solutions (Karup et al., 1994; Kankaanranta et al., 1996). We have earlier found that this compound inhibits neutrophil function (Moilanen et al., 1993) and has vasodilator, antiplatelet and fibrinolytic activities (Corell et al., 1994). NO donors have therapeutic potential in stable and unstable angina, coronary vasospasm, myocardial infarction, and congestive heart failure (Moncada and Higgs, 1995). These acute clinical conditions are often associated with gastric irritation or ulceration. However, the gastrointestinal safety profile of NO donors remains largely unknown. As there is evidence to suggest that NO may have either protecting or

* Corresponding author. Medical School, University of Tampere, P.O. Box 607, FIN-33101 Tampere, Finland. Tel.: +358-3-215-8081; fax: +358-3-215-8082; E-mail: blhaka@uta.fi

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00455-0

damaging effects on gastric mucosa, the present study was designed to find out how the potent NO-releasing mesoionic oxatriazole derivative GEA 3162 affects gastric mucosal damage induced by ethanol in rats as compared with the effect of the peroxynitrite-releasing compound SIN-1. The involvement of vascular effects and prostaglandin E_2 release in the actions of these NO donors was also evaluated.

2. Material and methods

2.1. Animal preparation, gastric ulceration and blood pressure measurement

Male Wistar rats, weighing 200–300 g were fasted for 24 h in cages with a raised floor. Rats were anesthetized with urethane (1.25 g/kg) and the right carotid artery and left jugular vein were cannulated with a catheter for blood pressure measurement and intravenous (i.v.) drug administration, respectively. Mean systemic arterial blood pressure was measured continuously via the right carotid artery catheter, which was connected to a pressure transducer and chart recorder system (model 7D polygraph, Grass Instrument, Quincy, MA). To ensure free breathing, a tracheostomy was made. The anesthetized rats were treated with GEA 3162 (GEA, Copenhagen, Denmark) or SIN-1 (GEA) intragastrically (i.g.) or intravenously (i.v.) 10 min before induction of gastric hemorrhagic damage by intragastric administration of one ml of 94% (v:v) ethanol. Animals were killed by an i.v. air bolus 5 min after instillation of ethanol. The stomachs were excised, opened along the greater curvature, rinsed with saline, pinned flat on a soft board with the mucosa facing upward and photographed. The area of hemorrhagic mucosal damage in the corpus region was measured with computerized planimetry by an independent observer. The study was approved by the Animal Experimentation Committee of the University of Tampere, Tampere, Finland.

2.2. Prostaglandin E_2 assays

Two samples (5 mm^2) from the damaged and nondamaged area of gastric mucosa together with the stomach wall were taken using a punch-holder. The samples were incubated in Dulbecco's phosphate-buffered saline for 30 min at 37°C. Prostaglandin E_2 released into the incubation medium was determined by radioimmunoassay (Institute of Isotopes of Hungary Academy of Sciences, Budapest, Hungary). The detection limit of the assay was 2.5 ng/ml.

2.3. Statistical analysis

Data are expressed as the means ± S.E. of (n) experiments. Statistical significance was calculated by analysis of variance supported by Dunnett's multiple comparison test. Differences were considered significant when $P < 0.05$.

3. Results

3.1. Effect of GEA 3162 and SIN-1 on ethanol-induced gastric ulceration

Intragastrically administered GEA 3162 and SIN-1 had biphasic concentration-dependent effects on the mucosal damage caused by ethanol (Fig. 1). GEA 3162 (0.3 mg/kg; i.g.) significantly reduced the area of ulceration induced by ethanol (Fig. 1). Increasing the dose to 1.0 mg/kg (i.g.) did not reduce but rather increased the damage area. Similarly, SIN-1 (1.0 mg/kg; i.g.) significantly reduced the gastric ulcer area (Fig. 1), but with a higher dose of SIN-1 (10 mg/kg; i.g.), the damage was significantly greater than in the saline control. Neither GEA 3162 nor SIN-1 administered intravenously had any effect on the mucosal lesions induced by ethanol (Fig. 1).

