

Full Length Research Paper

Protective effect of sildenafil against cysteamine induced duodenal ulcer in Wistar rats

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The aim of the current study was to investigate the possible protective effect of sildenafil (SIL) on cysteamine induced peptic ulcer in Wistar rats. Rats were randomly divided into five groups; six animals each. Normal control group; in which animals received an aqueous solution of Tween 80 (1 ml/kg) as a vehicle, two doses orally at an interval of 4 h. SIL group, in which animals received 25 mg/kg SIL orally 30 min before vehicle administration. Cysteamine group; in which duodenal ulceration was induced by two oral doses of cysteamine-HCl (450 mg/kg in 10% aqueous solution) at an interval of 4 h. Cysteamine+omepeazole group; in which animals were pretreated with 20 mg/kg omeprazole orally 30 min before cysteamine. Cysteamine+SIL group; in which animals were pretreated with 25 mg/kg SIL orally, 30 min before cysteamine. Twenty-four hours after the last dose of vehicle or cysteamine, rats were euthanized and the duodena were removed to determine the number of ulcers, ulcer surface area, ulcer score and ulcer index as well as superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), myeloperoxidase (MPO), thiobarbituric acid reactive substances (TBARS) in tissue homogenates referring to the malondialdehyde, reduced glutathione (GSH) and oxidized glutathione (GSSG). The results of the present study showed that SIL treatment decreased the number of ulcers, the ulcer surface area, the ulcer score and the ulcer index in the cysteamine induced duodenal ulcer. Moreover, SIL ameliorated the biochemical changes that were induced by cysteamine. In conclusion, SIL attenuates experimentally induced peptic ulceration using cysteamine partially through induction of nitrogen oxide (NO) and antioxidant effect which may be useful in the treatment of cystinosis.

Key words: Cysteamine, cystinosis, sildenafil, duodenal ulcer, rats.

INTRODUCTION

Peptic ulcer disease is an ulcerative gastrointestinal disease affecting the stomach and duodenum and causes a high rate of morbidity (Blanton et al., 2001). Duodenal ulcer is the most common type of peptic ulcer where discontinuity in the gastric mucosa is commonly observed. It is clear that gastric acid and pepsin secretion are

necessary in its pathogenesis, however, factors related to mucosal resistance particularly the production of gastrointestinal mucus and secretions of bicarbonate are also important (Katzung, 2001).

Cysteamine hydrochloride has been found to be the most potent agent for inducing duodenal ulcer, and

cysteamine induced duodenal ulcer in animals is now used to study the antiulcer activity of drugs (Szabo, 1978; Minaiyan et al., 2005). The cysteamine used in experimental studies has been found to concentrate in the duodenum (Nakamura et al., 2008). Its ulcerogenic effect may be due to the generation of reactive oxygen species (ROS), the decreasing defense activity of superoxide dismutase (SOD) and increasing duodenal endothelin-1 concentration, which are all associated with decreased duodenal mucosal blood flow (Jeitner and Lawrence, 2001; Khornenko et al., 2003). Oxidative stress, enhanced free-radical levels, and an impaired in-cell antioxidant pool are important factors underlying the pathophysiologic mechanisms in a variety of diseases (Khornenko et al., 2003).

In addition, intragastric nitric oxide (NO) has been shown to stimulate gastric blood flow and mucus generation (Bjorne et al., 2004) and to modulate secretory functions (Holm et al., 2000). NO and cyclic guanosine monophosphate (cGMP) were shown to protect parietal cells from ethanol-induced cytotoxicity (Yanaka et al., 1995). Moreover, NO/cGMP pathway was proven to protect endothelial cells against cellular damage in various tissues (Polte et al., 1997).

Sildenafil (SIL) increases the effects of cGMP by blocking the phosphodiesterase-type 5 (PDE-5), which inactivates intracellular cGMP, the second messenger in the NO signaling pathway (Chuang et al., 1998). Recently, several studies have shown that, in addition to treating erectile dysfunction, sildenafil can prevent or decrease tissue injury. Two previous studies demonstrated the beneficial anti-inflammatory effect of SIL on acetic acid-induced acute colitis and bleomycin-induced lung fibrosis models in rats via prevention of lipid peroxidation, oxidant generation, cytokine production and neutrophil accumulation (Iseri et al., 2009; Yildirim et al., 2010).

Sildenafil has been shown previously to protect against both indomethacin-induced gastropathy (Santos et al., 2005) and ethanol-induced gastric damage (Medeiros et al., 2008). However, its protective effect against cysteamine-induced duodenal ulcer has not been investigated. So, the aim of the current study is to investigate the possible protective effect of SIL on cysteamine induced peptic ulcer in Wistar rats.

