Attenuation of Cold Restraint Stress-Induced Gastric Lesions by Sildenafil in Rats

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Abstract

Aim: To investigate the protective effect of sildenafil citrate, a selective inhibitor of cyclic guanosine monophosphate-specific phosphodiesterase, on gastric mucosal damage induced by cold restraint stress (CRS) in rats. Further, the study was extended to investigate some possible mechanisms underlying this effect.

Material and Methods: Forty rats were randomly divided into 4 groups. Normal control, CRS group, rats received saline restrained and maintained at 4°C for 3h., sildenafil group rats received sildenafil (10mg/kg, p.o.) 30min Before CRS and fourth group received L-NAME 15min. Before+sildenafil before CRS.

Results: The results indicated that cold stress induced marked ulceration in the gastric mucosa, in addition to an increase in gastric acidity as compared to saline group (p<0.05). Furthermore, stress showed reduced glutathione, whereas lipid peroxides were elevated in the stomach homogenate. Pretreatment with sildenafil (10mg/kg p.o.) significantly reduced ulcer index, gastric mucosal malondialdehyde concentration compared with CRS without treatment rats. On the other hand, sildenafil (10mg/kg p.o.) provided increasing tissue NO (p<0.05). On the other hand, Pretreatment with L-NAME [N (G)-nitro-L-arginine methyl ester], a NO synthase inhibitor, partly altered the protection showed by sildenafil.

Conclusion: The present work concluded that sildenafil can protect the gastric mucosa against the aggressive effect of cold stress via increasing NO and inhibiting lipid peroxidation. Therefore, sildenafil might be helpful in preventing the gastric adverse effects of stress induced ulcer in a clinical setting.

Key Words: Sildenafil citrate — Gastric ulcer — Nitric oxide — Lipid peroxidation.

Introduction

PEPTIC ulcers are multi-etiology, frequently recurrent and widespread chronic disease [11]. There are many antiulcerogenic drugs, among which the most effective is omperazole. However, these drugs do not always provide an effective treatment of the ulcer.

Sildenafil citrate (SC) is currently used in the treatment of functional impotence; it increases the effect of the guanosine cyclin 3', 5'-monophosphate (cGMP), which displays an inhibitory effect on the smooth muscle cells of the arterioles supplying the human corpus cavernosum. The effect of sildenafil citrate is due to blockade of the phosphodiesterase type 5, which inactivates the intracellular GMP stimulated by nitric oxide (NO) [2,3].

It is well established that nitric oxide (NO) exerts gastro-protective activity mostly due to the maintenance of blood flow around the ulcer [4]. Accumulating evidence from both animal and human studies indicates that NO plays key roles in normal wound repair. The beneficial effects of NO on wound repair may be attributed to its functional influences on angiogenesis and inflammation [5]. Moreover, increased oxidative stress level is implicated in the pathogenesis of gastric ulcer [6].

Cold restraint stress (CRS) is frequently used and clinically relevant experimental model for induction of acute gastric ulcer [7]. The major factors involved in the development of stress ulcers include an increase in gastric acid secretion and pepsin activity [6,8], and a decrease in mucosal protection due to the reduction in mucus secretion, mucosal blood flow, and NO as well as prostaglandin levels [7].

Some previous studies demonstrated that sildenafil prevents indomethacin-induced gastropathy, possibly by reducing leukocyte adherence and maintaining gastric blood flow [9,10]. In addition, Meideiros, et al. [iii reported that sildenafil
also dramatically reduced alcohol-induced gastric damage in rats.

Therefore, the current study focused on investigating the protective effects of sildenafil citrate on gastric ulcer induced by CRS in rats. Gastric protection was evaluated by calculating the ulcer index and determining gastric mucosal (MDA; as an indicator of lipid peroxidation), reduced glutathione (GSH) and NO levels. In addition, to determine whether sildenafil citrate-induced NO production contributes to the likelihood of sildenafil's gastroprotection, the effect of sildenafil citrate in the presence of the NO synthase inhibitor (L-NAME) was studied.

Material and Methods

Animals:

White male albino rats weighing 180-200g were used after one week for proper acclimatization to the animal house conditions (12h lighting cycle and 25±2°C temperature) and had free access to standard rodent chow and water. All experimental procedures were conducted according to the ethical standards approved by the Institutional Ethics Committee guidelines for animal care and use, Benha University, Egypt.

