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The effects of sildenafil on the functional and structural changes of ileum induced by intestinal ischemia–reperfusion in rats

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ABSTRACT

There is evidence demonstrating the protective effect of cGMP-specific phosphodiesterase type 5 (PDE5) inhibitors against ischemic injury in certain tissues. In this study, sildenafil, a potent inhibitor of PDE5, was tested for its beneficial effects in the prevention of disrupted ileal contractility and damage to tissue caused by intestinal ischemia–reperfusion in rats. Male Sprague–Dawley rats were divided into four groups: sham-operated; sham-operated with sildenafil pretreatment; ischemia–reperfusion with vehicle pretreatment; and ischemia–reperfusion with sildenafil pretreatment. The superior mesenteric artery was occluded for 45 min to induce ischemia. The clamp was then removed for a 60 min period of reperfusion. Sildenafil (1 mg/kg, i.v.) or saline was administered prior to the surgical procedure in the ischemia–reperfusion and sham-operated groups. Isometric contractions of the ileal segments in response to acetylcholine or electrical field stimulation (120 V, 2 ms pulse for 5 s, 1–20 Hz) were recorded. Additionally, levels of thiobarbituric acid reactive substances and myeloperoxidase activity were measured in addition to a histopathological examination of the ileal tissue. The contractions induced by both acetylcholine and electrical field stimulations were markedly inhibited after ischemia–reperfusion. Sildenafil pretreatment (1 mg/kg, i.v.) abolished the inhibition of responses to acetylcholine. The increased levels of thiobarbituric acid reactive substances and myeloperoxidase activity caused by ischemia–reperfusion were reversed to control levels with sildenafil pretreatment. Intestinal ischemia–reperfusion caused severe ischemic injury in rat ileum, which was prevented by sildenafil. These results suggest that sildenafil pretreatment has a protective effect against ileal dysfunction and damage induced by intestinal ischemia–reperfusion in the rat.

1. Introduction

1.1. Ischemia and reperfusion

Intestinal ischemia and reperfusion has a critical role in many diseases associated with high mortality and morbidity, such as acute ischemic colitis, arterial thrombi and embolism (Cappell, 1998a,b; Homer-Vanniasinkam et al., 1997). During ischemia, an intracellular loss of ATP disrupts cellular homeostasis (Grace, 1994). After ischemia, the flow of oxygen to tissue further exacerbates the injury in a process termed, 'oxygen paradox' (McCord, 1985; Parks and Granger, 1986). The injury developed as a result of ischemia–reperfusion that is associated with oxygen free radical production. There is a complex interplay among endothelial activation, inflammatory cell recruitment, and the production of reactive oxygen species. In health, there exists a balance between the formation of these oxidizing chemical species and their effective removal by protective antioxidant mechanisms. Oxidative stress is the shifting of this balance towards increased production of reactive oxygen species, and this constitutes one of the important mechanisms of endothelial dysfunction (Girn et al., 2007). In addition to vasodilatory effects, nitric oxide (NO) has an important role in limiting neutrophil and platelet adhesion, aggregation and activation. Normally, NO effectively scavenges the low intracellular levels of superoxide and minimizes the adhesive interactions between leukocytes and the endothelial cell surface (Carden and Granger, 2000; Wink and Mitchell, 1998). However, after ischemia–reperfusion, the balance shifts towards higher levels of superoxide. This has been referred to as the "nitric oxide–superoxide imbalance theory of reperfusion-induced microvascular dysfunction" (Carden and Granger, 2000).

Several endogenous mechanisms exist to inhibit ischemia–reperfusion lesions and many drugs have also shown protective effects. The protection mechanisms against ischemia–reperfusion-induced injury are multifactorial and still have yet to be clearly defined. A number of mechanisms have been proposed, including the elimination of free radicals, inhibition of free radical production, neutrophilic inhibition

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and reduction of lipid peroxidation. However, none of the treatments, by themselves, have proved effective in limiting oxidative damage (Grace, 1994).

