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The nitric oxide donor cis-[Ru(bpy)$_2$(SO$_3$)NO](PF$_6$) increases gastric mucosa protection in mice – Involvement of the soluble guanylate cyclase/K$_{ATP}$ pathway

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ABSTRACT

Here, we have evaluated the protective effect of the NO donor cis-[Ru(bpy)$_2$(SO$_3$)NO](PF$_6$) (FOR0810) in experimental models of gastric damage induced by naproxen or ethanol in mice, and the involvement of soluble guanylate cyclase (sGC) and ATP-sensitive K$^+$ channels (K$_{ATP}$) in these events. Swiss mice were pre-treated with saline, ODQ (a soluble guanylate cyclase inhibitor; 10 mg kg^{-1}) or glibenclamide (a K$_{ATP}$ channels blocker; 10 mg kg^{-1}). After either 30 min or 1 h, FOR0810 (3 mg kg^{-1}) was administered. At the end of 30 min, the animals received naproxen (300 mg kg^{-1}) by gavage. After 6 h, the animals were sacrificed and gastric damage, myeloperoxidase (MPO) activity, and TNF-α and IL-1β gastric concentrations were evaluated. In addition, the effects of FOR0810 on naproxen-induced mesenteric leukocyte adherence were determined by intravital microscopy. Other groups, were pre-treated with saline, ODQ or glibenclamide. After either 30 min or 1 h, FOR0810 was administered. At the end of 30 min, the animals received 50% ethanol by gavage. After 1 h, the animals were sacrificed, and gastric damage, gastric reduced glutathione (GSH) concentration and malondialdehyde (MDA) levels were determined. In naproxen-induced gastric damage, FOR0810 prevented gastric injury, decreased gastric MPO activity and leukocyte adherence, associated with a decrease in TNFα and IL-1β gastric concentrations. FOR0810 also prevented ethanol-induced gastric damage by increase in GSH levels and decrease in MDA levels. ODQ and glibenclamide completely reversed FOR0810's ability to prevent gastric damage by either naproxen or ethanol. We infer that FOR0810 prevented gastric damage through the activation of both sGC and K$_{ATP}$ channels, which triggered a decrease in both free radical and cytokine production via the blocking of neutrophil adhesion and infiltration.

1. Introduction

The gastric mucosa is exposed to various noxious substances and resists damage from hydrochloric acid, hot and cold foodstuffs with different osmolarities, anti-inflammatory drugs (NSAIDs) and ethanol [1]. In the gastrointestinal tract, the use of NSAIDs and ethanol decreases gastroprotective mechanisms, such as mucus secretion and blood flow [2] resulting in mucosal injury and gastrointestinal bleeding [3,4], by different mechanisms. Naproxen increases the levels of inflammatory mediators and induces neutrophil migration to the gastric mucosa, which are the more important mechanisms to the NSAID-induced gastric injury [5]. On the other hand in ethanol-induced gastric damage, the most important effects are an increase in reactive oxygen species generation and a decrease in endogenous anti-oxidant defense mechanisms [6,7].

Nitric oxide (NO) is a very important mediator of gastric mucosal defense [8]. Many effects of NO on the gastrointestinal tract seem to depend on the activation of the NO/cGMP/K$_{ATP}$ pathway [4,9]. The chemical properties of this molecule enable the synthesis of a wide variety of NO-donors, each with a different rates of NO release [1,8]. In recent years, new metal complexes have been investigated regarding their ability to work as NO donors, including nitrosyl ruthenium complexes [10–14].

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The chemical properties of nitrosyl ruthenium complexes have come under on increased scrutiny, because of the role that these transition metal complexes can play in NO-related biological processes, as well as the possibility of producing thermodynamically stable species [15,16]. Current studies have focused on the development of a pharmacological compound capable of releasing NO in a controlled manner within biological tissues. In this context, nitrosyl ruthenium complexes are promising NO donor agents and offer several advantages for biological applications of NO [10,17].

Recently, our group synthesized the potential metallopharmaceutical compound cis-[Ru(bpy)$_2$(SO$_3$)NO](PF$_6$), which is a potent vasodilator capable of releasing intracellular NO and activating soluble guanylate cyclase (sGC) [10,16]. This compound is here called FOR0810. This NO donor releases nitric oxide through chemical, electrochemical and photochemical reactions [16]. In biological media, this complex releases NO following activation by biological reducing agents [12]. The FOR0810 compound exerts several biological effects, such as antihypertensive effect [10], trypanocidial activities in vitro and in vivo [18], beneficial effects in a model of ischemia and reperfusion [13] and antinociceptive effect [14]. Given the chemical properties of the cis-[Ru(bpy)$_2$(SO$_3$)NO](PF$_6$) compound, the aim of the present study was to investigate the effect of this NO-donor complex in experimental models of gastric injury induced by either naproxen or ethanol in mice, and to determine the involvement of the enzyme soluble guanylate cyclase and ATP-sensitive K^+ channels in this process.

2. Materials and methods

2.1. Animals

Swiss mice (weight 25–30 g) fasted for 18–24 h before the experiments. Animals were housed in cages inside a temperature controlled room and received food and water ad libitum. All animal treatments and surgical procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals, National Institutes of Health (Bethesda, MD, USA) and were approved by the local ethics committee (Protocol 33/10).