3.2. Effect of GEA 3162 and SIN-1 on blood pressure

Even at the highest concentration, GEA 3162 (1 mg/kg) administered i.g. did not change mean arterial blood pressure (MAP) as compared with saline control up to 10 min after administration (ΔMAP 2.4 ± 4.4 mm Hg; $n = 5$). In contrast, SIN-1 (10 mg/kg) administered i.g. lowered blood pressure during the 10 min follow-up (ΔMAP −28.2 ± 6.4 mm Hg; $n = 5$). While saline administered i.v. caused a small increase (maximally by 10.7 ± 5.2 mm Hg; $n = 5$) in blood pressure, GEA 3162 (1 mg/kg; i.v.) lowered the mean arterial pressure by 28.0 ± 4.5 mm Hg ($n = 5$) in the first minute but blood pressure returned to the saline control level 2 min later (ΔMAP 15.0 ± 6.3 mm Hg from baseline; $n = 5$). In contrast, SIN-1 (10 mg/kg;

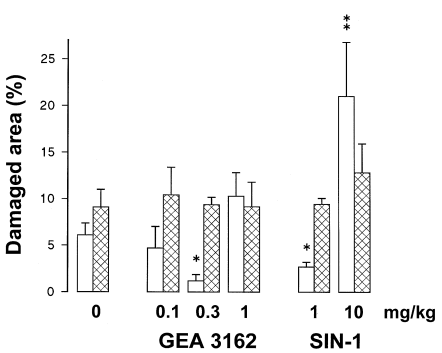

Fig. 1. The effects of GEA 3162 and SIN-1 on ethanol-induced (5 min) gastric hemorrhagic lesions. Results are expressed as the percentage of damaged area in the corpus region of the stomach, as analyzed with computerized planimetry. Saline control and NO donors were added 10 min before ethanol either intragastrically (i.g.) (open bars) or intravenously (i.v.) (cross-hatched bars). The * indicates $P < 0.05$ and ** $P < 0.01$ as compared with the respective saline control. Mean ± S.E., $n = 5–8$.

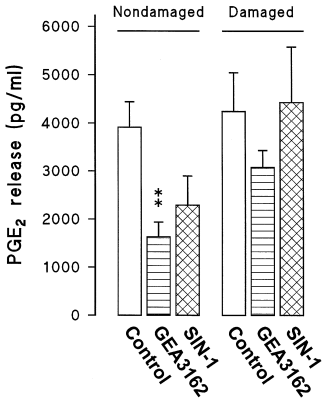

Fig. 2. Effect of GEA 3162 (0.3 mg/kg) and SIN-1 (1 mg/kg) administered i.g. on prostaglandin E_2 release in gastric mucosal tissue pieces (5 mm^2) obtained from nondamaged or damaged areas of the stomach after induction of ulceration with ethanol. The tissue pieces were incubated in Dulbecco's phosphate-buffered saline for 30 min at 37°C. Results are means ± S.E., $n = 4–6$. The ** indicates $P < 0.01$ as compared with the respective saline control.

i.v.) lowered the blood pressure already from the first minute after administration and the maximal ΔMAP was -28.8 ± 6.5 mm Hg; $n = 5$. At lower NO donor concentrations, the changes in MAP were smaller but qualitatively similar (data not shown).

3.3. Effect of GEA 3162 and SIN-1 on prostaglandin E_2 synthesis in gastric mucosa

There was no difference in prostaglandin E_2 release between the macroscopically healthy and hemorrhagic damaged areas of the stomach in saline-treated rats with ethanol-induced gastric ulcers (Fig. 2). For comparison, a cyclooxygenase inhibitor, acetylsalicylic acid (60 mg/kg; i.g.), reduced prostaglandin E_2 release to negligible levels (> 99% inhibition) in both the nondamaged and damaged areas of the stomach. GEA 3162 (0.3 mg/kg; i.g.) reduced prostaglandin E_2 release in the nondamaged area but did not significantly affect the damaged area of the stomach. Similarly, SIN-1 (1 mg/kg; i.g.) reduced prostaglandin E_2 release in the nondamaged area of the stomach while prostaglandin E_2 release in the damaged area remained unchanged. The prostaglandin E_2 release in rats treated with GEA 3162 (0.3 mg/kg; i.v.) and SIN-1 (1 mg/kg; i.v.) was at the same level as in saline treated rats ($n = 3–6$; data not shown).