1.2. Sildenafil

Sildenafil is a phosphodiesterase type 5 (PDE5) inhibitor and a drug developed for erectile dysfunction (Corbin et al., 2002). The inhibition of PDE5 increases cGMP levels. cGMP has many physiological roles such as smooth muscle relaxation and prevention of thrombocyte aggregation. cGMP is metabolized to 5′-GMP by the enzyme, type 5 phosphodiesterase. The dilation of vascular smooth muscle is regulated by a fine balance of this metabolite (Corbin and Francis, 1999; Lucas et al., 2000). Besides the treatment of erectile dysfunction, sildenafil possesses beneficial effects when used for cardiovascular disease, endothelial dysfunction and acute pulmonary hypertension (Reffelmann and Kloner, 2003). It has been reported that sildenafil provides a cardioprotective effect against ischemia–reperfusion injury in the heart of dog, rabbit, rat and mouse (Kukreja et al., 2005). This effect has been attributed to a pharmacological preconditioning mechanism (Kukreja et al., 2004). Additionally, sildenafil demonstrates a potent endothelial protection effect via opening of K_{ATP} channels in human radial arteries (Gori et al., 2005). It has also been reported that sildenafil therapy improves oxyhaemoglobin saturation and exercise tolerance in children with pulmonary hypertension (Karatza et al., 2005).

Since the mechanisms of ischemia–reperfusion injury have not been completely elucidated, many studies have been endeavored in an attempt to find an ideal therapy for mesenteric ischemia–reperfusion injury (Poussios et al., 2003). However, the effects of sildenafil on ileal dysfunction and structural changes induced by ischemia–reperfusion remain to be established. We set out to test the effects of sildenafil on ileal contractility, the level of thiobarbituric acid reactive substances (an index of lipid peroxidation) and tissue myeloperoxidase activity (an index of the degree of neutrophil accumulation) using a rat intestinal ischemia–reperfusion model. Additionally, the effect of sildenafil on ischemic ileal lesions was investigated histologically.

2. Materials and methods

2.1. Animals

The study protocol for the use of experimental animals was approved by the Animal Care Committee of Hacettepe University. All animals were divided into four groups: sham-operated; sham sildenafil; ischemia–reperfusion vehicle; and ischemia–reperfusion sildenafil. Male Sprague–Dawley rats (230–300 g) were anesthetized with urethane (1.25 g/kg, i.p.). In the ischemia–reperfusion groups, the superior mesenteric artery was dissected and occluded with an atraumatic vascular clamp for 45 min. Reperfusion was allowed for 60 min after the ischemic period. The same procedure was applied for sham groups without clamping the superior mesenteric artery. Sildenafil (1 mg/kg, i.v., in a volume of 1 ml/kg) or saline (1 ml/kg) vehicle was administered to rats through the tail vein 20 min before occlusion of the superior mesenteric artery.

2.2. Functional responses in ileal segments

Ileal segments (3–4 cm) obtained from all groups and from the same portion of the intestine (20–30 cm proximal to ileocaecal valve) were dissected, cleaned and suspended in an organ bath (20 ml) filled with warmed (37 °C) and aerated (95% O_2 and 5% CO_2 gas mixture) Tyrode solution containing the following composition: (mmol/l) 137 NaCl, 2 KCl, 0.9 $CaCl_2$, 1.2 $MgCl_2$, 11.9 $NaHCO_3$, 0.4 NaH_2PO_4, 5.6 glucose. An initial resting tension of 1 g was applied to the tissue for 60 min. After this equilibration period, acetylcholine (10^{-8}–10^{-4} M) responses were obtained, or electrical field stimulation was applied using an electrical stimulator (Model S48, Grass Instruments Co.) and a stimulation isolation unit (Grass SIU5). The 5 s trains of pulses with 2 ms duration at 1–20 Hz and 120 V were delivered to the electrodes. Isometric contractions were displayed on a Grass polygraph (model 7B) by means of a force-displacement transducer (FT03). At the end of each experiment, wet tissue weight was measured and the responses were evaluated as response (g)/wet tissue weight (g).

