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RESEARCH PAPER

Role of the NO/cGMP/K_{ATP} pathway in the protective effects of sildenafil against ethanol-induced gastric damage in rats

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Background and purpose: Sildenafil is a selective inhibitor of cGMP-specific phosphodiesterase. Sildenafil, acting via NO-dependent mechanisms, prevents indomethacin-induced gastropathy. Activation of ATP-sensitive potassium channels (K_{ATP}) is involved in gastric defence. Our objective was to evaluate the role of the NO/cGMP/K_{ATP} pathway in the protective effects of sildenafil against ethanol-induced gastric damage.
Experimental approach: Rats were treated with L-NAME (1 or 3 mg kg^{-1}, i.p.) or with L-arginine (200 mg kg^{-1}, i.p.) + L-NAME (3 mg kg^{-1}, i.p.), the guanylate cyclase inhibitor, ODQ (10 mg kg^{-1}, i.p.), glibenclamide (0.1, 0.3, 1 or 3 mg kg^{-1}, i.p.) or with glibenclamide (1 mg kg^{-1}, i.p.) + diazoxide (3 mg kg^{-1}, i.p.). After thirty minutes, the rats received sildenafil (1 mg kg^{-1}, by gavage), followed by intragastric instillation of absolute ethanol (4 ml kg^{-1}) to induce gastric damage. One hour later, gastric damage (haemorrhagic or ulcerative lesions) was measured with a planimetry programme. Samples of stomach were also taken for histopathological assessment and for assays of tissue glutathione and haemoglobin.
Key results: Sildenafil significantly reduced ethanol-induced gastric damage in rats. L-NAME alone, without L-arginine, significantly reversed the protection afforded by sildenafil. Inhibition of guanylate cyclase by ODQ completely abolished the gastric protective effect of sildenafil against ethanol-induced gastric damage. Glibenclamide alone reversed sildenafil's gastric protective effect. However, glibenclamide plus diazoxide did not alter the effects of sildenafil.
Conclusions: Sildenafil had a protective effect against ethanol-induced gastric damage through the activation of the NO/cGMP/K_{ATP} pathway.
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Keywords: sildenafil; gastric damage; ethanol; nitric oxide; cGMP; K_{ATP} channels

Abbreviations: GSH, reduced glutathione; K_{ATP}, ATP-sensitive potassium channels; ODQ, 1*H*-[1,2,4] oxadiazolo[4,3-a] quinoxaline-1-one

Introduction

Ethanol ingestion causes acute gastric mucosal lesions in humans (Gottfried *et al*., 1978; Laine and Weinstein, 1988). Ethanol-induced gastric damage is characterized by mucosal oedema, subepithelial haemorrhage, cellular exfoliation and inflammatory cell infiltration (Guslandi, 1987). It has been shown that ethanol induces gastric mucosal injury through the release of inflammatory mediators, which induce vasoconstriction/ischaemia and then cell death (Szabo *et al*., 1985).

Nitric oxide (NO) is a crucial mediator of gastrointestinal mucosal defence (Muscara and Wallace, 1999) and induces arterial smooth muscle relaxation through stimulation of guanylate cyclase, with a consequent increase in the formation of cGMP (Reffelmann and Kloner, 2003). NO and cGMP protect parietal cells from ethanol-induced cytotoxicity (Yanaka *et al*., 1995), and the NO/cGMP pathway protects endothelial cells against cellular damage in various tissues (Polte *et al*., 1997). NO and cGMP can activate several targets including different types of K^+ channels (Archer *et al*., 1994; Bolotina *et al*., 1994; Carriers *et al*., 1997). Recently, it was demonstrated that the activation of

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ATP-sensitive potassium channels (K_{ATP}) is involved in gastric defence (Peskar *et al*., 2002; Gomes *et al*., 2006).

Sildenafil increases the effects of cGMP by blocking the phosphodiesterase-type 5, which inactivates intracellular cGMP (Chuang *et al*., 1998; Gibson, 2001). Recently, we demonstrated that sildenafil protected against both indomethacin-induced gastropathy (Santos *et al*., 2005) and ethanol-induced gastric damage (Medeiros *et al*., in press). However, the mechanisms of this latter effect of sildenafil were not completely elucidated.