4. Discussion

In low doses, the NO donors GEA 3162 and SIN-1, when administered i.g., were found to have a protective effect on the gastric damage induced by ethanol. The results confirm earlier observations showing that low-to-moderate amounts of NO released from NO donors protect the gastric mucosa in models of gastric injury (Lopez-Belmonte et al., 1993; Wallace and Chin, 1997). The present study extends these data by showing that the two NO donors tested had a biphasic action on ethanol-induced mucosal lesions when given i.g., being protective at low doses, and aggravating the damage at higher doses, and were ineffective when given intravenously. The actions could not be explained by changes in blood pressure or prostaglandin E_2 production in the gastric mucosa.

The NO released into the bloodstream from NO donors causes both local and systemic vasodilation and lowers blood pressure. In the first minute after i.v. administration, GEA 3162 lowered blood pressure significantly and SIN-1 had a similar action with a somewhat slower onset. This suggests that GEA 3162 releases NO at a faster rate than SIN-1 in the blood as described earlier in vitro (Kankaanranta et al., 1996). Thus, if GEA 3162 were absorbed one would expect vasodilatation and a reduction of blood pressure. However, blood pressure remained unaltered after intragastric administration of high doses of GEA 3162. This is consistent with the earlier reported low oral bioavailibity of some NO-releasing mesoionic oxatriazole derivatives (Karup et al., 1994). Thus, the results suggest that GEA 3162 is not significantly absorbed and that the gastroprotective effects are not due to intravascular release of NO. SIN-1 given i.g. lowered blood pressure, suggesting that it is absorbed orally. However, SIN-1 caused qualitatively similar gastroprotective effects as GEA 3162, further suggesting that the gastric effect is not explained by the vascular actions of these compounds. This is also supported by the finding that intravenously given GEA 3162 or SIN-1 did not protect gastric mucosa from acute ethanol-induced ulceration.

Our results show that the NO donors GEA 3162 and SIN-1 have a protective action at low doses and an aggravating effect at higher doses on ethanol-induced ischemia-reperfusion injury in gastric mucosa. Acute intestinal ischemia-reperfusion syndrome is associated with endothelial dysfunction characterized by enhanced superoxide release and reduced NO production detectable minutes after reperfusion (Lefer and Lefer, 1999). Replacement of the lowered endogenously produced NO by NO donors is the likely explanation of the protective actions of the low doses of GEA 3162 and SIN-1 seen in the present study. There are experimental data to support the involvement of various cellular and molecular mechanisms in the protective action of NO in gastric mucosa during ischemia-reperfusion injury, i.e. (1) improvement of tissue perfusion by vasodilatation, (2) scavenging of the superoxide radical and restoration of the glutathione content in gastric cells, (3) maintenance of mucus secretion, and (4) inhibition of platelet and neutrophil adhesion (Kim and Kim, 1998; Lefer and Lefer, 1999). Higher amounts of GEA 3162 and SIN-1 exacerbated the damage induced by ethanol. Close-arterial infusion of 2–4-fold therapeutic doses of *S*-

nitroso-*N*-acetylpenicillamine and nitroprusside have been found to cause extensive hemorrhagic mucosal damage (Lopez-Belmonte et al., 1993; Lamarque and Whittle, 1995a). An important mechanism for NO-induced cytotoxicity is its reaction with superoxide ($O_2^{\cdot -}$) to produce peroxynitrite ($ONOO^-$) (Crow and Beckman, 1995). This potent oxidizing agent can initiate lipid peroxidation, oxidize proteins and DNA and nitrate amino acids, and thus produce cell membrane damage (Crow and Beckman, 1995). SIN-1 releases NO together with superoxide anion, leading to the formation of peroxynitrite. GEA 3162 does not produce measurable amounts of superoxide anion and peroxynitrite as compared with SIN-1 (Holm et al., 1998). During ischemia-reperfusion injury, enhanced production of $O_2^{\cdot -}$ is evident (Wada et al., 1998). Thus, $O_2^{\cdot -}$ of cellular origin and NO derived from NO donors might form peroxynitrite under these conditions. This is supported by the findings of Lamarque and Whittle (1995b) that local superoxide dismutase and a hydroxyl radical scavenger, dimethylthiourea, reduced the gastric mucosal damage induced by local intra-arterial infusion of NO donors. The same authors reported lipid peroxidation after local intra-arterial infusion of high doses of NO donors, suggesting the involvement of peroxynitrite in the gastric mucosal damage (Lamarque and Whittle, 1996). In addition, immunohistochemical evidence has recently been presented to support the role of peroxynitrite in the pathogenesis of ischemia-reperfusion injury in the gastric mucosa (Wada et al., 1998). Thus, peroxynitrite might mediate the NO donor-induced gastrotoxicity found in the present study. However, at low concentrations peroxynitrite has been reported to protect against ischemia-reperfusion injury due to decomposition to NO (Lefer et al., 1997), which could explain the beneficial action of low doses of SIN-1 seen in the present study.