2.3. Determination of lipid peroxidation

Thiobarbituric acid reactive substances, an index of lipid peroxidation, were measured by the method described by Mihara and Uchiyama (1978). Ileal tissue samples (3–4 cm in length, 20–25 cm proximal to ileocaecal valve) obtained from each group were cleaned and stored immediately at −80 °C until experimentation. Upon thawing, tissue samples were homogenized in 50 mmol/l potassium phosphate buffer (PB; pH 6.0) at a volume that was 10 times the tissue weight using a homogenizer (Ultra Turrax). The homogenate (0.5 ml) was mixed with 3 ml of 1% phosphoric acid and 1 ml of 0–67% thiobarbituric acid (TBA) was subsequently added. Tubes were placed into boiling water for 45 min. After cooling the tubes, thiobarbituric acid reactive substances were extracted in n-butanol and the absorbance was measured at 532 nm. Taking the molar absorptivity of the TBA–MDA complex to be $1.56 \times 10^{-5}\ M^{-1}\ cm^{-1}$, tissue levels of lipid peroxidase (in terms of thiobarbituric acid reactive substances) were calculated as nanomoles per gram of wet tissue.

2.4. Measurement of tissue myeloperoxidase activity

Myeloperoxidase activity, an index of the degree of neutrophil accumulation, was measured in intestinal tissue samples by assaying myeloperoxidase activity as previously described (Bradley et al., 1982; Suzuki et al., 1983). Briefly, ileal tissue samples (3–4 cm in length, 20–25 cm proximal to the ileocaecal valve) obtained from each group were cleaned and stored immediately at −80 °C until experimentation. Upon thawing, each sample was homogenized in ice-cold 50 mmol/l potassium phosphate buffer (PB; pH 6.0) at a volume that was 10 times the tissue weight using a motor-driven homogenizer (Ultra Turrax).

The homogenate (1 ml) was centrifuged at 10,000 *g* for 15 min at 4 °C. The homogenized tissue pellet was then suspended in 50 mmol/l PB containing 0.5% hexadecyltrimethylammonium bromide (HETAB) and then homogenized again at 5000 *g* for 2 min. Aliquots of supernatant (0.1 ml) were added to 2.9 ml of the reaction mixture containing 0.167 mg/ml o-dianisidine and 20 mmol/l H_2O_2 solution, which were prepared in 50 mmol/l of PB. After adding the aliquot to the mixture, the change in absorbance at 460 nm was measured for 5 min. One unit of myeloperoxidase activity was defined as that degrading 1 μmol of peroxide per min at 25 °C. The activity was then normalized as units per mg of wet tissue (U/mg).

2.5. Pathologic examination of ileum

For macroscopic examination, the entire small intestine (20–30 cm proximal to ileacaecal valve) obtained from all groups was removed, cleaned, and stabilized on a metric carton. Afterwards, the whole length (cm) of the intestine was measured. Macroscopically, areas with a pink–red color change in the small intestine were determined ischemic regions. The length (cm) of the ischemic areas was also measured and calculated as a percentage of the whole length of the entire intestine.

For microscopic examination, the ileal segments (4–5 cm) obtained from all groups and from the same portion of the intestine (20–30 cm proximal to the ileocaecal valve) were taken for microscopic examination, which were opened longitudinally by

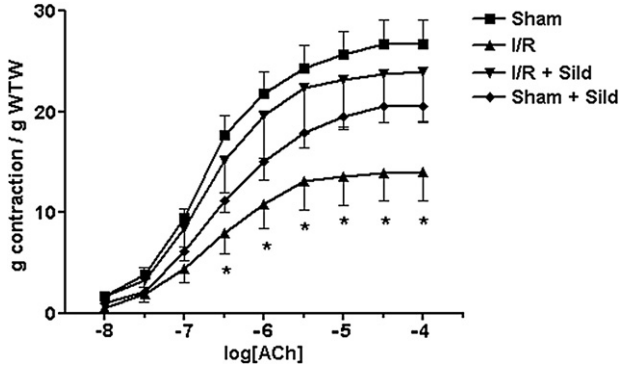

Fig. 1. The effects of intestinal ischemia–reperfusion (I/R, 45 min/60 min) and sildenafil (sild; 1 mg/kg, i.v.) pretreatment on acetylcholine-induced contractions in ileum isolated from rats ($n=5$–7). WTW: wet tissue weight. *$P<0.05$ vs. control (sham) and vs. I/R + sild.