In the present study, we have evaluated the role of the NO/cGMP/K_{ATP} pathway in the protective effect of sildenafil against ethanol-induced gastric damage in rats.

Methods

Animals

All animal treatments and surgical procedures were performed in accordance to the Guide for Care and Use of Laboratory Animals, the National Institutes of Health (Bethesda, MD, USA) and were approved by the local University ethics committee. Male Wistar rats (220–270 g) were fasted 18–24 h before the experiments. The animals were housed in cages in temperature-controlled rooms and received water and food *ad libitum*.

Effect of sildenafil on ethanol-induced gastric damage

Rats were treated with saline or sildenafil (1 mg kg^{-1}) by gavage. After 30 min, absolute ethanol (4 ml kg^{-1}) was administered by gavage, as described previously (Robert, 1979). The control group received only saline. One hour later, the animals were killed and their stomachs rapidly removed, opened through an incision along the greater curvature and pinned out on a wax block. The haemorrhagic or ulcerative lesions were measured using a computer planimetry program (Image J). A sample of the corpus region of each stomach was fixed in 10% formalin for subsequent histopathological assessment. Other full-thickness pieces of the gastric corpus were then weighed, frozen and stored at −70 °C until assayed for glutathione (Sedlak and Lindsay, 1968) and haemoglobin (Souza *et al*., 2004).

Role of NO in the protective effect of sildenafil

To study the role of NO in the protective effects of sildenafil, the animals were treated with an inhibitor of NOS, N_{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME) (1 or 3 mg kg^{-1}, i.p.) or with L-arginine (200 mg kg^{-1}, i.p.) + L-NAME (3 mg kg^{-1}, i.p.). After 30 min, the rats received sildenafil (1 mg kg^{-1}, by gavage). Thirty minutes later, gastric damage was induced by intragastric instillation of absolute ethanol (4 ml kg^{-1}, by gavage). The control group received only vehicles. In other groups of rats, L-arginine (200 mg kg^{-1}, i.p.) or L-NAME (3 mg kg-1, i.p.) alone was also injected followed by saline injections before absolute ethanol administration. One hour later, gastric damage was determined as described above. Finally, a sample of the corpus region of each stomach was fixed in 10% formalin for subsequent histopathological assessment, and other full-thickness pieces of the gastric corpus were weighed, frozen and stored at −70 °C until glutathione and haemoglobin assays.

Role of cGMP in the protective effect of sildenafil

To study the role of cGMP in sildenafil's protective effect, the animals were treated with an inhibitor of guanylate cyclase, 1*H*-[1,2,4]oxadiazolo[4,3-a]quinoxaline-1-one (ODQ; 10 mg kg^{-1}, i.p.). After 30 min, the rats received sildenafil (1 mg kg^{-1}, by gavage). Thirty minutes later, gastric damage was induced by intragastric instillation of absolute ethanol (4 ml kg^{-1}, by gavage). The control group received only saline or dimethylsulphoxide 0.01% + ethanol. One hour later, gastric damage was determined as described above. Finally, a sample of the corpus region of each stomach was fixed in 10% formalin for subsequent histopathological assessment, and other full-thickness pieces of the gastric corpus were weighed, frozen and stored at −70 °C until glutathione and haemoglobin assays.

Role of K_{ATP} channels in the protective effect of sildenafil

To study the role of K_{ATP} in sildenafil's protective effect, the animals were treated with glibenclamide (0.1, 0.3, 1 or 3 mg kg^{-1}, i.p.) or with glibenclamide (1 mg kg^{-1}, i.p.) + diazoxide (3 mg kg^{-1}, i.p.). After 30 min, the rats received sildenafil (1 mg kg^{-1}, by gavage). Thirty minutes later, gastric damage was induced by intragastric instillation of absolute ethanol (4 ml kg^{-1}, by gavage). The control group received only saline, NaOH 0.01 N or NaOH 0.05 N. In other groups of rats, glibenclamide (3 mg kg^{-1}, i.p.) or diazoxide (3 mg kg^{-1}, i.p.) alone was also injected, followed by saline injections before absolute ethanol administration. One hour later, gastric damage was determined as described above. Finally, a sample of the corpus region of each stomach was fixed in 10% formalin for subsequent histopathological assessment, and other full-thickness pieces of the gastric corpus were weighed, frozen and stored at −70 °C until glutathione and haemoglobin assays.