Induction of gastric ulceration:

Rats were deprived of food for 24h prior to the experiment in mesh-bottomed cages to minimize coprophagia but allowed free access to water except for the last hour before the experiment. Rats were restrained by fixing the four limbs to a wooden board and placed in a refrigerator at 4°C for 3h. The door of the refrigerator was opened every 0.5h for inspection and follow-up [12] and maintained for 3h [13]. All experiments were performed in Benha University-Pharmacology department from Oct. 2012 — Feb. 2013 during the same time of the day to avoid diurnal variations of putative regulators of gastric functions.

Experimental procedures:

Animals were divided into 4 groups of six animals each:

- The first group: Served as the control group.
- The second group: Received saline 30h before CRS-induced ulcer.
- The third group: Was treated with sildenafil (10mg/kg, p.o.) dissolved in saline 30min before CRS (The dose was selected based on our preliminary experiments) [14].
- The fourth group: Was given the NO synthase inhibitor L-NAME (50mg/kg, i.p.) dissolved in saline 15min before sildenafil administration [14].

Animals were sacrificed 3h after CRS and gastric ulcer index has been calculated according to the method of Hemmer, et al. [15]. For biochemical study, 1 g gastric tissue was removed, rinsed in ice-cold distilled water, and immediately placed in 5ml of 1.15% KCL containing 0.2% Triton X-100 and homogenized.

Analysis of gastric mucosa:

Lipid peroxidation was determined as thiobarbituric acid reacting substance and is expressed as equivalents of MDA, using 1, 1, 3, 3—tetramethoxypropane as standard [16]. Gastric mucosal GSH concentration was measured using commercially available colorimetric assay kit (Biodiagnostic, Egypt). NO level was measured as total nitrite/nitrate, the stable degradation products of NO, by reduction of nitrate into nitrite using copperized cadmium, followed by color development with Griess reagent in acidic medium [17].

Statistical analysis:

The data are expressed as mean±SEM. Statistical analysis was performed by one-way ANOVA followed by Tukey-Kramar post analysis test for multiple comparisons with p<0.05 being considered as statistically significant.

Results

- Effects of cold restraint stress-induced gastric lesions:

  The exposure to cold-restraint stress (4°C) for 3h produced significant damage in the gastric mucosa of normal rats (Fig. 1). There are significant reduction in nitrite/nitrate and GSH and increase in MDA concentrations in CRS-treated group (Table 1).

- Effects of sildenafil on cold restraint stress-induced gastric lesions:

  Pretreatment with sildenafil citrate significantly protected rats from CRS-induced gastric ulceration (Figs. 1,2). In addition, sildenafil citrate significantly increased nitrite/nitrate and GSH and reduced MDA concentrations (Table 1). In contrast, L-NAME partly attenuated the gastroprotective activity of sildenafil (Fig. 1 & Table 1).
Fig. (1): (A): Photomicrographs showing stomach of negative control animal. (B) CSR-control group showing multiple ulcers. (C) Sildenafil pretreatment group showing significant decrease of ulcer index. (D) L+NAME 30min. Before sildenafil in CRS rats partly attenuated the gastroprotective effect of sildenafil in rats.

Fig. (2): Effect of sildenafil citrate pretretatment on ulcer index in cold restraint stress (CRS)-induced gastric ulcer in rats. Data are represented as means±SEM of six rats. a,b,c,p<0.05 compared with control, CRS and sildenafil citrate (CS), respectively, L-NAME=N (G)-nitro-L-arginine methyl ester.

Table (1): Effect of sildenafil citrate (CS), alone and pulse N (G)-nitro-L-arginine methyl ester (L-NAME), on gastric mucosal malondialdehyde, reduced glutathione, and nitrite/nitrate levels in cold restraint stress (CRS)-induced gastric ulcer in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Malondialdehyde (nmol/g tissue)</th>
<th>Reduced glutathione (tgmol/g tissue)</th>
<th>Nitrite/nitrate (nmol/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28.9±2.2</td>
<td>55±3</td>
<td>268±10.4</td>
</tr>
<tr>
<td>CRS</td>
<td>37.2±0.7a</td>
<td>31.7±1.2a</td>
<td>198±7.7a</td>
</tr>
<tr>
<td>SC</td>
<td>28.6±1.9b</td>
<td>40.7±0.9ab</td>
<td>277±8.2b</td>
</tr>
<tr>
<td>SC+</td>
<td>32.7±1.6</td>
<td>36.7±2.7a</td>
<td>226±7.9ab</td>
</tr>
<tr>
<td>L-NAME</td>
<td></td>
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</tr>
</tbody>
</table>

Data are represented as means±SEM of six rats. (a,b,c)p<0.05 compared with control, CRS and SC, respectively.