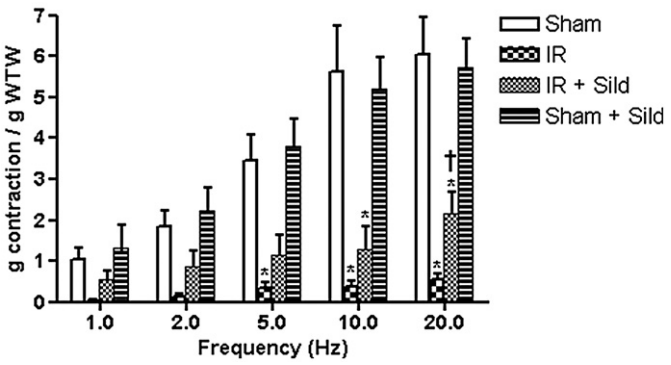

Fig. 3. The effects of intestinal ischemia–reperfusion (I/R, 45 min/60 min) and sildenafil (sild; 1 mg/kg, i.v.) pretreatment on electrical field stimulation (120 V, 2 ms pulse duration for 5 s, 1–20 Hz)-induced responses in ileum isolated from rats ($n=5$–7). WTW: wet tissue weight. *$P<0.05$ vs. control (sham), † $P<0.05$ vs. I/R.

scissors and washed with isotonic saline solution. The segments were immediately placed into a 4% formalin solution for fixation. Sections 4–5 μm in thickness were obtained from paraffinized tissue after tissue processing. All sections were stained with hematoxylin and eosin (HE) solution. After staining, two samples from each segment were examined using a light microscope.

Histological examination of reperfused intestinal tissue was performed using a previously described staging method (Hierholzer et al., 1999). A semi-quantitative histological evaluation of intestinal injury was graded on a scale ranging from 0 to 5:

Grade 0: Normal structure.
Grade 1: Sloughing of surface epithelium, mild mucosal damage.
Grade 2: Loss of one-third of mucosal crypts, moderate damage.
Grade 3: Loss of two-thirds of mucosal crypts, extensive damage.
Grade 4: Mural infarct, mucosal and submucosal necrosis was present.
Grade 5: Transmural infarct, necrosis in areas throughout the thickness of the intestinal wall.

2.6. Drugs

Acetylcholine-hydrochloride (Sigma Chemical Co., St. Louis, MO, USA), sildenafil-citrate (Eczacibasi, Istanbul, Turkey), urethane (Merck Sharp & Dohme, Rahway, NJ, USA), potassium phosphate (Sigma), phosphoric acid (Sigma), thiobarbituric acid (Sigma), hexadecyltrimethylammonium bromide (Sigma), o-dianisidine (Sigma), H_2O_2 solution were used. All drugs were dissolved in distilled water, except for sildenafil, which was dissolved in saline.

2.7. Statistical analyses

The results were expressed as means ± S.E.M. Isometric responses obtained from ileal segments were given as g contraction or g relaxation/g wet tissue. For comparisons between groups, ANOVA for repeated measurements of concentration response curves was used. An ANOVA and *post hoc* Bonferroni test, when needed, for frequency responses were employed. The student's *t* test was used to analyze thiobarbituric acid reactive substances, myeloperoxidase and intestinal macroscopic values. The Kruskal–Wallis and then Mann–Whitney *U* tests were applied to assess pathological score values. Differences were considered to be statistically significant when *P* values less than 0.05 were obtained.

3. Results

3.1. Functional responses in isolated ileal segments

Acetylcholine (10^{-8}–10^{-4} M) produced concentration-dependent contractions in the rat isolated ileum segments (Fig. 1). Intestinal ischemia–reperfusion significantly decreased the contractions induced by acetylcholine (Fig. 1), which were reversed to control response levels with sildenafil pretreatment (1 mg/kg, i.v.) (Fig. 1). In the control group, no significant effect on contractile responses was observed as a result of sildenafil pretreatment (Fig. 1).

Electrical field stimulation-induced contractions in the rat isolated ileum (1–20 Hz, 120 V, 2 ms pulse duration for 5 s) produced frequency-dependent contractions in the ileum (Fig. 2). Intestinal ischemia–reperfusion significantly reduced the contractions induced by electrical field stimulation; however, at 1 and 2 Hz, there was no reduction in contractility (Fig. 3). Sildenafil pretreatment (1 mg/kg, i.v.) increased the reduced response resulting from ischemia–reperfusion. However, its effect was found to be significant only in response to 20 Hz stimulation (Fig. 3). Sildenafil alone had no effect on the control group's response to electrical field stimulation (Fig. 3).