MATERIALS AND METHODS

Drugs and chemicals

Sildenafil (Viagra; Pfizer, NY, USA) tablets (50 mg) were obtained from commercially available sources and dissolved in tap water. Drug solution was administered to the animals in a volume of 0.5 ml by means of an orogastric tube. Omeprazole (Losec, 20 mg tablets) was a kind gift from AstraZeneca, Riyadh, Saudi Arabia. Superoxide dismutase (SOD) kit (Ransod) and glutathione peroxidase (GSH-PX) kit (Ransel) were purchased from (Randox

Laboratories Ltd., Crumlin Co. Antrim, UK). Cysteamine-HCl and all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Animals

Male adult Wistar rats, weighing 200 to 250 g were obtained from the animal facility of King Fahd Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. They were used in the study according to the guidelines of the Biochemical and Research Ethics Committee at King Abdulaziz University, in accordance with the National Institutes of Health (NIH) guidelines. Animals were kept on wire mesh floors to prevent coprophagy and were housed in a well-ventilated, temperature-controlled room at $22 \pm 2^\circ\text{C}$ with a 12 h light-dark cycle. The food consisted of the normal rat chow and water was provided *ad libitum*. Care was taken to avoid stressful conditions. All experimental procedures were performed between 8 and 12 a.m to avoid diurnal variations of putative regulators of gastric functions.

Experimental design

Duodenal induced peptic ulcer

Rats were fasted for 24 h prior to the experiment and randomly divided into five groups; six animals each:

1. Normal control group; in which animals received an aqueous solution of Tween 80 (1 ml/kg) as a vehicle, two doses orally at an interval of 4 h.
2. SIL group; in which animals received 25 mg/kg SIL orally, 30 min before vehicle administration.
3. Cysteamine group; in which duodenal ulceration was induced by two oral doses of cysteamine-HCl (450 mg/kg in 10% aqueous solution) at an interval of 4 h according to the previously described method by Szabo et al. (1979).
4. Cysteamine+omeprazole group; in which animals were pretreated with 20 mg/kg omeprazole orally, 30 min before cysteamine (Desai et al., 1997).
5. Cysteamine+SIL group; in which animals were pretreated with 25 mg/kg SIL orally, 30 min before cysteamine. The dose of SIL was chosen according to the previous study by Karakoyun et al. (2011) which revealed efficacy as anti-inflammatory and antiapoptotic in an experimentally induced colitis.

Twenty-four hours after the last dose of vehicle or cysteamine, rats were euthanized with an ether overdose and the duodena (5 cm in length) were removed carefully. The duodenum was incised along the antimesenteric side and rinsed with saline; ulcers were examined under 5-fold binocular magnification to assess lesions. The number of ulcers, ulcer surface area, ulcer score and ulcer index were determined. Ulcer intensities were scored using a 4-point scale (0 = no lesion, 1 = superficial mucosal erosion, 2 = deep ulcer or transmural ulcer, 3 = perforated or penetrated ulcer) (Szabo et al., 1979; Vogel, 1997). The ulcer index was calculated by the following equation:

$$U_i = U_N + U_S + U_A \times 0.1$$

Where U_i is the ulcer index, U_N is the ulcer number, U_S is the ulcer score and U_A is the ulcer surface area for each duodenum. The ulcer surface area was estimated using transport tapes fixed on

Table 1. The effect of sildenafil (SIL) on cysteamine induced alterations in ulcer score, ulcer surface area and ulcer index.

Parameter	Ulcer score	Ulcer surface area (mm ²)	Ulcer index
Normal group	0	0	0
SIL group	0	0	0
Cysteamine group	2.32±0.20*	58.24±4.21*	12.30±1.10*
Omeprazole + cysteamine group	0.70±0.13* [#]	16.14±1.50* [#]	3.42±0.46* [#]
SIL + cysteamine treated group	0.80±0.11* [#]	20.12±2.02* [#]	5.46±0.62* [#]

Data are the mean ± SD of 6 rats. **p* < 0.05 versus corresponding control group. [#]*p* < 0.05 versus corresponding cysteamine group.

a light, transparent sheet. The tape transport was divided into cells each 1 mm² in area; the number of cells was counted and the ulcer area was thus measured for each duodenum (Szabo et al., 1979).

Biochemical assays in duodenal tissues

Duodenal tissue samples were weighed and 0.5 g samples were homogenized and the homogenates were centrifuged for 15 min at 17,000 rpm.

The supernatants were collected and kept frozen at -80°C for subsequent biochemical studies. SOD activity in duodenal tissue was assayed as described in the Randox-Ransod enzyme kit. SOD activity was determined according to the method of Sun et al. (1988). The principle of the method is based on the inhibition of nitroblue-tetrazolium reduction by the xanthine-xanthine oxidase system as a superoxide generator. Values are expressed as U/mg protein.