2.2. Drugs and solutions

The drugs and reagents used in the study were ethanol and naproxen, as well as ODQ (1H-1,2,4oxadiazolo [4,3-a]quinoxaline-1-one), and glibenclamide, which were purchased from Sigma-Aldrich (St. Louis, MO, USA). Vehicle solutions consisted of carboxymethylcellulose 0.5% or saline. Glibenclamide was dissolved in 0.01N NaOH containing 4% glucose, ODQ was dissolved, in 1% DMSO, and naproxen was dissolved, in 0.5% carboxymethylcellulose (CMC).

2.3. Synthesis of the ruthenium NO donor (cis-[Ru(bpy)$_{2(SO3)NO](PF6))}$

The ruthenium NO donor (cis[Ru(bpy)$_2$(SO$_3$)NO](PF$_6$)), FOR0810, was synthesized and purified at the Department of Organic and Inorganic Chemistry of the Federal University of Ceara as described previously [16].

2.4. Determination of the levels of nitrite/nitrate (NOX) in gastric juice

To evaluate NO liberation, Swiss mice were pre-treated by gavage with FOR0810 (NO donor; 3 mg kg^{-1}, p.o.) or Rut (NO carrier; 2.3 mg kg^{-1}, p.o.) and subjected to pylorus ligation surgery. After 4 h, the animals were sacrificed, and gastric juice was collected to determine the levels of nitrite/nitrate (NOx), as described elsewhere [19].

2.5. Effect of FOR0810 on gastric damage models

Mice were treated with carboxymethylcellulose (CMC) 0.5%, FOR0810 (3 mg kg^{-1}) or Rut (2.3 mg kg^{-1}) by gavage. After 30 min, naproxen (300 mg kg^{-1}, p.o.) was administered by gavage to all groups. Six hours later the animals were sacrificed and the stomach was rapidly removed, opened and pinned to a wax block. Hemorrhagic or ulcerative lesions were measured using an analog caliper. The gastric damage score (lesion index) was subsequently calculated as the sum of the lengths of all linear lesions, which were measured by a single observer (A.P.M.S). A sample of the corpus region of each stomach was fixed in 10% formalin for subsequent histopathological assessment. Other full thickness specimens of the gastric corpus were then weighed, frozen and stored at −70 °C until assayed for myeloperoxidase (MPO) activity as previously described [19], and for determination of TNF-α and IL-1β gastric concentrations as previously described [20]. Additionally, the effect of FOR0810 (3 mg kg^{-1}) on naproxen-induced mesenteric leukocyte adherence was determined by intravital microscopy.

Other groups were pre-treated with saline, FOR0810 (3 mg kg^{-1}) or Rut (2.3 mg kg^{-1}). At the end of 30 min, the animals received 50% ethanol (0.5 ml 25 g^{-1}) by gavage as described by Medeiros [7]. After 1 h, the animals were sacrificed and their stomachs were rapidly removed, opened through an incision along the greater curvature, and pinned to a wax block. Hemorrhagic or ulcerative lesions were measured using a computer planimetry program (Image J; National Institutes of Health). A sample of the corpus region of each stomach was fixed in 10% formalin for subsequent histopathological assessment. Other full-thickness specimens of the gastric corpus were then weighed, frozen and stored at −70 °C until assayed for either reduced glutathione (GSH) (Sedlak and Lindsay) [21] or malondialdehyde (MDA) (Mihara and Uchiyama) [22].

2.6. Involvement of the soluble guanylate cyclase/K_{ATP} pathway in the protective effect of FOR0810

Swiss mice (25–30 g) were pre-treated with saline, ODQ (sGC inhibitor, 10 mg kg^{-1}, p.o.) or glibenclamide (GLIB, K_{ATP} blocker, 10 mg kg^{-1}, i.p.). After either 30 min or 1 h, FOR0810 (3 mg kg^{-1}, p.o.) was administered. At the end of 30 min, the animals received naproxen (300 mg kg^{-1}) by gavage. After 6 h, the animals were sacrificed, and gastric damage (macroscopic) and myeloperoxidase (MPO) activity were evaluated. The other group was pre-treated either ODQ (10 mg kg^{-1}, p.o.) or glibenclamide (GLIB 10 mg kg^{-1}, i.p.). After either 30 min or 1 h, FOR0810 (3 mg kg^{-1}, p.o.) was administered. At the end of 30 min, the animals received 50% ethanol (0.5 ml 25 g^{-1}) by gavage. After 1 h, the animals were sacrificed and gastric damage (macroscopic), reduced glutathione (GSH) and malondialdehyde (MDA) levels were determined.

2.7. Histological evaluation of gastric damage

For histological assessment, the glandular stomach was fixed in 10% neutral-buffered formalin solution, sectioned and embedded in paraffin. Four-micrometer-thick sections were deparaffinized, stained with hematoxylin and eosin, and examined under light in a blinded manner by an experienced histologist (P.M.G Soares), using Laine's scores [23]. In brief, a 1 cm length of each histological section was assessed for epithelial cell loss (a score of 0–3), edema in the upper mucosa (a score of 0–4), hemorrhagic damage (a score of 0–4), and the presence of inflammatory cells (a score of 0–3).