3.2. Biochemical results in the ileum

3.2.1. Lipid peroxidation

Thiobarbituric acid reactive substances significantly increased after intestinal ischemia–reperfusion (Fig. 4). Sildenafil pretreatment

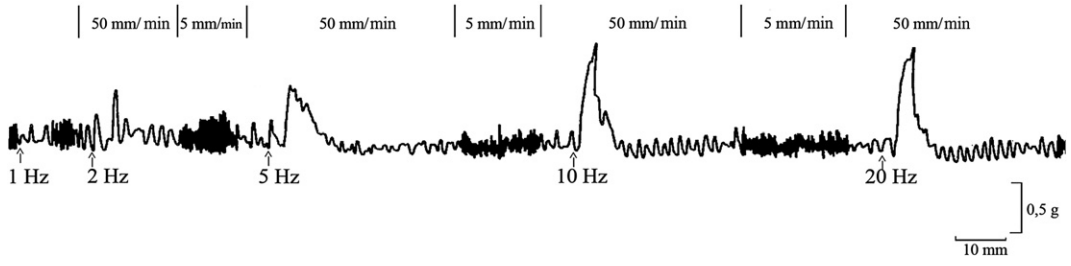

Fig. 2. Tracing showing the contractions produced by electrical field stimulation (120 V, 2 ms pulse duration for 5 s, 1–20 Hz) in ileum isolated from rats.

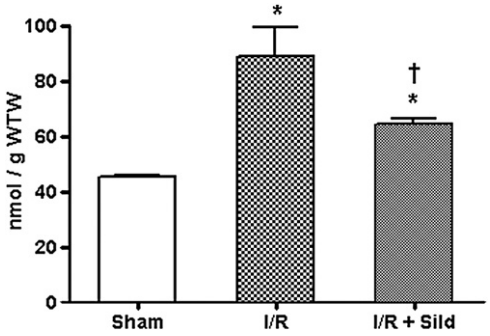

Fig. 4. The effects of intestinal ischemia–reperfusion (I/R, 45 min/60 min) and sildenafil pretreatment (sild; 1 mg/kg, i.v.) on the levels of thiobarbituric acid reactive substances in ileum isolated from rats ($n = 10$–14). WTW: wet tissue weight. *$P<0.05$ vs. control (sham); †$P<0.05$ vs. I/R.

(1 mg/kg, i.v.) significantly prevented this increase (Fig. 4). No change in responses was observed in the sildenafil control group (data not shown).

3.2.2. Myeloperoxidase activity

Intestinal ischemia–reperfusion significantly increased myeloperoxidase values in the ileum (Fig. 5). Sildenafil pretreatment (1 mg /kg, i.v) was found to be partially effective in returning the myeloperoxidase values to control levels. Normal responses (control) were not affected by sildenafil alone (data not shown).

3.3. Histopathological findings

3.3.1. Macroscopic examination

Intestinal ischemia–reperfusion caused marked ischemic injury in the rat ileum (78.5 ± 2.9%), which was significantly reduced (48.8 ± 3.7%) by sildenafil pretreatment (1 mg/kg, i.v., Fig. 6).

3.3.2. Microscopic examination

Photomicrographs taken from ileal slices of rats exposed to ischemia–reperfusion indicated intestinal damage occurring at different levels, from sloughing of surface epithelium (1) to transmural infarct (5), as semi-quantitatively scored by light microscope examination (Fig. 7). Mesenteric ischemia–reperfusion caused severe structural damage to the ileum (4.1 ± 0.3, Fig. 8). Sildenafil pretreatment (1 mg/kg, i.v.) significantly reduced ischemic injury (2.8 ± 0.2, Fig. 8); the mural infarct observed after ischemia–reperfusion was limited only to the villi in this experimental group (Fig. 8).