The biphasic effects of NO donors were seen only when the drugs were given intragastrically, but not when they were given intravenously. In aqueous solutions, both GEA 3162 and SIN-1 release NO at a fast rate (Kankaanranta et al., 1996). This is reflected by the prompt decrease in blood pressure when the compounds were given intravenously. The half-life of NO in aqueous solutions is 1–10 s (Crow and Beckman, 1995). This suggests that NO released from intravenously given NO donors is rapidly metabolized and is not delivered to the gastric mucosa at concentrations high enough to produce effects similar to those seen when the NO donors are given intragastrically. The extensive reduction in blood pressure produced by intravenously given NO donors prevented the use of higher doses to clarify this matter further.

Suppression of prostaglandin synthesis via inhibition of the enzyme cyclooxygenase is a key mechanism underlying gastric ulceration caused by nonsteroidal anti-inflammatory drugs (Wallace and Chin, 1997). Prostaglandins exert their cytoprotective actions by stimulating mucus and bicarbonate secretion, maintaining mucosal blood flow, and enhancing the resistance of epithelial cells to injury induced by cytotoxins (Wallace and Chin, 1997). It has been proposed that NO upregulates prostaglandin production in some cell types (Franchi et al., 1994). Thus, the gastroprotective effects of NO donors could be due to increased production of prostaglandins in the stomach. However, GEA 3162 and SIN-1 at oral gastroprotective doses did not increase but rather decreased prostaglandin E_2 release. This is in accordance with the results showing that NO may also downregulate prostaglandin production (Kosonen et al., 1998). Thus, we were able to exclude prostaglandin E_2 as the mediator of the gastroprotective effect of the NO donors.

In conclusion, the NO donors GEA 3162 and SIN-1 were found to have protective effects against ethanol-induced gastric ulceration at low doses but to exacerbate the mucosal damage at high doses. The results suggest that these effects are not mediated via modulation of blood pressure or gastric prostaglandin synthesis. The relatively small difference between the gastroprotective and damaging doses suggests that there is a narrow gastric safety margin for orally administered NO donors, and this is especially the case for GEA 3162.

Acknowledgements

This work was supported by the Academy of Finland (EM), The Finnish Cultural Foundation (HK), The Finnish Foundation for Gastroenterological Research (HK), and the Medical Research Fund of Tampere University Hospital (EM, HK).

References

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

Corell, T., Pedersen, S.B., Lissau, B., Moilanen, E., Metsä-Ketelä, T., Kankaanranta, H., Vuorinen, P., Vapaatalo, H., Rydell, E., Andersson, R., Marcinkiewicz, E., Korbut, R., Gryglewski, R.J., 1994. Pharmacology of mesoionic oxatriazole derivatives in blood, cardiovascular and respiratory systems. Pol. J. Pharmacol. 46, 553–566.

Crow, J.P., Beckman, J.S., 1995. The role of peroxynitrite in nitric oxide-mediated toxicity. Curr. Top. Microbiol. Immunol. 196, 57–73.

Franchi, A.M., Chaud, M., Rettori, V., Suburo, A., McCann, S.M., Gimeno, M., 1994. Role of nitric oxide in eicosanoid synthesis and uterine motility in estrogen-treated rat uteri. Proc. Natl. Acad. Sci. U.S.A. 91, 539–543.

Holm, P., Kankaanranta, H., Metsä-Ketelä, T., Moilanen, E., 1998. Radical releasing properties of nitric oxide donors GEA 3162, SIN-1 and *S*-nitroso-*N*-acetylpenicillamine. Eur. J. Pharmacol. 346, 97–102.