Histological assessment

For histological assessment, the glandular stomach was fixed in 10% neutral-buffered formalin solution, sectioned and embedded in paraffin. Four-micrometre-thick sections (4 μm thick) were deparaffinized, stained with haematoxylin and eosin and then examined under a light microscope. The specimens were assessed according to the criteria of Laine and Weinstein (1988). In brief, a 1 cm length of each histological section was assessed for epithelial cell loss (a score of 0–3), oedema in the upper mucosa (a score of 0–4), haemorrhagic damage (a score of 0–4) and presence of inflammatory cells (a score of 0–3). The sections were assessed by an experienced pathologist without the knowledge of the treatments.

GSH assay

The reduced glutathione (GSH) content of stomach tissues as non-protein sulphydryls was estimated according to the method described by Sedlak and Lindsay (1968). A glandular segment from each stomach was homogenized in 5 ml of ice-cold 0.02 M EDTA solution (1 ml per 100 mg tissue). Aliquots (400 μl) of tissue homogenate were mixed with 320 μl of distilled water and 80 μl of 50% (w/v) trichloroacetic acid in glass tubes and centrifuged at $1800 \times g$ for 15 min. The supernatants (400 μl) were mixed with 800 μl Tris buffer (0.4 M, pH 8.9), and 20 μl 5,5-dithio-bis(2-nitrobenzoic acid) (0.01 M) were added. After shaking the reaction mixture, its absorbance was measured at 412 nm within 5 min of the addition of 5,5-dithio-bis(2-nitrobenzoic acid) against a blank with no homogenate. Glutathione concentration was read off a standard curve and expressed as μg GSH per g wet tissue.

Haemoglobin assay

The presence of haemorrhage in the gastric mucosa was determined by haemoglobin assay, using the cyanomethaemoglobin method (Bioclin, Belo Horizonte, MG, Brazil). This standard haemoglobin kit (Bioclin) contains the colour reagent for haemoglobin detection (Drabkin's reagent). A glandular segment of the stomach was homogenized in Drabkin's reagent (100 mg tissue per ml reagent). Shortly afterwards, the samples were centrifuged at $10\,000 \times g$ for 10 min. The supernatants were then removed, filtered using a 0.22 μm filter and centrifuged at $10\,000 \times g$ for 10 min. Absorbance was measured at 540 nm, and haemoglobin concentration was read off a standard curve and expressed as mg Hb per g wet tissue.

Statistical analysis

All values are expressed as means ± s.e.mean. ANOVA and Student–Newman–Keuls test were used to determine the statistical significance of differences between groups. For histological assessment, the Kruskal–Wallis nonparametric test was used, followed by Dunn's test for multiple comparisons. Differences were considered to be significant when $P \leqslant 0.05$.

Results

Sildenafil protected ethanol-induced macroscopic (Figure 1) and microscopic gastric damage (Table 1). From the microscopic analysis, sildenafil decreased haemorrhagic damage, oedema and epithelial cell loss induced by ethanol (Table 1). Figure 2 shows that ethanol administration induced a disruption of the superficial region of the gastric gland with epithelial cell loss and intense haemorrhage. Conversely, we did not observe these alterations in rats treated with ethanol + sildenafil. Furthermore, sildenafil treatment also reversed the increase in haemoglobin concentration and the decrease in GSH concentration in the gastric mucosa induced by ethanol, in a dose-dependent manner (Table 2).