Glutathione peroxidase (GSH-PX) activity was assessed spectrophotometrically as described in the Randox-Ransod enzyme kit. GSH-Px activity was assessed spectrophotometrically according to the method of Paglia and Valentine (1967) and expressed as U/mg protein. Myeloperoxidase (MPO) activity was determined by the method of Wei and Frenkel (1993). The principle of the assay is based on using 4-aminoantipyrene/phenol solution as the substrate for MPO-mediated oxidation by H₂O₂ and recording the changes in absorbance at 510 nm. Values are expressed as mU/g protein. Lipid peroxidation was determined by measuring thiobarbituric acid reactive substances (TBARS) in tissue homogenates referring to the malondialdehyde (MDA) standard calibration curve according to the method of Mihara and Uchiyama (1978). Values are expressed as nmol/g protein. Reduced glutathione (GSH) content and oxidized glutathione (GSSG) level were assessed according to the modified method of Ellman (1959) and values are expressed as nmol/mg protein.

Statistical analysis

The data were analyzed statistically by t-test and expressed as mean ± standard deviations (SD). Student t-test was used to check the statistical significance of the observed differences in mean values. All calculations were performed using Software GraphPad InStat, Version 5 (GraphPad Software Inc., La Jolla, CA, USA). Statistical significance was determined as *p* value below 0.05.

RESULTS

No ulcer or erosions were observed in normal or SIL rats

except for mild hyperemia in the SIL group indicating that SIL had no interference with experimental outputs. Cysteamine has induced peptic ulceration in rats that was revealed by a significant increase in ulcer score, ulcer surface area and ulcer index. These changes were significantly reduced in ulcer induced rats pretreated with either omeprazole or SIL (Table 1). Moreover, cysteamine significantly decreased both SOD and GSH-Px activity and increased MPO activity. These changes were significantly ameliorated in ulcer induced rats pretreated with either omeprazole or SIL (Table 2). In addition, cysteamine significantly increased both TBARS and GSSG compared with the control group and decreased GSH levels. These changes were ameliorated to near normal levels in ulcer induced rats pretreated with either omeprazole or SIL (Table 2 and Figure 1).

DISCUSSION

The only existing treatment for cystinosis is the aminothiols cysteamine, a drug that reduces intracellular cystine levels (Pisoni et al., 1995). Oral cysteamine therapy, if administered early in the course of the disease, delays the progression of renal insufficiency (Markello et al., 1993), however, it may cause digestive intolerance especially duodenal ulcer (Gahl et al., 1987). On the other hand, SIL has shown previously to protect against gastric damage induced by either indomethacin (Santos et al., 2005) or ethanol (Medeiros et al., 2008).

In the present study, cysteamine was used as a cytotoxic agent to induce duodenal ulcer which was evident by an increase in the ulcer score, ulcer surface area and ulcer index. The cysteamine model of duodenal ulceration in rats was first described by Selye and Szabo (1973). The mechanism of pathogenesis in the cysteamine-induced duodenal ulcer model may be due to a hypersecretion of gastric acid, deterioration of mucosal resistance and promotion of gastric emptying (Szabo et al., 1979; Lichtenberger et al., 1977; Briden et al., 1985). Moreover, the mechanism may involve the generation of hydroxyl radical, and it has been suggested that

Table 2. The effect of sildenafil (SIL) on cysteamine induced alterations in duodenal superoxide dismutase (SOD), glutathione peroxidase (GSH-PX) and myeloperoxidase (MPO).

Parameter	SOD (U/mg protein)	GSH-PX (U/mg protein)	MPO (mU/g protein)
Normal group	5.2±0.42	8.4±0.80	6.6±0.46
SIL group	5.8±0.48	8.6±0.78	6.8±0.42
Cysteamine group	0.9±0.08*	4.8±0.42*	10.2±0.81*
Omeprazole + cysteamine group	1.8±0.02 [#]	5.2±0.52	8.8±0.83
SIL + cysteamine treated group	1.2±0.01 [#]	7.9±0.65 [#]	7.2 ± 0.63 [#]

Data are the mean ± SD of 6 rats.**p* < 0.05 versus corresponding control group; [#]*p* < 0.05 versus corresponding cysteamine group.