4. Discussion

This study reveals that sildenafil pretreatment prevents ileal dysfunction and demonstrates a protective effect against ileal damage

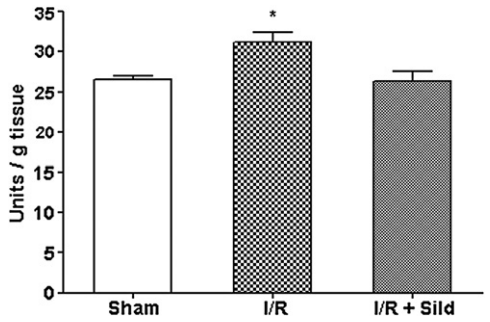

Fig. 5. The effects of intestinal ischemia–reperfusion (I/R, 45 min/60 min) and sildenafil pretreatment (sild; 1 mg/kg, i.v.) on myeloperoxidase activity in ileum isolated from rats ($n = 7$–13). *$P<0.05$ vs. control (sham).

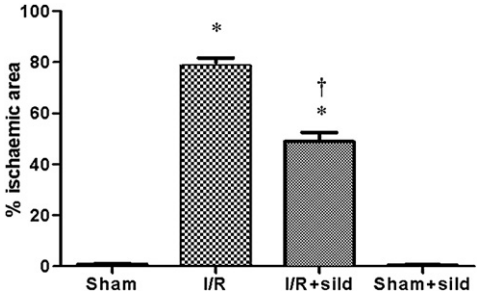

Fig. 6. Macroscopic observation of the effects of intestinal ischemia–reperfusion (I/R, 45 min/60 min) and sildenafil pretreatment (sild; 1 mg/kg, i.v.) on the ileum of rats ($n = 9$–11). *$P<0.05$ vs. control (sham); †$P<0.05$ vs. I/R.

resulting from intestinal ischemia–reperfusion. We used 1 mg/kg (i.v.) of sildenafil in our experiments and found that this dose was effective in protecting against ileal injury due to mesenteric ischemia–reperfusion in the rat. The dose chosen was within the range of doses used in animal experiments. Kukreja et al. (2005) have reported a 0.5 mg/kg (i.v.) dose of sildenafil to be protective against ischemia–reperfusion injury in rabbits. This dose has been chosen to simulate the serum levels of sildenafil in a 70 kg patient that has taken an oral dose of 100 mg of sildenafil. In contrast, Reffelmann and Kloner (2003) have failed to show a decrease in myocardial necrosis following ischemia–reperfusion in the rabbit model using an i.v. dose of 1.45 mg/kg of sildenafil. This level is similar to the upper range of