Pretreatment with L-NAME alone, without L-arginine, significantly reversed the sildenafil-mediated protection in both macroscopic and microscopic assessments (Figure 3 and Table 1). Co-treatment with L-NAME and sildenafil also increased haemoglobin concentration and microscopic haemorrhagic damage score and decreased GSH concentration

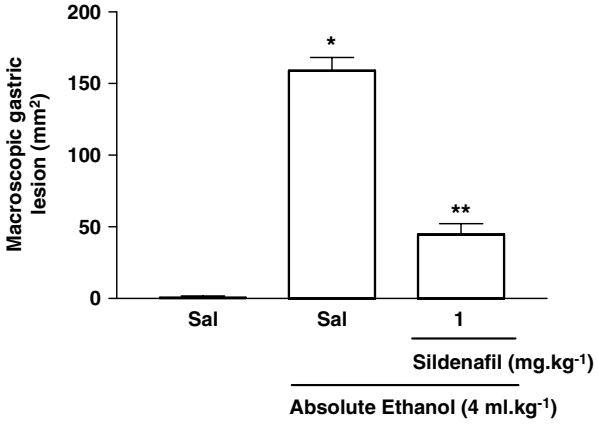

Figure 1 Sildenafil prevents ethanol-induced gastric damage. The rats were treated with saline (Sal) or sildenafil. Thirty minutes later, absolute ethanol (4 ml kg^{-1}) was administered. The control group was treated with saline only. The total area of macroscopic gastric lesions was determined after 1 h. The results are expressed as the means ± s.e.mean of at least five rats per group. *$P<0.05$; **$P<0.01$, when compared to the ethanol group; ANOVA and Newman–Keuls test.

Table 1 Effect of L-NAME (3 mg kg^{-1}), L-arginine (200 mg kg^{-1}), ODQ (10 mg kg^{-1}), glibenclamide (1 mg kg^{-1}) and diazoxide (3 mg kg^{-1}) on protective effects of sildenafil (1 mg kg^{-1}) against ethanol-induced microscopic damage in gastric mucosa

Experimental group (N = *7*)	*Haemorrhagic damage (score 0–4)*	*Oedema (score 0–4)*	*Epithelial cell loss (score 0–3)*	*Inflammatory cells (score 0–3)*	*Total (scores 14)*
Saline	0	0	0	0	0
Ethanol	3 (1–4)	2 (2–4)	1.5 (1–3)	1 (0–1)	10 (8–12)
Ethanol + sildenafil	1 (0–2)*	1 (0–2)*	1 (0–1)*	1 (0–1)	6 (1–7)*
Ethanol + sildenafil + L-NAME	2 (1–4)$^{\#}$	2 (2–3)	1.5 (1–2)	1 (0–1)	7 (6–10)
Ethanol + sildenafil + L-NAME + L-arginine	1 (0–1)^{a}	1 (0–2)^{a}	1 (1–2)	0 (0–1)	5 (1–7)^{a}
Ethanol + sildenafil + ODQ	3 (0–4)$^{\#}$	4 (0–4)$^{\#}$	1 (0–2)	0 (0–1)	11 (2–13)
Ethanol + sildenafil + glibenclamide	2 (1–4)$^{\#}$	4 (3–4)$^{\#}$	2 (1–3)	1 (0–1)	11 (9–12)
Ethanol + sildenafil + glibenclamide + diazoxide	1 (0–4)	1 (0–3)^{b}	1 (0–2)	0 (0–1)	5 (3–10)^{b}

Data shown are medians with minimum and maximal scores shown in brackets.
Abbreviations: L-NAME, N_{ω}-nitro-L-arginine methyl ester hydrochloride; ODQ, 1*H*-[1,2,4]oxadiazolo[4,3-a]quinoxaline-1-one.
*$P<0.05$, vs ethanol group; $^{\#}P<0.05$, vs ethanol + sildenafil group; $^{a}P<0.05$, vs ethanol + sildenafil + L-NAME group; $^{b}P<0.05$, vs ethanol + sildenafil + glibenclamide group. Kruskal–Wallis nonparametric test, followed by Dunn's test was used for multiple comparisons for histological assessment.

