Pentoxifylline and prostaglandin E1 action on ischemia and reperfusion of small intestine tissue in rats. An immunohistochemical study[1]

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ABSTRACT

PURPOSE: To investigate the action of pentoxifylline (PTX) and prostaglandin E1 (PGE1) on ischemia and reperfusion of small intestine tissue in rats, using immunohistochemical analysis.

METHODS: Thirty-five Wistar rats were distributed as follows: group A (n=10): subjected to intestinal ischemia and reperfusion for 60 min, with no drugs; group B (n=10): PTX given during tissue ischemia and reperfusion; group C (n=10): PGE1 given during tissue ischemia and reperfusion; group D (n=5): sham. A segment of the small intestine was excised from each euthanized animal and subjected to immunohistochemical examination.

RESULTS: Mean number of cells expressing anti-FAS ligand in the crypts was highest in Group A (78.9 ± 17.3), followed by groups B (16.7 ± 2.8), C (11.3 ± 1.8), and D (2.5 ± 0.9), with very significant differences between groups ($p<0.0001$).

CONCLUSIONS: The use of pentoxifylline or prostaglandin E1 proved beneficial during tissue reperfusion. The immunohistochemical results demonstrated a decrease in apoptotic cells, while protecting other intestinal epithelium cells against death after reperfusion, allowing these cells to renew the epithelial tissue.

Key words: Ischemia. Reperfusion. Intestine, Small. Pentoxifylline. Alprostadil. Fas Ligand Protein. Immunohistochemistry. Apoptosis. Rats.

Introduction

Sudden interruption of arterial blood flow causes tissue ischemia, which triggers an array of metabolic changes in the ischemic territory, making tissue repair dependent on early restoration of blood flow[1]. Yet, reperfusion, while necessary to reverse the ischemic condition, can worsen the lesions formed during the ischemic period. The extent of damage caused by ischemia depends on location of the arterial occlusion, availability of collateral circulation, oxygen demand by the tissues involved, and time elapsed between ischemia and reperfusion[2,3].

A systematic review of the literature[4] demonstrated the importance of elucidating biochemical processes for a better comprehension of the reactions triggered after ischemia and reperfusion. More recently, this body of published data has led to the use of drugs capable of minimizing the deleterious effects of ischemia-reperfusion on small intestine tissues[4].

Owing to their antioxidative ability, pentoxifylline (PTX) and prostaglandin E1 (PGE1) directly inhibit superoxide anions, which indirectly block the action of xanthine oxidase. In addition, they appear to decrease inflammatory response by reducing neutrophil migration, as well as other mechanisms that participate in inflammation, involving, for instance, the release of cytokines, tumor necrosis factor, platelet activating factor, and endothelin, a potent vasoconstrictor. In addition, PGE1 has strong vasodilating action and plays a role in activating fibrinolysis and modulating cell proliferation, as well as in fibrogenesis, angiogenesis, and tissue fibrinolysis[11,12].

Considering these aspects, the purpose of the present study was to perform an immunohistochemical evaluation of PTX and PGE1 ability to attenuate lesions in the small intestine of rats subjected to ischemia and reperfusion. Anti-FAS ligand was employed as a biomarker of cell death by apoptosis.

Methods

The research was approved by the Committee for Ethics in Research in Humans, UFMS (permit 308).

Thirty-five adult male albino Wistar rats (*Rattus norvegicus*) of 260g mean weight were assigned to four groups (GA-GD), as follows: GA, n=10; GB, n=10; GC, n=10; and GD, n=5. The animals in GA were subjected to 60 min ischemia followed by 60 min reperfusion (as described in the next topic), but were given no test drugs[13]; GB animals underwent ischemia and reperfusion with PTX at 40 mg kg^{-1} distributed as 20 mg kg^{-1} given 3 min before the beginning of ischemia and 20 mg kg^{-1} given 3 min before reperfusion; GC animals underwent ischemia and reperfusion with PGE1 at 5 µg kg^{-1} distributed as 2.5 µg kg^{-1} given 3 min before the beginning of ischemia and 2.5 µg kg^{-1} given 3 min before reperfusion; GD animals (sham group) were subjected to all experimental procedures—*i.e.*, analgesia, anesthesia, and laparotomy—except clamping of the mesenteric artery or drug use.