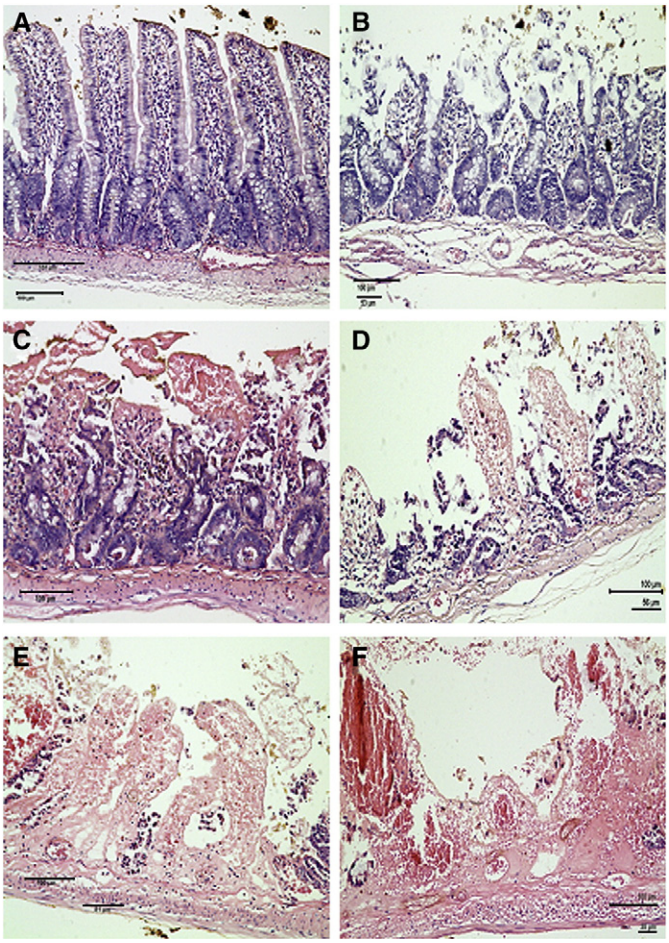

Fig. 7. Microphotographs showing intestinal damage and semi-quantitative evaluation of intestinal damage by light microscope, in rat ileum. 0: Normal (A); 1: sloughing of the surface epithelium (B); 2: necrosis in one-third of villi (C); 3: necrosis in two-thirds of villi (D); 4: mural infarct (E); 5: transmural infarct (F).

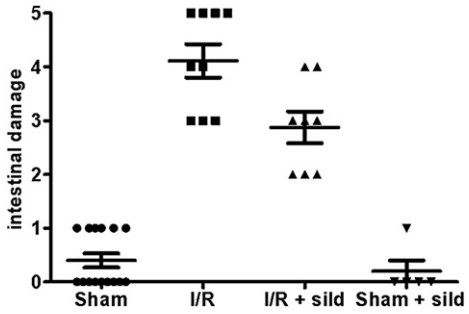

Fig. 8. The effect of sildenafil pretreatment (sild; 1 mg/kg, i.v.) on intestinal damage induced by ischemia–reperfusion (I/R, 45 min/60 min) in the rat ileum. Intestinal damage was semi-quantitatively evaluated by light microscope examination ($n = 5$–15). I/R and I/R + sild were significant vs. control (sham). I/R + sild was significant vs. I/R.

sildenafil used in patients treated for erectile dysfunction. The doses of 0.7 mg/kg and 1.5 mg/kg of sildenafil were used in our preliminary experiments; however, 0.7 mg/kg was found to be ineffective, and 1.5 mg/kg was lethal in 60% of the animals.

Ischemia–reperfusion causes damage to the ilium which can include a disruption of electrical field stimulation-induced activity and contractile response (Hassoun et al., 2001; Takahashi et al., 2001). Similarly, it was found in an anaesthetized rat that intestinal motor responsiveness to pharmacological/electrical stimulation was significantly suppressed after mesenteric ischemia–reperfusion (Ballabeni et al., 2002). Our findings also indicate that the responses of the ileum to acetylcholine and electrical stimulation were significantly depressed after intestinal ischemia–reperfusion.

The presence of oxygen free radicals (Takahashi et al., 2001), inflammatory mediators, extravasated leukocytes (Hassoun et al., 2001) and a defect in NO metabolism (Hassoun et al., 2001) may also contribute to the muscle dysfunction observed after ischemia–reperfusion. Ileal muscle and nerve cells are very vulnerable to ischemia. In intestinal tissue exposed to ischemia, energy depletion occurs in the cells (Takahashi et al., 2001). Furthermore, during the reperfusion period, the generation of oxygen free radicals interferes with the function of cells by disrupting ionic homeostasis (Takahashi et al., 2001). A number of experimental studies have tested various pharmacological agents in conjunction with surgical revascularization in hopes of attenuating motor alterations and reperfusion injury of the intestinal mucosa (Poussios et al., 2003).

In our study, sildenafil pretreatment prevented ischemia–reperfusion-induced impairment of acetylcholine responses. Kukreja et al. (2005) proposed the following mechanism for sildenafil cardioprotection in ischemia–reperfusion: The vasodilatory effect of sildenafil could release endogenous mediators such as adenosine and/or bradykinin and may trigger a signaling pathway by activation of kinases (protein kinase C), which results in nitric oxide synthase phosphorylation and eventually nitric oxide production. Nitric oxide may potentially activate guanylate cyclase, resulting in elevated cyclic GMP levels. cGMP may activate protein kinase G (PKG), resulting in cardioprotective effects. Decreased NO production by ischemia–reperfusion has been suggested to contribute to disrupted smooth muscle contractility (Kurose et al., 1994; Sayan et al., 2008). Sildenafil might be effective in promoting normal smooth muscle contractility by producing NO. The observed partial improvement in the responses to electrical field stimulation suggests that sildenafil was more effective in preventing muscular dysfunction and less effective at preventing neurotransmission damage induced by ischemia–reperfusion.