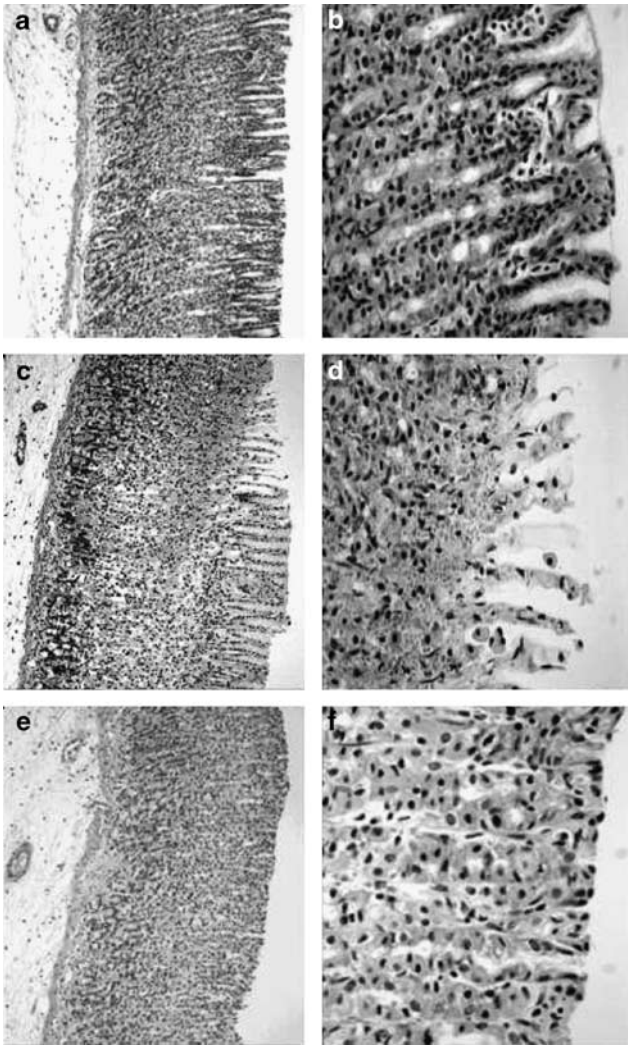

Figure 2 Photomicrographs of gastric mucosa (magnification $\times 100$ for (**a**, **c** and **e**); $\times 400$ for **b**, **d** and **e**). (**a** and **b**) Normal rats; (**c** and **d**) animals treated with absolute ethanol, showing disruption of the superficial region of the gastric gland with epithelial cell loss and intense haemorrhage. (**e** and **f**) Animals treated with absolute ethanol + sildenafil (1 mg kg^{-1}), showing preservation of gastric mucosa. Quantitative results from these assessments are shown in Table 1.

in gastric tissue, when compared to sildenafil alone in the treatment of ethanol-induced gastric damage (Tables 1 and 2). L-Arginine was able to reverse the L-NAME effect on macroscopic damage, microscopic damage, haemoglobin and GSH changes.

In the present study, we investigated whether cGMP participated in sildenafil's gastroprotective effect against ethanol-induced gastric damage. Pretreatment with an inhibitor of cGMP formation, ODQ, significantly reversed sildenafil-mediated protection in macroscopic and microscopic evaluation (Figure 4). Furthermore, ODQ plus