2.8. Determination of neutrophil adhesion to the mesenteric microcirculation by intravital microscopy

Intravital microscopy was used to establish the extent of leukocyte adhesion to mesenteric microcirculation in mice groups treated with vehicle (0.5% CMC) alone, naproxen (300 mg kg^{-1}) alone and FOR0810 (3 mg kg^{-1}) or Rut (2.3 mg kg^{-1}) plus naproxen. Leukocyte parameters were examined as previously described [24] with adaptations. Leukocyte adhesion was investigated 3 h after treatment. Cells were counted in a recorded image, using four different fields for each animal to avoid sampling variability. The data were then averaged for each animal.

2.9. Statistical analysis

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

3. Results

3.1. Effects of FOR0810 compound on the levels of nitrite/nitrate in gastric juice

The content of nitrite/nitrate in gastric juice of mice is shown in Fig. 1. Compared with the control group (0.883 ± 0.35 μM) a single dose of 3 mg kg^{-1} of FOR0810 induced an increase in nitrite/nitrate levels (3.025 ± 0.87 μM). Furthermore, the treatment of animals with ruthenium complex alone (without bound NO) did not alter nitrite/nitrate levels compared with the control group (0.52 ± 0.29 μM).

3.2. Effects of FOR0810 compound on naproxen and ethanol-induced gastric damage

In Fig. 2, we confirmed that treating animals with either naproxen or ethanol led to the formation of macroscopic gastric lesions (naproxen = 11.37 ± 1.15 mm; ethanol = 136.5 ± 10.19 mm^2). Moreover, treatment with FOR0810 (3 mg kg^{-1}) reduced naproxen and ethanol-induced gastric damage (naproxen = 5.11 ± 1.09 mm; ethanol = 7.67 ± 5.0 mm^2, respectively). However, the ruthenium compound alone (without bound NO) did not have protective effect in either model tested. The gastroprotective effect of FOR0810 was confirmed by histological analysis (Table 1 and Fig. 3). Microscopic mucosal analysis revealed that naproxen increased epithelial cell loss, edema, hemorrhagic damage and inflammatory cells infiltration, and ethanol increased edema, epithelial cell loss, and hemorrhagic damage, but did not cause inflammatory cells infiltration. In contrast, pretreatment with FOR0810 significantly decreased the edema, hemorrhage and loss of epithelial cells induced by naproxen or ethanol, and also decreased the inflammatory cells infiltration induced by naproxen. Therefore, analysis of these micro and macroscopic findings revealed an excellent correlation between compound use and treatment outcome, confirming the efficacy of FOR0810. These protective effects were not observed with ruthenium complex alone (without bound NO) (Table 1 and Fig. 3); reinforcing NO is essential for this activity.

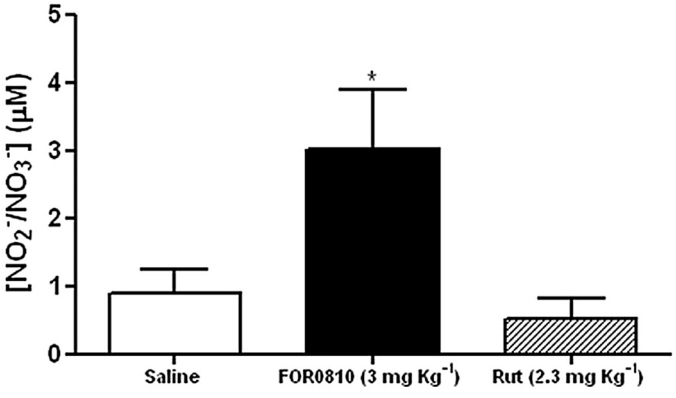

Fig. 1. Levels of nitrite/nitrate (NOx) in gastric juice of mice. Swiss mice were pre-treated by gavage with FOR0810 (3 mg kg^{-1}) or Rut (3 mg kg^{-1}) and subjected to pylorus ligation surgery; after 4 h, the animals were sacrificed, and gastric juice was collected to determine the levels of nitrite/nitrate (NOx). The results are expressed as the mean ± SEM of at least six mice per group. *$P < 0.05$ versus saline group (ANOVA and Newman–Keuls method). FOR0810 (NO donor) or Rut (NO carrier).

3.3. Effects of FOR0810 compound on naproxen-induced mucosal inflammation

Naproxen produced a marked increase in neutrophil infiltration (124.4 ± 11.4 neutrophil/mg of tissue) (Fig. 4A). This increase was reduced by pre-treatment with FOR0810 (67.3 ± 9.7 neutrophil/mg of tissue). The administration of FOR0810 also reduced neutrophil adherence to endothelial cells (Fig. 4B) compared with the naproxen group, suggesting that its gastroprotective action may involve inhibition of neutrophil infiltration.