Tissue ischemia and reperfusion procedures

All animals were anesthetized had the ventral skin aseptically prepared while lying on a dorsal position and were subjected to median xyphopubic laparotomy for access to the superior mesenteric artery. In GA, GB, and GC animals, this artery was clamped and the abdomen was sutured with nylon 3-0 monofilament (Mononylon®). The artery remained clamped for 60 min. Anesthetization was subsequently repeated in the same dose, the abdominal cavity was opened by cutting the sutures, the small intestine was exposed, the vascular clamp was removed to restore blood flow, and the intestine was repositioned and allowed to reperfuse for 60 min.

Tissue collection for immunohistochemistry

After reperfusion, all animals were again anesthetized for suture removal and resection of a roughly 2 cm-long segment of the ileum, 5 cm proximal to the ileocecal junction. The segment was washed with saline solution and 10% formaldehyde and fixed in 10% buffered formalin for subsequent immunohistochemical processing. The animals were then euthanized by aortic exsanguination while under anesthesia.

Immunohistochemical processing

Immunohistochemical processing (using the avidin-biotin complex method, ABC) was carried out at the Screenlab Laboratório, Campo Grande-MS, using primary anti-FAS ligand antibody (epitope terminus N20; Santa Cruz, product SC834, lot A1812, 1:100 dilution), LSAB secondary antibody (Dako, product K0609, lot 10055833), and DAB color developer (diamino + benzidine; Dako, product K3468, lot 10029914, 1:10 dilution), with immunopositivity in the expression of the anti-FAS ligand biomarker shown as brown staining.

Microscopy fields were randomly selected so as to contain four crypts each, designated a-d from left to right (Figure 1). The brown-stained cells (indicating expression of anti-FAS

ligand) were visually counted. The mean number of stained cells in the crypts was calculated for each animal and then for each group, for statistical treatment.

Statistical analysis

Data were tabulated using Microsoft Excel 2007 software and statistically analyzed with BioEstat 5.0 software.

The Kolmogorov–Smirnov test was applied, followed by analysis of variance (ANOVA) with Tukey's post-test. A significance level of $p \leq 0.05$ was adopted.

Results

Table 1 shows the results obtained.

The average number of cells expressing anti-FAS ligand in the crypts was highest in GA animals (78.9 ± 17.3), followed by those in GB (16.7 ± 2.8), GC (11.3 ± 1.8), and GD (2.5 ± 0.9), with very significant differences across groups ($p<0.0001$) (Figures 1 and 2).

TABLE 1 - Inter-group statistical analysis of the immunohistochemical examination of crypts in the small intestine mucosa of rats subjected to ischemia and reperfusion.

	GA	GB	GC	GD	Inter-group ANOVA	Tukey's test	p-value
Crypt *a*	70.1±22.4	16.8±3.2	11.7±1.8	2.4±0.5	<0.0001	GA *vs*. GB	<0.01
Crypt *b*	80.8±13.6	16.0±2.7	11.0±1.6	2.2±1.6		GA *vs*. GC	<0.01
Crypt *c*	79.7±19.8	16.7±3.1	11.6±1.8	2.4±0.5		GA *vs*. GD	<0.01
Crypt *d*	84.8±9.1	17.3±2.3	10.8±1.9	2.8±0.8		GB *vs*. GC	<0.05
						GB *vs*. GD	<0.01
						GC *vs*. GD	<0.01
Overall means	78.9±17.3	16.7±2.8	11.3±1.8	2.5±0.9			
Intra-group ANOVA	0.2781	0.7876	0.6249	0.8247			
	NS	NS	NS	NS			