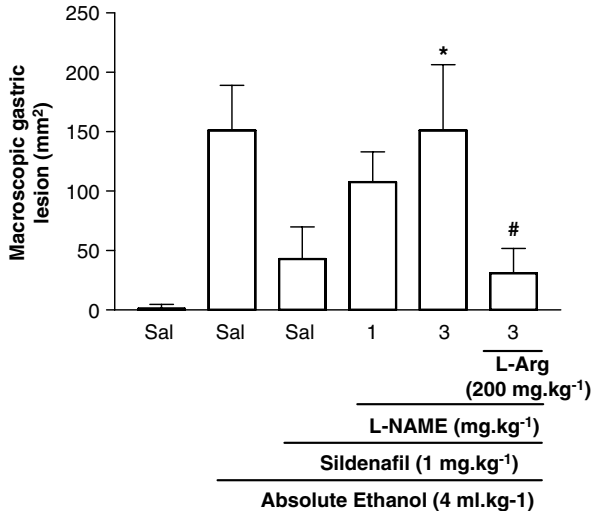

Figure 3 Effect of L-NAME and L-arginine pretreatment on the protective effect of sildenafil against ethanol-induced gastric damage. L-NAME (1, 3 mg kg^{-1}, i.p.) was injected 30 min before sildenafil (1 mg kg^{-1}) or saline (sal) injection. Another group received the same treatment except that L-arginine (L-ARG, 200 mg kg^{-1}, i.p.) was administered 5 min before L-NAME injection (3 mg kg^{-1}). Thirty minutes later, absolute ethanol (4 ml kg^{-1}) was administered. The control group was treated with saline only. Macroscopic gastric lesions were determined after 1 h. Results are expressed as means ± s.e.mean for groups of six rats. *$P<0.05$, statistically significant differences between the sildenafil-treated group and the sildenafil + L-NAME group; $^{\#}P<0.05$, statistically significant differences between the L-NAME-treated group and the L-NAME + L-arginine-treated group; ANOVA and Newman–Keuls test. L-NAME, N_{ω}-nitro-L-arginine methyl ester hydrochloride.

Table 2 Effect of L-NAME, ODQ and glibenclamide pretreatment on sildenafil's protective activity against ethanol-induced increase in Hb and decrease in GSH concentration in gastric mucosa

Experimental group	*Hb (mg per g tissue)*	*GSH (μg per g tissue)*
Saline	15.7 ± 4.5	145.4 ± 26.3
Ethanol	37.9 ± 5.1*	78.7 ± 9.5*
Ethanol + sildenafil	19.3 ± 3.5$^{\#}$	143.6 ± 15.7$^{\#}$
Ethanol + sildenafil + L-NAME	32.3 ± 2.8^{a}	77.6 ± 19.0^{a}
Ethanol + sildenafil + L-NAME + L-arginine	15.6 ± 1.5^{b}	127.5 ± 14.5^{b}
Ethanol + sildenafil + ODQ	27.4 ± 1.2^{a}	41.5 ± 16.7^{a}
Ethanol + sildenafil + glibenclamide	32.8 ± 1.5^{a}	35.4 ± 13.7^{a}
Ethanol + sildenafil + glibenclamide + diazoxide	12.2 ± 2.9^{c}	91.4 ± 5.4^{c}

Abbreviations: GSH, reduced glutathione; Hb, haemoglobin; L-NAME, N_{ω}-nitro-L-arginine methyl ester hydrochloride; ODQ, 1*H*-[1,2,4]oxadiazolo[4,3-a] quinoxaline-1-one.
Data shown are means ± s.e.mean ($n=7$).
*$P<0.05$, vs saline group; $^{\#}P<0.05$, vs ethanol group; $^{a}P<0.05$, vs ethanol + sildenafil group; $^{b}P<0.05$, vs ethanol + sildenafil + L-NAME group; $^{c}P<0.05$, when compared with ethanol + sildenafil + glibenclamide group.

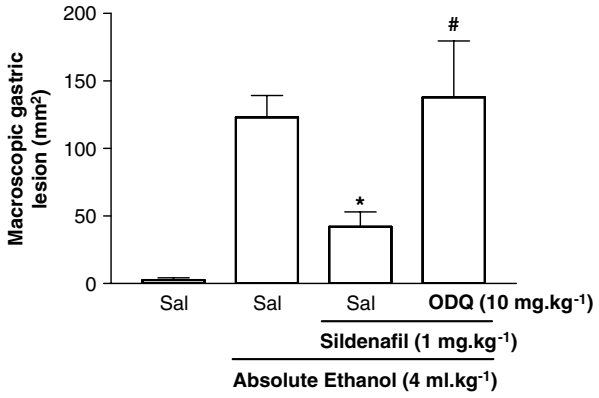

Figure 4 Effect of pretreatment with ODQ on the protective effect of sildenafil against ethanol-induced gastric damage. ODQ (10 mg kg^{-1}, i.p.) was injected 30 min before sildenafil (1 mg kg^{-1}) or saline (sal) injection. Thirty minutes later, absolute ethanol (4 ml kg^{-1}) was administered. The control group was treated with saline only. Macroscopic gastric lesions were determined after 1 h. Results are expressed as means ± s.e.mean for groups of six rats. *$P<0.05$, statistically significant differences between the sildenafil-treated group and the absolute ethanol group; $^{\#}P<0.05$, statistically significant differences between the sildenafil-treated group and the sildenafil + ODQ-treated group; ANOVA and Newman–Keuls test. ODQ, 1*H*-[1,2,4]oxadiazolo[4,3-a]quinoxaline-1-one.

