European Journal of Pharmacology 610 (2009) 87–92

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Pulmonary, Gastrointestinal and Urogenital Pharmacology

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

Güray Soydan [a], Cenk Sökmensüer [b], Kamer Kılınç [c], Meral Tuncer [a,*]

[a] Department of Pharmacology, Hacettepe University, Ankara 06100, Turkey
[b] Department of Pathology, Hacettepe University, Ankara 06100, Turkey
[c] Department of Biochemistry Faculty of Medicine, Hacettepe University, Ankara 06100, Turkey

ARTICLE INFO

Article history:
Received 3 December 2008
Received in revised form 26 February 2009
Accepted 10 March 2009
Available online 20 March 2009

Keywords:
Sildenafil
Intestinal ischemia–reperfusion
Electrical field stimulation
Ileal injury
Thiobarbituric acid reactive substance
Myeloperoxidase

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

* Corresponding author. Department of Pharmacology, Hacettepe University, Sıhhiye, 06100 Ankara, Turkey. Tel.: +90 312 305 10 86; fax: +90 312 310 53 12.
E-mail address: mtuncer@hacettepe.edu.tr (M. Tuncer).

0014-2999/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2009.03.038

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