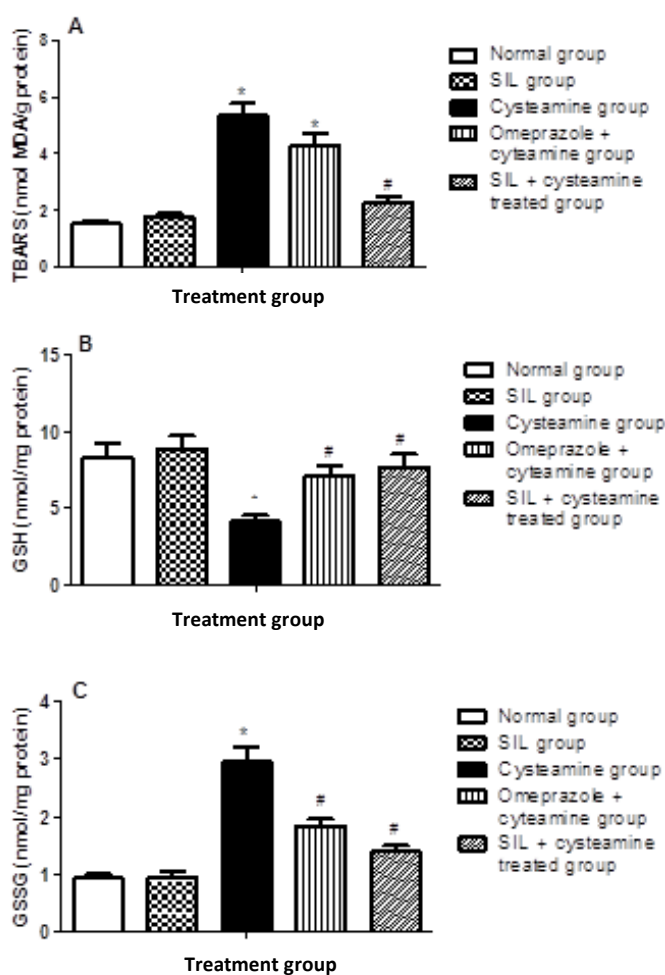


Figure 1. The effect of sildenafil (SIL) on cysteamine induced alterations in thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), and oxidized glutathione (GSSG). Data are the mean ± SD of 6 rats.**p* < 0.05 versus corresponding control group [#]*p* < 0.05 versus corresponding cysteamine group.

antioxidants can inhibit cysteamine-induced duodenal ulcer (Khomenko et al., 2003). Its ulcerogenic effect may

be due to the generation of ROS decreasing defense activity of SOD (Jeitner and Lawrence, 2001; Khomenko

et al., 2003). This is in accordance with the present study which revealed a decrease in the SOD activity in the cysteamine treated group in addition to decrease in the GSH-PX and GSH, with an increase in the GSSG.

In the present study, cysteamine caused an elevation in duodenal MPO activity, indicating the presence of enhanced polymorph nuclear leukocyte recruitment in the inflamed tissue, while the increased duodenal TBARS level, an indicator of lipid peroxidation, verified the oxidative damage in the renal tissue (Klebanoff, 2005).

In the current study, pretreatment with SIL ameliorated the increase in the MPO activity which is consistent with Santos et al. (2005) who showed that SIL provides effective protection against indomethacin-induced gastropathy in rats via preventing the effect of indomethacin on gastric blood flow and MPO activity. Moreover, pretreatment with SIL reduced the ulcer indices and TBARS level and elevated the activities of SOD and GSH-PX. These results are consistent with those of Karakoyun et al. (2011) who concluded that SIL is beneficial in experimentally-induced rat colitis partially by nitric oxide-dependent mechanisms via the maintenance of oxidant-antioxidant status.

Sildenafil is a selective inhibitor of phosphodiesterase-5 (PDE5), which degrades cyclic guanosine monophosphate (cGMP), a downstream product in the NO-soluble guanylate cyclase cascade, in endothelial cells. Interestingly, sildenafil can induce iNOS and eNOS, and sildenafil-induced increases in NO have been associated with attenuation of doxorubicin-induced cardiotoxicity (Ockaili et al., 2002). NO plays an important role in the host defense and inflammation response. It also modulates several elements of gastric mucosal defense, 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 PDE-5. In addition, NO acts as an endogenous mediator of the gastroprotective effect of different anti-ulcer agents (Brzozowski et al., 1999).

Cystinosis is usually associated with renal tubular atrophy (Gubler et al., 1999). Cysteamine which is used in the treatment of cystinosis enters the lysosome by a specific transporter for aminothiols or aminosulfides (Pisoni et al., 1995). Lee et al (2009) suggested that SIL has a renoprotective effect and attenuates experimental cisplatin-induced nephrotoxicity by preventing apoptosis. This finding with the findings of the current study may endorse further clinical investigations in human to evaluate the possible usefulness of SIL with cyteamine in cases of cystinosis to prevent its possible ulcerogenic effect on the duodenum and prevent the detrimental effect of cystinosis on the kidney.

Conclusion

SIL attenuates experimentally induced peptic ulceration using cysteamine partially through induction of NO and antioxidant effect. Further investigations may be needed to evaluate the possible clinical effect of SIL in patients with cystinosis and its clinical protective effect against duodenal ulcer.

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