Pentoxifylline attenuates ischemia/reperfusion injury to the small intestine in the rat

Abstract There is a large body of evidence that neutrophils may play an important role in the mucosal injury that follows ischemia of the intestine. Pentoxifylline (PTF), a methylxanthine derivative, prevents leukocyte adherence to vascular endothelium and restores intestinal blood flow following hemorrhagic shock and sepsis. The purpose of this study was to evaluate the protective properties of PTF in an ischemia-reperfusion model of the intestine and whether its action is mediated through tissue neutrophils as assessed by myeloperoxidase (MPO) determination. Intestinal ischemia of either 1 or 2 h was induced in rats by clamping the superior mesenteric artery, followed by a 17-min reperfusion period. PTF (25 mg/kg) or saline solution was injected IP 10 min prior to ischemia. Multiple bowel samples were harvested at the end of the reperfusion period and evaluated for histology and tissue MPO. PTF significantly changed the resultant histologic damage to the intestinal mucosa exerted by prolonged ischemia of 1 and 2 h duration, although the beneficial effect of PTF in this animal model was independent of the number of tissue neutrophils as assessed by tissue MPO levels. Pretreatment with PTF can thus reduce the histologic damage caused by prolonged ischemia to the intestine.

Key words Pentoxifylline • Ischemia • Intestine • Animal model

Introduction

Intestinal ischemia is a clinical phenomenon affecting humans of all ages, especially the aged and the very young. It is implicated as one of the major factors in the pathogenesis of necrotizing enterocolitis in both humans and animal models [7, 24]. There is a large body of evidence that neutrophils may play an important role in the mucosal injury that follows ischemia of the intestine [22, 49]. This has led to extensive research efforts to develop pharmacological manipulations that would reduce the damage caused by intestinal ischemia, especially via attenuation of neutrophil adherence to the vascular endothelium [15].

Pentoxifylline (PTF) is a xanthine derivative that is a potent systemic as well as pulmonary vasodilator [32] and also has a proven cerebral-protective action [40]. Initially, investigators concentrated on its potential vasodilatory effects as a phosphodiesterase inhibitor [18], its capacity to improve red blood cell (RBC) deformability [11], and to its ability to decrease tumor necrosis factor-alpha (TNF-α) production [47]. Subsequent investigations demonstrated many other potential beneficial effects on metabolism that might be relevant in the postischemic setting, such as enhanced production of interleukin-6 [31], antagonism of interferon-gamma [23], inhibition of oxygen free-radical generation [8], diminution of neutrophil-mediated vasoconstriction [48], and an inhibitory effect on cytokine release and leukocyte activation that can attenuate the inflammatory process induced by the activated leukocyte/endothelial complex [10, 14, 44]. The aim of this study was to assess whether PTF would offer a protective effect on intestinal mucosa subjected to prolonged ischemia and whether this effect is mediated via recruited neutrophils.

Materials and methods

All studies were performed on male Sabra rats weighing 180–220 g that were randomly assigned to four study groups. All animal manipulations were performed in accordance with guidelines established by the Animal Welfare Committee at this institution. Animals were housed in individual cages and fed rat chow and water ad lib; they were fasted for 18 h before each procedure with water allowed ad lib. No antibiotics were used. The rats were anesthetized with diethyl ether. Rectal temperature was monitored and maintained at 37 °C.
Intestinal ischemia and reperfusion

Through a small midline incision the entire bowel was eviscerated. By retracting the bowel to the left, the superior mesenteric artery was easily identified. Total ischemia was induced by occluding the artery with a microvascular aneurysm clamp for either 1 or 2 h. Collateral arcades from the right colic artery and the jejunal arteries proximal to the site of occlusion were also clamped as proposed by Megison et al. [26]. Following the application of the clamps, the abdominal wall incision was closed and the rats were allowed to awaken and move freely in the cage. The vascular clamps were then removed with the animals under light ether anesthesia. Bowel specimens for histopathologic examination and myeloperoxidase (MPO) determination were harvested 17 min after removal of the clamps and termination of the ischemia. Three bowel specimens (each 1 cm in length) were harvested in each animal from the ileum starting from 4 cm proximal to the ileocecal valve and spaced 3 cm apart. Each specimen was halved; one part was fixed in formalin and later stained with hematoxilin-eosin, the other kept at 70 °C for later determination of tissue MPO.

Histology

The expected patchy distribution of tissue damage in ischemic injury to the bowel [28] dictated multiple sampling of the ileum and of each specimen. Each specimen was cut in five evenly-spaced places and evaluated separately for a total of 15 specimens per examined animal. Evaluation of the sections was performed in a blinded fashion by the same pathologist using previously published histologic criteria [41]. Each specimen was assigned one of five pathological grades (Fig. 1A–E): A (1 point): intact villous epithelium; B (2 points): epithelial denudation of the tips of the villi with some inflammation; C