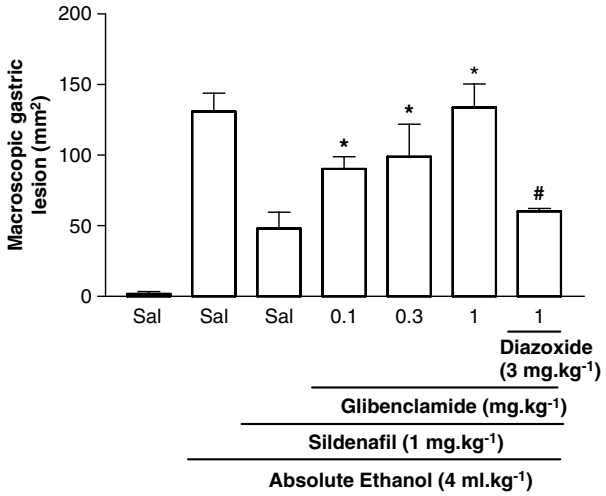

Figure 5 Effect of glibenclamide and diazoxide pretreatment on the protective effect of sildenafil on ethanol-induced gastric lesions in rats. Glibenclamide (0.1, 0.3, 1 mg kg^{-1}, i.p.) was injected 30 min before sildenafil (1 mg kg^{-1}) or saline (sal) injections. Another group was treated in the same way but administered diazoxide (3 mg kg^{-1}, i.p.) + glibenclamide (1 mg kg^{-1}). Thirty minutes later, absolute ethanol (4 ml kg^{-1}) was administered. The control group was treated with saline only. Macroscopic gastric lesions were determined after 1 h. Results are expressed as means ± s.e.mean for groups of six rats. *$P<0.05$, statistically significant differences between the sildenafil-treated group and the sildenafil + glibenclamide group; $^{\#}P<0.05$, statistically significant differences between the glibenclamide-treated group and the glibenclamide + diazoxide-treated group; ANOVA and Newman–Keuls test.

sildenafil treatment significantly increased microscopic haemorrhagic damage, oedema (Table 1) and haemoglobin concentration and decreased GSH concentration in gastric mucosa (Table 2), when compared to sildenafil alone in the treatment of ethanol-induced gastric damage (Table 2). Rats treated with dimethylsulphoxide (0.01%) plus absolute ethanol showed the same area of gastric damage (135.5 ± 29.02 mm^2) as in those treated with ethanol alone (158.9 ± 9.35 mm^2).

To assess the contribution of K_{ATP} channels in the protective effects of sildenafil, other groups of rats were pre-treated with glibenclamide alone or with diazoxide. In Figure 5 and Table 1, we can see that glibenclamide alone, without diazoxide, reversed sildenafil's gastroprotective effect against ethanol-induced macroscopic and microscopic gastric damage, with a maximal effect at a dose of 1 mg kg^{-1}. In addition, glibenclamide without diazoxide also reversed the protective effect of sildenafil against the microscopic haemorrhagic damage and oedema (Table 1), as well as the increase in haemoglobin concentration and the decrease in GSH concentration in the gastric mucosa induced by ethanol administration (Table 2). Gastric damage in rats treated with NaOH 0.01 N (144.6 ± 20.14 mm^2) or NaOH 0.05 N (135.6 ± 16.28 mm^2) plus absolute ethanol was not different from that in animals treated with ethanol alone (158.9 ± 9.35 mm^2).