Kankaanranta, H., Rydel, E., Petersson, A.S., Holm, P., Moilanen, E., Corel, T., Karup, G., Vuorinen, P., Pedersen, S.B., Wennmalm, Å., Metsä-Ketelä, T., 1996. Nitric oxide-donating properties of mesoionic 3-aryl substituted oxatriazole-5-imine derivatives. Br. J. Pharmacol. 117, 401–406.

Karup, G., Preikschat, H., Wilhelmsen, E.S., Pedersen, S.B., Marcinkiewicz, E., Cieslik, K., Gryglewski, R.J., 1994. Mesoionic

oxatriazole derivatives: a new group of NO donors. Pol. J. Pharmacol. 47, 541–552.

Kim, H., Kim, K.H., 1998. Role of nitric oxide in oxidative damage in isolated rabbit gastric cells exposed to hypoxia-reoxygenation. Dig. Dis. Sci. 43, 1042–1049.

Konturek, S.J., Brzozowski, T., Majka, J., Pytko-Polonczyk, J., Stachura, J., 1993. Inhibition of nitric oxide synthase delay healing of chronic gastric ulcers. Eur. J. Pharmacol. 239, 215–217.

Kosonen, O., Kankaanranta, H., Malo-Ranta, U., Ristimäki, A., Moilanen, E., 1998. Inhibition by nitric oxide-releasing compounds of prostacyclin production in human endothelial cells. Br. J. Pharmacol. 125, 247–254.

Lamarque, D., Whittle, B.J.R., 1995a. Involvement of superoxide and xanthine oxidase in neutrophil-independent rat gastric damage induced by NO donors. Br. J. Pharmacol. 116, 1843–1848.

Lamarque, D., Whittle, B.J., 1995b. Role of oxygen-derived metabolites in the gastric mucosal injury induced by nitric oxide donors. Eur. J. Pharmacol. 277, 187–194.

Lamarque, D., Whittle, B.J., 1996. Involvement of peroxynitrite in the lipid peroxidation induced by nitric oxide in rat gastric mucosa. Eur. J. Pharmacol. 313, R5–R7.

Lefer, A.M., Lefer, D.J., 1999. Nitric oxide: II. Nitric oxide protects in intestinal inflammation. Am. J. Physiol. 276, G572–G575.

Lefer, D.J., Scalia, R., Campbell, B., Nossuli, T., Hayward, R., Salamon, M., Grayson, J., Lefer, A.M., 1997. Peroxynitrite inhibits leukocyte–endothelial cell interactions and protects against ischemia-reperfusion injury in rats. J. Clin. Invest. 99, 684–691.

Lippe, I.T., Holzer, P., 1992. Participation of endothelium-derived nitric oxide but not prostacyclin in the gastric mucosal hyperemia due to acid back-diffusion. Br. J. Pharmacol. 105, 708–714.

Lopez-Belmonte, J., Whittle, B.J.R., Moncada, S., 1993. The actions of nitric oxide donors in the prevention or induction of injury to the rat gastric mucosa. Pharmacology 108, 73–78.

Moilanen, E., Vuorinen, P., Kankaanranta, H., Metsä-Ketelä, T., Vapaatalo, H., 1993. Inhibition by nitric oxide donors of human polymorphonuclear leucocyte functions. Br. J. Pharmacol. 109, 852–858.

Moncada, S., Higgs, E.A., 1995. Molecular mechanisms and therapeutic strategies related to nitric oxide. FASEB J. 9, 1319–1330.

Tepperman, B.L., Brown, J.F., Whittle, B.J.R., 1993. Nitric oxide synthase induction and intestinal epithelial cell viability in the rat. Am. J. Physiol. 256, G214–G218.

Wada, K., Kamisaki, Y., Ohkura, T., Kanda, G., Nakamoto, K., Kishimoto, Y., Ashida, K., Itoh, T., 1998. Direct measurement of nitric oxide release in gastric mucosa during ischemia-reperfusion in rats. Am. J. Physiol. 274, G465–G471.

Wallace, J.L., Chin, B.C., 1997. Inflammatory mediators in gastrointestinal defence and injury. Proc. Soc. Exp. Biol. Med. 214, 192–203.