NS: non-significant

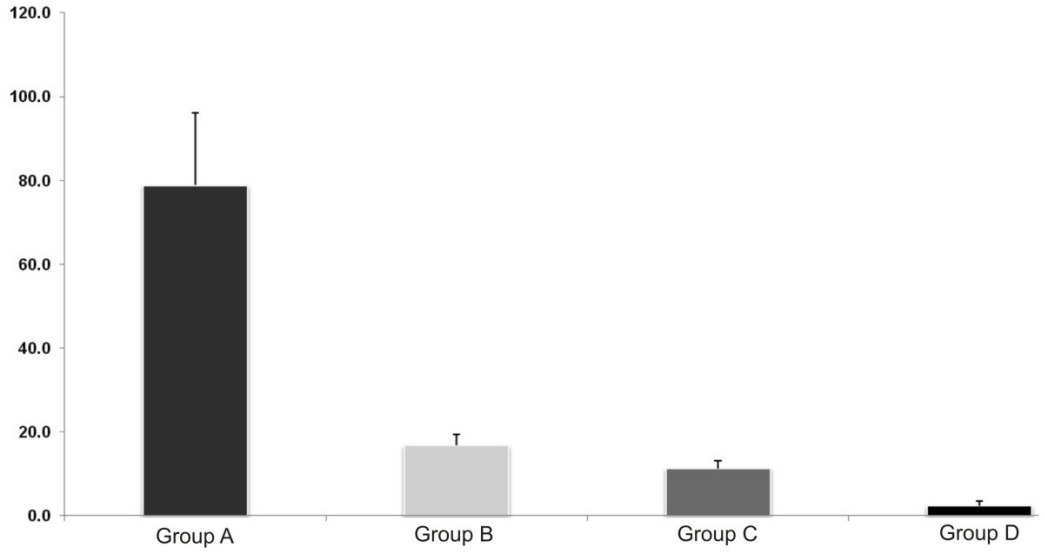

FIGURE 1 - Immunohistochemical evaluation of the small intestine mucosa of rats subjected to ischemia and reperfusion. Mean numbers of cells expressing anti-FAS ligand, by group. Values are means ± standard deviations.