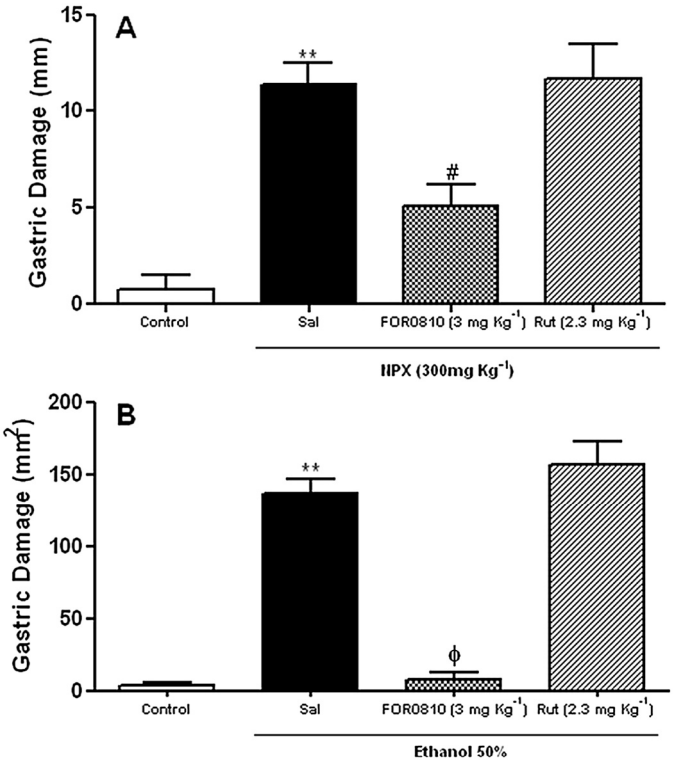

Fig. 2. FOR0810 reduces naproxen or ethanol-induced macroscopic gastric damage. Swiss mice were pre-treated by gavage with FOR0810 (3 mg kg^{-1}) 30 min before they received naproxen (300 mg kg^{-1}) by gavage. After 6 h, the animals were sacrificed and gastric damage (macroscopic) was determined (A). Another group was pre-treated by gavage with FOR0810 30 min before 50% ethanol (0.5 ml 25 g^{-1}, p.o.). After 1 h, the animals were sacrificed, and gastric damage (macroscopic) was determined (B). The results are expressed as the mean ± SEM of at least six mice per group. **$P < 0.01$ compared with the control group; #$P < 0.01$ compared with the naproxen group; ΦP < 0.001 compared with the ethanol group (ANOVA and Newman–Keuls method). Sal (Saline); NPX (Naproxen). FOR0810 (NO donor) or Rut (NO carrier).