Discussion

The current study showed that cold restraint stress resulted in significant gastric injury, significant in compare with control group. These findings are congruent with the observations of several researchers [17,18]. The pathogenic mechanisms responsible for stress-induced gastric mucosal lesions include disturbance of gastric mucosal microcirculation, alteration of gastric secretion, and abnormal gastric motility [3]. Levenstein, et al. [19] reported that stress increases gastric acid secretion leading to peptic ulcer in the presence of other risk factors. In addition, decreased prostaglandin synthesis [15] and enhancement of lipid peroxidation [13] are also involved in genesis of stress-induced ulcers.

In the present study, pretreatment with sildenafil citrate significantly reduces gastric ulcer index, gastric mucosal MDA level with concomitant increase in gastric mucosal concentrations of GSH and NO in compare with control group. This is in agreement with previous studies which demonstrated that sildenafil, by amplifying the effects of endogenous NO, prevents indomethacin-induced gastropathy, possibly by reducing leukocyte adherence and maintaining gastric blood flow [9,10]. A similar finding has been reported by Aydini, et al. [20].Medeiros, et al. demonstrate that sildenafil ameliorates ethanol-induced gastric haemorrhagic damage, oedema and epithelial cell loss. Similar kind of work with L-arginine, a precursor for NO formation, was earlier reported by Duffin, et al. [21].

NO modulates several elements of gastric mucosal defense, including blood flow [25], neutrophil adhesion [23,24] and mucus secretion [28]. 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 [26]. The present study has shown that sildenafil, acting via NO-dependent mechanisms, protects the stomach against cold restraint stress ulcer. These results were shown to be consistent with the hypothesis that the protective effect of sildenafil is mediated by the inhibition of cold stress induced leukocyte adhesion to vascular endothelium and by maintenance of gastric blood flow [9].

There are two types of NOS inhibitors that are eNOS (constitutive) and iNOS (inducible). Pharmacological studies proposed that NO produced
by Ca2+ dependent cNOS is cytoprotective, whereas NO produced by Ca2+ independent iNOS is cytotoxic. In normal gastric physiology cNOS is mainly active. Therefore, L-NAME pretreatment before stress exposure aggravated the ulcer formation due to inhibition of cytoprotective NO produced by cNOS. Stress results in drastic increase of iNOS activity. INOS activation results in increased production of cytotoxic NO. This increased cytotoxic NO level leads to ulcer formation. This describes the increased gastric NO level in terms of nitrite in stress on direct measurement. Similar kind of work with L-arginine, a precursor for NO formation, was earlier reported by Nishida, et al. [29].

Some studies have suggested that vasodilatation results in enhancement of the blood flow. Therefore, vasodilatation is important in the maintenance of gastric integrity and removing irritant to prevent the activation of inflammatory factors [27]. NO, a potent vasodilator agent is produced by NO synthase (NOS). NO appears to maintain the integrity of the gastric epithelium by accelerating the gastric ulcer healing. It is also involved in regulating the gastric mucosal blood flow and stimulating synthesis as well as secretion of mucus [28].

In the present study, the role of NO in the gastroprotective activity of sildenafil citrate was evaluated by using L-NAME. L-NAME is a non-specific NOS inhibitor. L-NAME pretreatment decreased the gastroprotective activity of sildenafil citrate against CRS. It can be predicted from the results that the NO production by NOS was decreased in stress group. This decrease in NO level caused ulcer formation. Sildenafil citrate restored the stress induced decrease in NO level and therefore, offered gastroprotective activity against stress. L-NAME pretreatment inhibited the restoration of stress-induced decrease in NO level by sildenafil citrate. Therefore, it reduced the gastroprotective activity of sildenafil citrate. Thus, it can be concluded that NO plays a significant role in the gastroprotective activity of sildenafil citrate.

In conclusion, We concluded that sildenafil can protect the gastric mucosa against the aggressive effect of cold stress via increasing NO and inhibiting lipid peroxidation. Therefore, sildenafil might be helpful in preventing the gastric adverse effects of stress induced ulcer in a clinical setting.

References