Discussion and conclusions

NO modulates several elements of gastric mucosal defence, including blood flow (Whittle *et al*., 1981), neutrophil adhesion (Kubes *et al*., 1991; May *et al*., 1991) and mucus secretion (Allen *et al*., 1993; Wallace and Miller, 2000). The effect of NO is, at least partially, mediated by an increase in cGMP content, and cGMP is normally broken down rapidly by phosphodiesterase-type 5. Sildenafil, a drug used to improve functional impotence, increases cGMP levels by blocking phosphodiesterase-type 5 (Moreland *et al*., 1998). Our group has shown that sildenafil, acting via NO-dependent mechanisms, protects the stomach against indomethacin-induced damage. These results were shown to be consistent with the hypothesis that the protective effect of sildenafil is mediated by the inhibition of indomethacin-induced leukocyte adhesion to vascular endothelium and by maintenance of gastric blood flow (Santos *et al*., 2005). Recently, we demonstrated that sildenafil also prevents ethanol-induced gastric damage (Medeiros *et al*., in press). In the present study, we evaluated the role of NO/cGMP/K_{ATP} pathway in sildenafil's gastroprotective effect.

Our results confirmed that ethanol administration at high concentrations caused severe macroscopic and microscopic gastric mucosal damage, with haemorrhage, oedema and epithelial cell loss (Guslandi, 1987; Laine and Weinstein, 1988). We also measured haemorrhagic damage using a commercial haemoglobin assay (Souza *et al*., 2004), and an increase in redox state of the tissue by measuring gastric GSH concentration during ethanol-induced gastric damage. Despite the fact that ethanol administration can increase inflammatory mediators and then induce neutrophil migration to the gastric mucosa, we did not observe an increase in inflammatory cell infiltration, probably because we killed the rats only 1 h after ethanol administration. Thus, sildenafil's gastroprotective effect against ethanol-induced gastric damage could not be explained by a decrease in

leukocyte adherence, which was observed in the protective effect of sildenafil against indomethacin-induced gastropathy. Another possibility is that the effect of sildenafil may be related to an increase in gastric blood flow, as sildenafil increased the gastric blood flow in normal, untreated rats and prevented the decrease in gastric blood flow induced by indomethacin (Santos *et al*., 2005).

Our findings suggested that sildenafil's defensive effect was a NO-dependent process. First, pretreatment with the non-selective NOS inhibitor (L-NAME) abolished the effect of sildenafil. Moreover, co-administration of L-arginine, a precursor of endogenous NO synthesis, restored sildenafil's protective effect against ethanol-induced gastric damage. Earlier work has demonstrated that NO donors reduced ethanol-induced gastric damage (Macnaughton *et al*., 1989), and our group has shown that sildenafil's protective effect against indomethacin-induced gastropathy was also a NO-dependent event (Santos *et al*., 2005).

Levels of cGMP are increased in response to the activation of soluble guanylate cyclase by NO. Using a pharmacological approach, we demonstrated that inhibition of soluble guanylate cyclase by ODQ reversed the protective effects of sildenafil against ethanol-induced gastric damage. Inhibition of guanylate cyclase also increased haemorrhage and GSH concentration in the gastric mucosa (Table 2). Our results are compatible with those of Brzozowski *et al*. (2000), who found that ODQ treatment completely abolished the protective effect of NO-releasing non-steroidal anti-inflammatory drugs against ethanol-induced gastric damage.

Recently, the participation of K_{ATP} channels in several models of gastric protection was described (Peskar *et al*., 2002; Gomes *et al*., 2006). However, the role of the K_{ATP} channels in the gastroprotective effect of sildenafil was not defined. We have shown here that blockade of K_{ATP} channels with glibenclamide alone, without diazoxide, reversed sildenafil's protective effect against ethanol-induced gastric damage. NO and cyclic GMP can activate different types of K^+ channels (Archer *et al*., 1994, Bolotina *et al*., 1994) and sildenafil induced a cardioprotective effect via the opening of K_{ATP} channels (Ockaili *et al*., 1999). Moreover, the antinociceptive effect of sildenafil was dependent on the activation of the NO/cGMP/K_{ATP} pathway (Vale *et al*., 2007).

In summary, our results indicate that sildenafil prevents ethanol-induced gastric damage. Although there are many mechanisms through which this effect can occur, our data supports the hypothesis that the activation of NO/cGMP/K_{ATP} pathway is of primary importance. Sildenafil may have utility as a protective agent against ethanol-induced gastropathy in a clinical setting.

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Conflict of interest

The authors state no conflict of interest.

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