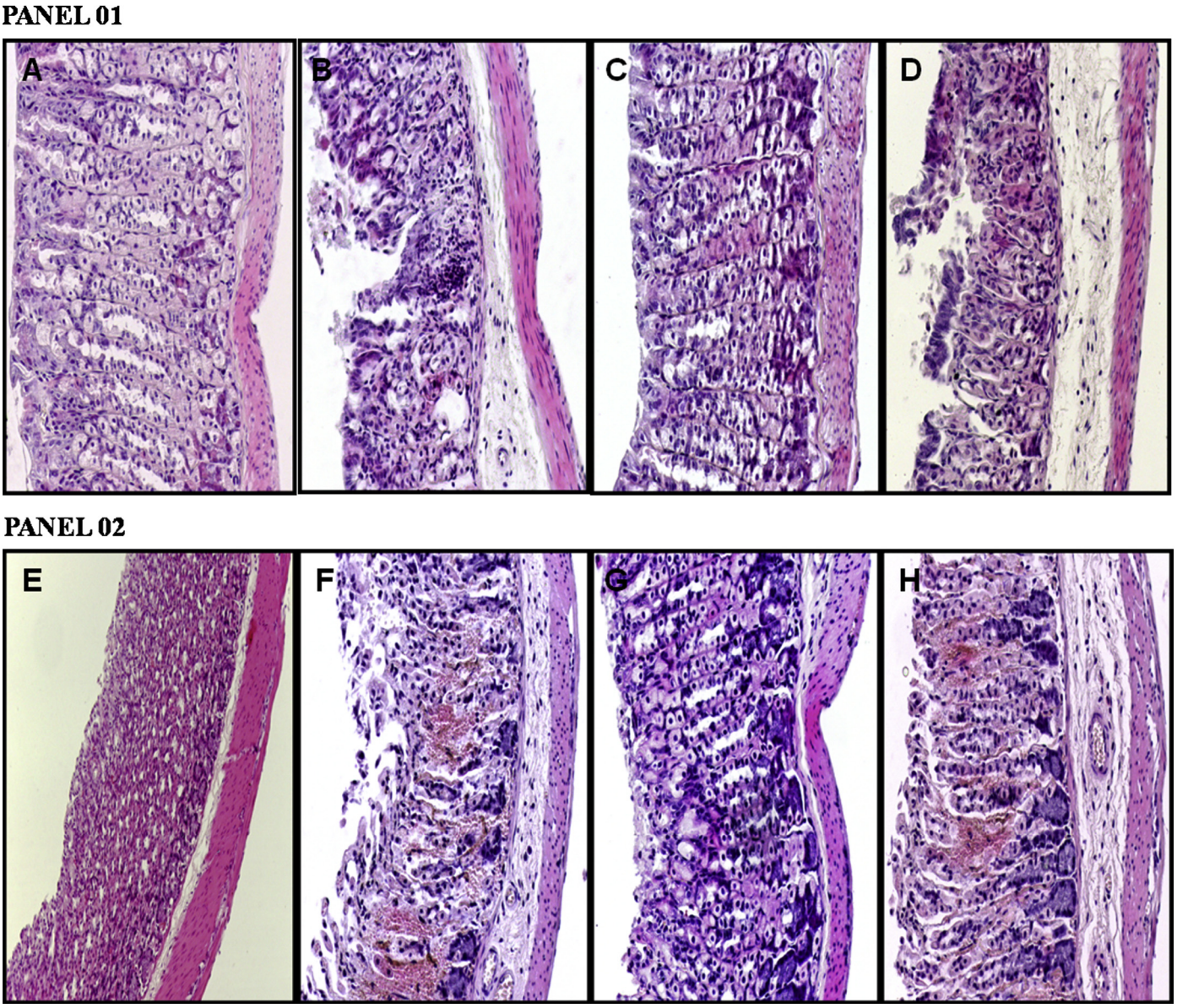

Fig. 3. Photomicrographs of gastric mucosa (magnification ×100). Panel 01 – (A) Normal mice; (B) animals treated with naproxen (300 mg kg^{-1}), showing disruption of the superficial region of the gastric gland with associated epithelial cell loss and intense inflammatory infiltration; (C) animals treated with naproxen (300 mg kg^{-1}) + FOR0810 (3 mg kg^{-1}), showing preservation of gastric mucosa; (D) animals treated with naproxen (300 mg kg^{-1}) + Ruthenium (2.3 mg kg^{-1}), showing disruption of the superficial region of the gastric gland with associated epithelial cell loss and intense inflammatory infiltration. Panel 02 – (E) Normal mice; (F) animals treated with ethanol 50%, showing disruption of the superficial region of the gastric gland with associated epithelial cell loss and intense hemorrhage; (G) animals treated with ethanol 50% + FOR0810 (3 mg kg^{-1}), showing preservation of gastric mucosa; (H) animals treated with ethanol 50% + Ruthenium (2.3 mg kg^{-1}), showing disruption of the superficial region of the gastric gland with associated epithelial cell loss and intense hemorrhage. Quantitative results from these assessments are shown in Table 1. NPX (Naproxen). FOR0810 (NO donor) or Rut (NO carrier).

As shown in Fig. 4C, the administration of naproxen induced a marked increase in TNF-α and IL1-β concentrations in gastric mucosa (5.33 ± 0.37 and 2.01 ± 0.11 pg/g of tissue). Pre-treatment with FOR0810 reduced TNF-α (3.24 ± 0.21 pg/g of tissue) and IL1-β (1.30 ± 0.09 pg/g of tissue) levels significantly. No changes in these parameters were observed in animals treated with ruthenium complex alone (without bound NO).

3.4. Role of cGMP and K_{ATP} channels in the protective effect of FOR0810 compound against naproxen-induced gastric damage

In the present study, we investigated whether cGMP and K_{ATP} channels participated in the gastroprotective effects of FOR0810 against naproxen-induced gastric damage (Fig. 5A). Pre-treatment with ODQ (sGC inhibitor) or glibenclamide (blocker of K_{ATP} channels), significantly reversed FOR0810 induced gastroprotection against naproxen-induced macroscopic lesions and neutrophil infiltration of gastric mucosa (Fig. 5A and B, respectively). These results suggest that the endogenous NO/cGMP/K_{ATP} pathway is involved in naproxen induced gastric damage.

3.5. Role of cGMP and K_{ATP} channels in the protective effect of FOR0810 compound against ethanol-induced gastric damage

We investigated whether cGMP and K_{ATP} channels participated in the gastroprotective effects of FOR0810 against ethanol-induced

Table 1
Effect of FOR0810 compound on naproxen or 50% ethanol-induced gastric microscopic damage.

Experimental group (n = 6)	Hemorrhagic damage (score 0–4)	Edema (score 0–4)	Epithelial cell loss (score 0–3)	Inflammatory cells (score 0–3)	Total (score 0–14)
CMC	0	0	0	0	0
NPX (300 mg kg^{-1})	2 (1–4)	2 (0–3)	2 (1–3)	2 (1–3)	7 (4–10)
NPX + FOR0810 (3 mg kg^{-1})	0 (0–1)*	0.5 (0–1)*	1 (0–1)*	0 (0–1)*	1.5 (0–3)*
NPX+ Rut (2.3 mg kg^{-1})	1 (1–2)	2 (1–3)	2 (1–3)	2 (1–3)	6 (3–10)
Saline	0	0	0	0	0
Ethanol	2 (2–4)	2 (2–3)	2 (2–3)	0	6 (4–11)
Ethanol + FOR0810 (3 mg kg^{-1})	0 (0–1)**	0 (0–1)**	0.5 (0–1)**	0	0 (0–1)**
Ethanol + Rut (2.3 mg kg^{-1})	2 (2–4)	2 (2–3)	2 (1–2)	0	7 (6–8)

The table shows median values followed by minimum and maximum scores. The Kruskal–Wallis nonparametric test, followed by Dunn's test, was used for multiple comparisons in this histological assessment.
* $P < 0.05$ vs. NPX group.
** $P < 0.05$ vs. ethanol group.
CMC (carboxymethylcellulose); NPX (Naproxen). FOR0810 (NO donor) or Rut (NO carrier).

gastropathy (Fig. 6A). As seen with naproxen, pre-treatment with ODQ or glibenclamide, significantly reversed FOR0810 induced gastroprotection against ethanol-induced macroscopic lesions.

Administration of ethanol also resulted in reduced glutathione levels and an increased MDA concentration (Fig. 6B and C), compared with the control group. FOR0810 treatments reversed the effects of ethanol on these biochemical parameters. However, pre-treatment with either ODQ or glibenclamide significantly reversed the FOR0810's effect on reduced glutathione and MDA levels.