Potential strategies to prevent ischemia–reperfusion injury include three different modalities: (1) nitric oxide supplementation; (2) antioxidant molecules; and (3) neutrophil–endothelial cell blockade strategies. It is clear that there is a marked functional deficit in endothelium-derived NO following reperfusion of ischemic tissues. NO donors have been demonstrated to attenuate leukocyte–endothelial cell adhesion, platelet–leukocyte aggregation, mast cell degranulation, and increased vascular permeability under a number of different inflammatory conditions including intestine, mesentery, heart, lung, skeletal muscle, kidney and whole body ischemia–reperfusion injury (Gaboury et al., 1993; Kubes et al., 1991; Kurose et al., 1994; Lopez-Neblina et al., 1994; Lopez-Neblina et al., 1996). Briefly, in addition to the production of oxygen free radicals after ischemia–reperfusion, which destroy a wide variety of biological molecules by lipid peroxidation (Halliwell et al., 1992), the recruitment and activation of neutrophils in the intestine are important pathological events of intestinal ischemia–reperfusion.

The positive effects of sildenafil were further supported by biochemical and pathological findings. In our study, intestinal thiobarbituric acid reactive substances levels were increased following ischemia–reperfusion, indicating that the production of oxygen free radicals was augmented and lipid peroxidation was induced. Sildenafil pretreatment appeared to be protective against destructive events observed in ischemia–reperfusion by inhibiting lipid peroxidation. This was confirmed by a reduction in the levels of thiobarbituric acid reactive substances. Sildenafil abolished the increase in levels of thiobarbituric acid reactive substances and inhibited lipid peroxidation. These observations may be attributed to the ability of sildenafil to induce NO production and the capability of NO to scavenge oxygen free radicals (as discussed above).

Similarly, the activity of myeloperoxidase, one of the indicators of neutrophil infiltration, was increased by ischemia–reperfusion and partially reversed to control levels with sildenafil pretreatment. These findings were important in explaining the mechanism of action of sildenafil protection against ischemia–reperfusion damage. It has been shown that the amount of thiobarbituric acid reactive substances and myeloperoxidase activity are increased in many inflammatory conditions such as ischemia–reperfusion (Hayward and Lefer, 1998). The occlusion and subsequent reperfusion of a vessel triggers an inflammatory response in the exposed tissue that is characterized by neutrophil infiltration (Lucchesi, 1990). Oxygen free radicals and activated neutrophils have all been correlated to the pathogenesis of intestinal ischemia–reperfusion and related motor alterations. Our results suggest that sildenafil improves the disrupted physiological responses by decreasing oxidative damage and leukocyte infiltration in ileal segments by increasing NO production.

An inadequate supply of oxygen during the ischemia process has consequences in terms of cellular respiration. The lack of oxygen causes an accumulation of vital metabolites involved cellular respiration, resulting in cellular injury (Granger et al., 1986). With the progression of ischemia, the accumulation of these metabolites results in progressive cellular alterations that ultimately result in necrosis. Intestinal ischemia induces a spectrum of injury that, depending on the severity and duration, ranges from relatively subtle changes in mucosal capillary permeability to gross transmural infarction (Hierholzer et al., 1999). It has been proposed that two basic events induce intestinal tissue injury in ischemic states: hypoxia during the ischemic period and the generation of oxygen free radicals with reperfusion (Haglund et al., 1987). In the present study, the most extensive histopathological changes were detected in the ischemia–reperfusion group. These pathological alterations were alleviated in animals pretreated with sildenafil. Indeed, the morphological changes in the ileal sections of sildenafil-pretreated rats were similar to those observed in sections obtained from the sham-operated control animals. The beneficial effect of sildenafil in preventing structural ileal damage was consistent with its other positive actions observed in this study.

There are a number of clinical scenarios where PDE5 inhibitors, including sildenafil, can be developed for future use in the protection against ischemia–reperfusion injury. For example, PDE5 inhibitors could potentially be used to protect the brain (Zhang et al., 2002), liver

and other organs against ischemic injury; however, further studies are necessary for a better understanding of the protective effects of sildenafil.

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