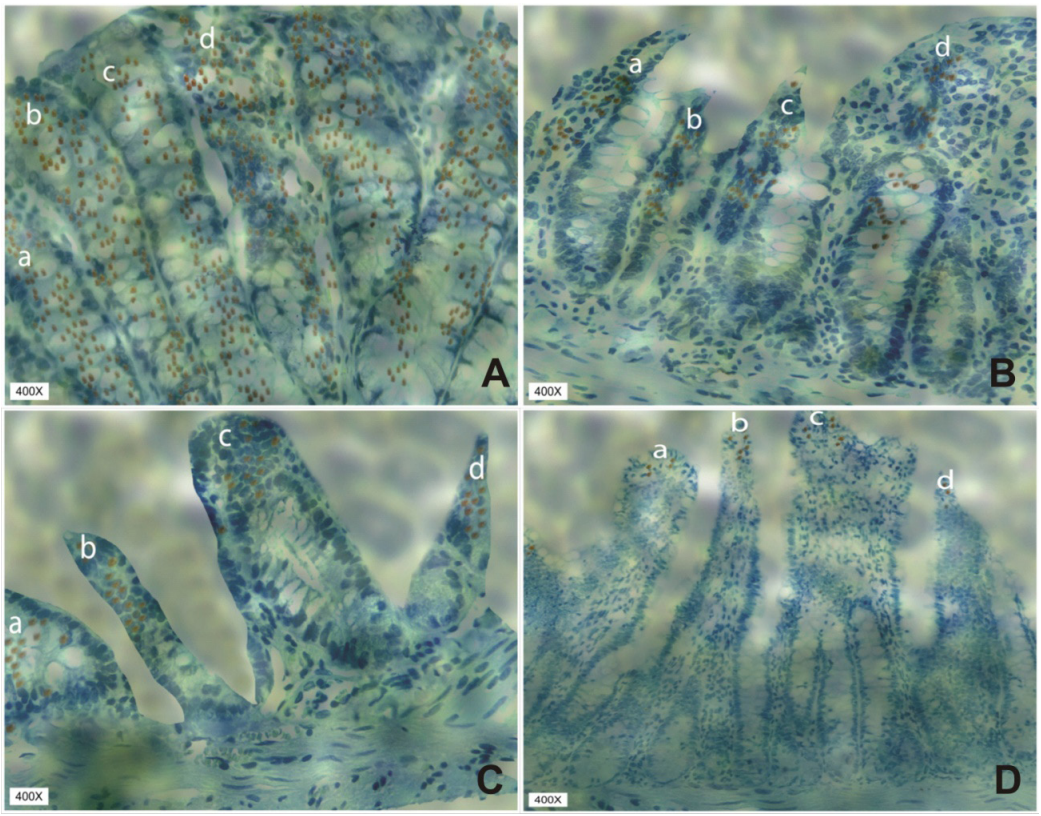

FIGURE 2 - Photomicrographs of stained cells in the crypts, by group (A-D). The crypts visible in each randomly selected field were assigned lowercase letters (A-D) from left to right. In each field, the cells expressing anti-FAS ligand were counted.

Discussion

In cases of ischemia of the small intestine, prompt restoration of blood flow is crucial, yet it can lead to further damage to the lesions formed during ischemia. Animal models capable of reproducing the ischemic conditions found in humans have been investigated, in an effort to elucidate the pathophysiology of ischemia-reperfusion and develop alternative therapies[14,15]. In the present investigation, obstruction of the superior mesenteric artery alone was the approach chosen to simulate the conditions of acute ischemia of the human intestinal artery, of frequent occurrence in clinical practice.

Damage control in cases of ischemia-reperfusion of the small intestine after acute arterial ischemia or intestinal transplant require drugs capable of reducing the release of reactive oxygen species, limiting neutrophil-mediated inflammatory response and curbing the release of tumor necrosis factor and all mediators of the complex inflammatory response involved in this phenomenon. Previous studies conducted by our group, involving histological examination of slides stained with hematoxylin-eosin and statistical treatment of scores based on the criteria set by Chiu *et al*.[16] demonstrated that PTX and PGE1, separately administered to different groups, attenuated intestinal mucosa lesions caused by tissue ischemia-reperfusion[13].

In the present study, immunohistochemical examination revealed positive expression of anti-FAS ligand, demonstrating it to be an efficient marker of cell apoptosis. In the control group (GA), cell apoptosis and high expression of the ligand predominated, corroborating the findings of other investigations[17-19].

In contrast, use of PTX or PGE1 lowered anti-FAS ligand expression and promoted epithelial renewal, demonstrating the favorable effects of these drugs against cell apoptosis in cases of ischemia-reperfusion.

In the sham group (GD), anti-FAS ligand expression was low, possibly indicating the occurrence of physiological epithelial renewal, more frequent at epithelial villus tips[17,19,20].

PGE1 led to more favorable results than PTX, both in histological and immunohistochemical terms, despite the absence of significant differences. This superior performance of PGE1 may stem from properties such as fibrinolysis, angiogenesis, and strong vasodilation, which appear to mitigate the pathophysiological lesions caused by ischemia-reperfusion.

Conclusions

Pentoxifylline and prostaglandin E1 attenuated mucosal lesions caused by ischemia-reperfusion of the small intestine

of rats. Anti-FAS ligand proved an effective marker for the immunohistochemical identification of cell death by apoptosis in the small intestine of rats.

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