4. Discussion

In this study, we demonstrated that the FOR0810 compound has protective effects against gastric lesions induced by either naproxen or ethanol, both macroscopically and microscopically. Both the significant reduction in histologic scores and the preservation of stomach epithelium in samples from animals treated with FOR0810 indicate a protective action of these agents. It has previously been shown that the FOR0810 complex releases NO through

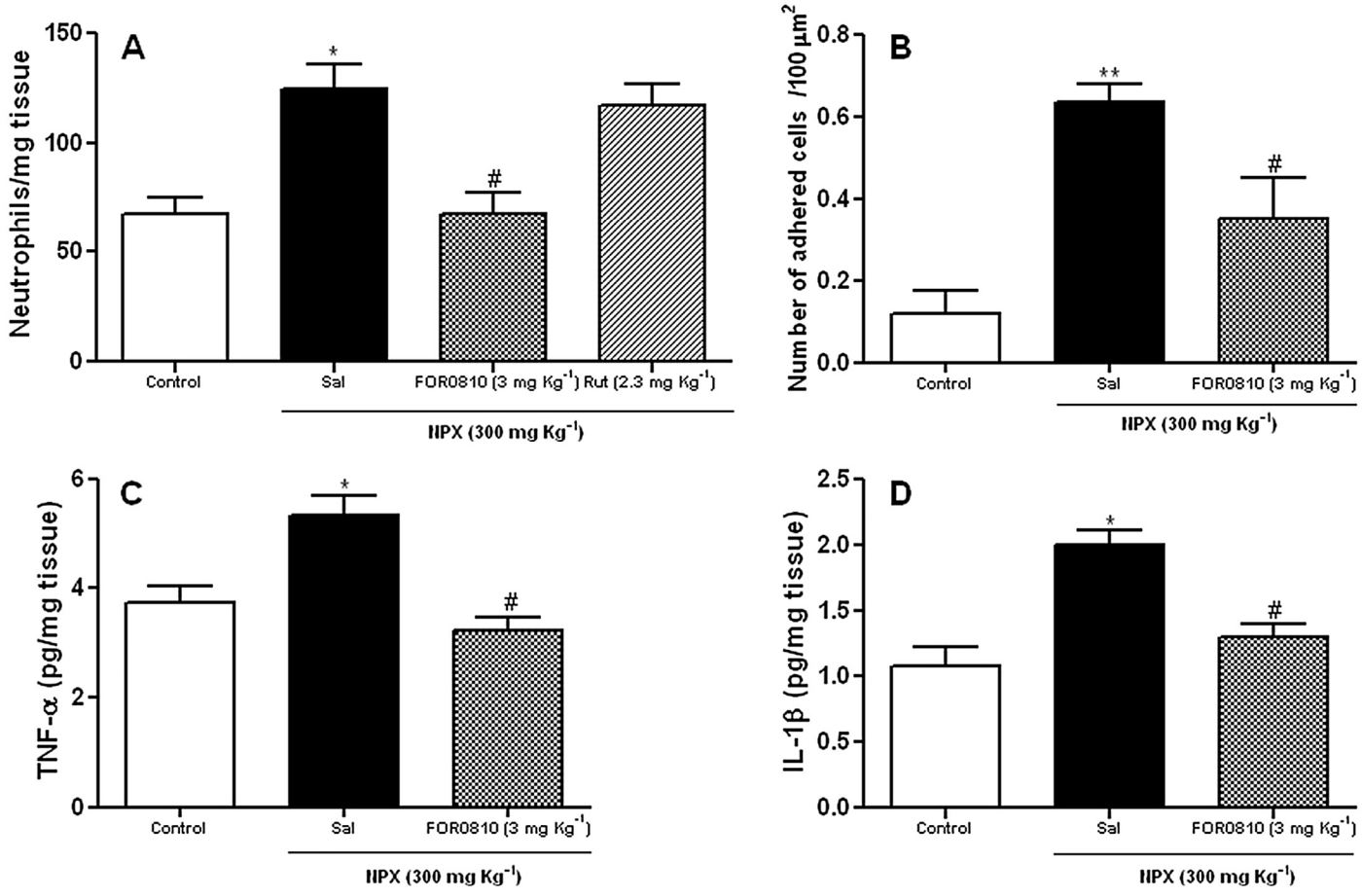

Fig. 4. Effect of FOR0810 compound on increased levels of TNF-α, IL-1β, MPO activity and neutrophil adhesion induced by naproxen in mice. Gastric mucosal segments were obtained to assay MPO activity and, levels of TNF-α and IL-1β. The effect of FOR0810 (3 mg kg^{-1}) on naproxen (300 mg kg^{-1})-induced mesenteric leukocyte adherence as determined by intravital microscopy. The results are expressed as the mean ± SEM of at least six mice per group. *$P < 0.05$; **$P < 0.01$ compared with the control group; #$P < 0.01$ compared with the naproxen group (ANOVA and Newman–Keuls method). Sal (Saline); NPX (Naproxen). FOR0810 (NO donor) or Rut (NO carrier).

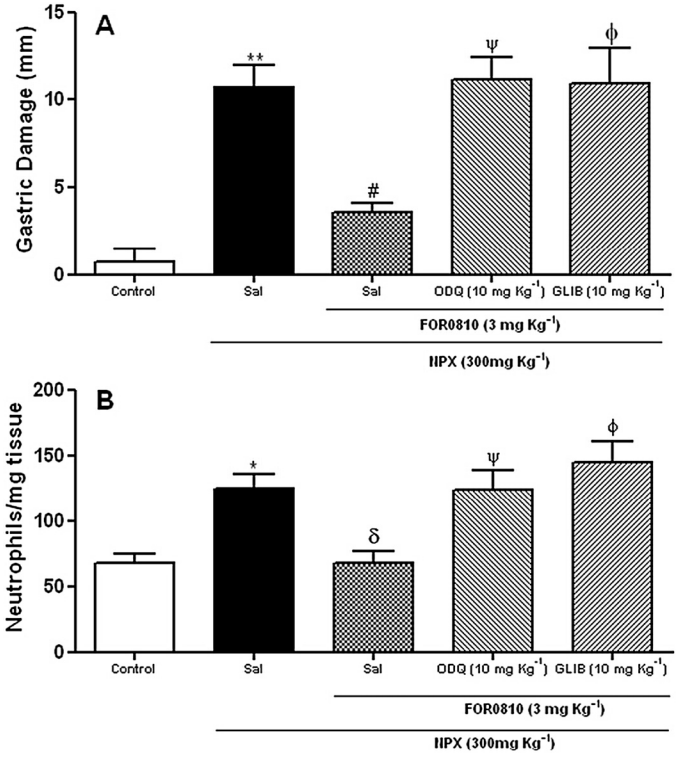

Fig. 5. Effect of ODQ and Glibenclamide (GLIB) pre-treatment on the protective effect of FOR0810 compound against naproxen-induced gastric damage. Mice were pre-treated with either ODQ or GLIB. After either 30 min or 1 h, FOR0810 (3 mg kg^{-1}) was administered. At the end of 30 min, the animals received naproxen (300 mg kg^{-1}) by gavage. After 6 h, the animals were sacrificed, and gastric damage (macroscopic) was determined. Gastric mucosal segments were obtained to assay MPO activity. The results are expressed as the mean ± SEM of at least six mice per group. *P < 0.05; **P < 0.01 compared with the control group; #P < 0.01; δP< 0.05 compared with the naproxen group; ΨP< 0.05; ΦP< 0.05 compared with the FOR0810 group (ANOVA and Newman–Keuls method). Sal (Saline); NPX (Naproxen). FOR0810 (NO donor) or Rut (NO carrier).

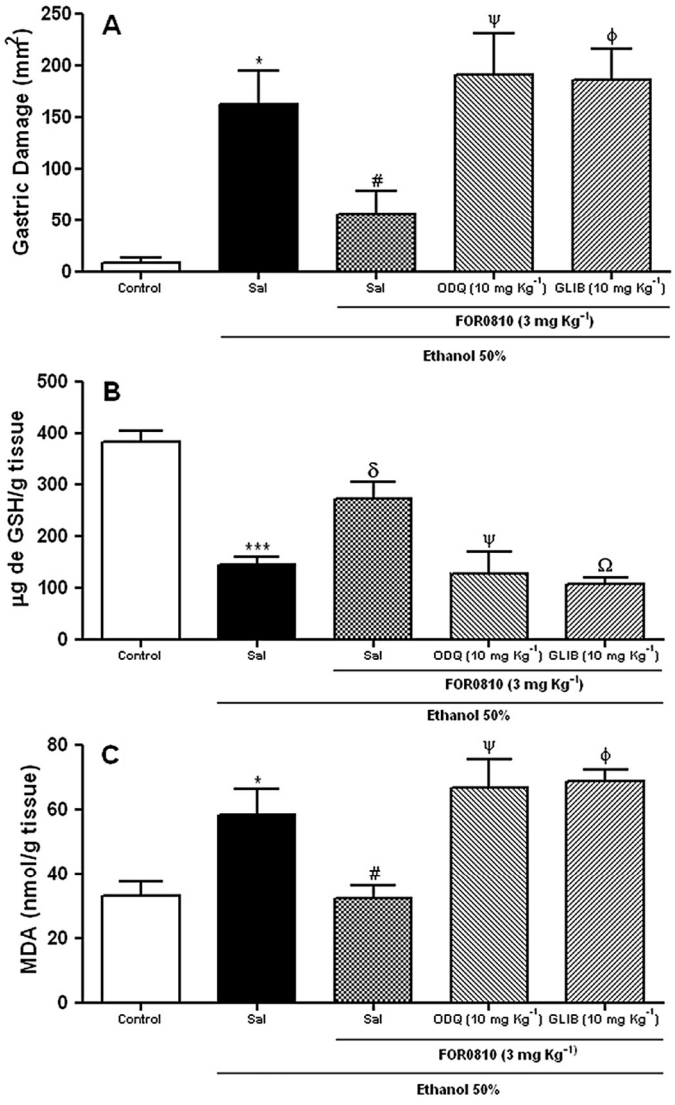

Fig. 6. Effect of ODQ and glibenclamide (GLIB) pretreatment on the protective effect of FOR0810 compound against ethanol-induced gastric damage. Mice were pre-treated with either ODQ or GLIB. After either 30 min or 1 h, FOR0810 (3 mg kg^{-1}) was administered. At the end of 30 min, the animals received 50% ethanol (0.5 ml $25g^{-1}$) by gavage. After 1 h, the animals were sacrificed and gastric damage (macroscopic) was determined. Gastric mucosal segments were obtained for GSH and MDA assays. The results are expressed as the mean ± SEM of at least six mice per group. *P < 0.05; ***P < 0.001 compared with the control group; #P < 0.05; δP < 0.01 compared with the ethanol group; ΨP < 0.05; ΦP < 0.05; ΩP < 0.001 compared with the FOR0810 group (ANOVA and Newman–Keuls method). Sal (Saline), FOR0810 (NO donor) or Rut (NO carrier).

electrochemical, photochemical and chemical processes [16]. The reactions of these complexes with sulfhydryl compounds, such as glutathione and cysteine, may result in the formation of an aqua species and free nitric oxide. In past years, much attention has been paid to nitrosyl-ruthenium compounds and their possible pharmacological uses, particularly due to their ability to promote rapid NO release as well as a low level of toxicity as demonstrated by Silva et al. These authors conducted in vivo toxicity studies with the compound FOR0810 (cis-$[Ru(bpy)_2(SO_3)NO](PF_6)$ and found that symptoms of toxicity were observed only with a dose higher than 500 µmol kg^{-1} that was superior than the dose used in our study (4.4 µmol kg^{-1}) [18]. To the best of our knowledge, the present study is the first to demonstrate that FOR0810 increases gastric defense.

Naproxen is known to increase the levels of inflammatory mediators and to induce neutrophil migration to the gastric mucosa, which is important in the development of gastric injury [5]. In the present investigation, it was demonstrated that FOR0810 prevented naproxen-induced gastric damage by decreasing the concentrations of both TNF-α and IL-1β, as well as reducing granulocyte infiltration into gastric tissue (measured by MPO assay). Our results are in accordance with the literature, which showed that other NO-releasing agents also protected against experimental NSAID-induced gastric damage [25,26], and NO donors also accelerated gastric ulcer healing in rats [27]. There is much evidences that NO inhibits the expression of adhesion molecules on endothelial cells, which is an important step in neutrophil adherence and migration to an inflammatory site [28–30]. We observed that our NO releasing agent (FOR0810) decreased naproxen-induced leukocyte adherence in postcapillary mesenteric venules, and also decreased neutrophil migration. There is also evidence that NO is a crucial mediator of gastric mucosal blood flow [31], and that NSAID induced gastric damage is associated with a reduction in mucosal blood flow [25,32]. We did not assess gastric blood flow in our study. However, Cerqueira et al. demonstrated that FOR0810 induced potent vasodilatation in pre-contracted aortic rings. We infer that the FOR0810 increases gastric blood flow, and protects the stomach from damage by naproxen [10].

Another important model to study in the development of new agents for gastric protection is ethanol-induced gastric damage. The most important effects of ethanol-induced gastropathy are an increase in reactive oxygen species generation and a decrease in

endogenous anti-oxidant defense mechanisms [6,7]. In our study, to avoid the effect of neutrophil migration, mice were sacrificed only 1 h after ethanol administration. This model evaluates the effect of FOR0810 in changing the redox state associated with ethanol-induced gastropathy. Our study demonstrated that FOR0810 prevented ethanol-induced gastric damage by decreasing lipid peroxidation (measured by MDA assay), as well as by increasing reduced glutathione concentration. We inferred that the protective effect of FOR0810 administration may be explained by a resultant increase in gastric reduced glutathione concentration. Another possibility is that an increase in reduced glutathione levels may be secondary to a decrease in the free radical production. The literature showed that other NO-releasing agents exerted gastroprotective activity via an attenuation of lipid peroxidation by suppression of ROS [33]. Preliminary studies have showed FOR0810 exhibited moderate superoxide scavenging activity (unpublished data). Another possibility was that this FOR0810 effect is indirect and works by increasing blood flow in gastric mucosa.

Using a pharmacological approach, we investigated the possible role of the sGC/KATP pathway in FOR0810's gastroprotective effect against naproxen or ethanol-induced damage. Our group previously showed the involvement of this pathway in gastric defense [4]. We demonstrated that inhibition of soluble guanylate cyclase completely abolished the protective effect of FOR0810 in two models of gastric damage. Our results are compatible with those obtained by Brzozowski et al. [34], who found that ODQ treatment completely abolished the protective effect of NO-releasing NSAIDs against ethanol-induced gastric damage. The participation of K_{ATP} channels in several models of gastric protection was previously described [4,9,35]. We also investigated the possible role of K_{ATP} channels in FOR0810 gastroprotective effects against naproxen or ethanol-induced gastric damage. We have shown that glibenclamide reversed the protective effect of FOR0810. Ferrari et al. showed that FOR0810 inhibits inflammatory hyperalgesia by activating the cGMP/PKG/K_{ATP} signaling pathway [14]. Utilizing the same mechanism, FOR0810 also induced vasorelaxant effects via the utilization of soluble guanylate cyclase and potassium channels [36,37].

In conclusion, our results suggested that FOR0810 prevented gastric damage through the activation of sGC and K_{ATP} channels, activities followed by a decrease in both free radical and cytokines production, and by the inhibition of neutrophil adhesion. These results may be of broader interest to readers because FOR0810 release of NO in biological systems is a process easily achievable and prompt for investigations. This compound is can be easily prepared in a pure form, while its stability, and its degradation products are inert and nontoxic, making it a very suitable agent for further pharmacological investigations [38].

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