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Effects of erythropoietin and pentoxifylline on the oxidant and antioxidant systems in the experimental short bowel syndrome

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In this study, we investigated the effects of erythropoietin (Epo), and pentoxifylline (Ptx) on the oxidant and antioxidant systems in the experimental short bowel syndrome. Spraque-Dawley rats were divided into four groups and all animals underwent 75% small bowel resection. Group E was treated with 500 IU kg^{-1} Epo subcutaneously (s.c.), group P with 50 mg kg^{-1} day^{-1} s.c. Ptx and group E + P with 500 IU kg^{-1} s.c. Epo plus 50 mg kg^{-1} day^{-1} s.c. Ptx for a period of 28 days. In group C, which is the control group, no drug treatment was given. At the end of 28 days the experimented rats were killed and ileum samples excised for biochemical and histopathological testing. Malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) levels were determined in ileum homogenates. When compared to group C, the MDA and GSH-Px levels were significantly decreased ($p < 0.05$), but SOD activitiy was not changed ($p > 0.05$) in groups P and E + P, whereas both MDA and SOD and also GSH-Px activities were not changed significantly in group E ($p > 0.05$). The average villous length, crypt depth, muscular thickness and mucosal length were measured in all groups. The average crypt depth and mucosal length were statistically higher in the group P than group C ($p < 0.001$, $p < 0.01$, respectively). In addition, the crypt depth was statistically higher in both E and E + P groups as compared to group C ($p < 0.001$, $p < 0.01$, respectively). Therefore, our study indicates that Ptx may be more effective than Epo in reducing lipid peroxidation. Moreover, we considered that Ptx may give this protective effect by inhibiting the free oxygen radicals to a greater extent than developing the antioxidant capacity. Copyright © 2002 John Wiley & Sons, Ltd.

KEY WORDS — short bowel syndrome; erythropoietin; pentoxifylline; surgerical stress

ABBREVIATIONS — SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; TBA, thiobarbituric acid; MDA, malondialdehyde; LSD, least significant difference; Epo, erythropoietin; Ptx, pentoxifylline; BHT, butylated hydroxytoluene

INTRODUCTION

Short bowel syndrome is often the result of extensive intestinal resection and includes several symptomatic and pathophysiological changes.[1] Untreated short bowel syndrome is characterized by diarrhoea, steatorrhea, weight loss, dehydration, malnutrition and failure to digest some specific foods.[2]

Erythropoietin (Epo), a 34.4-kD glycoprotein hormone, was identified as the humoral regulator of red blood cell production. Decreased tissue oxygen tension modulates Epo levels by increasing expression of the Epo gene.[3] Recent reports suggest that Epo receptors are present in fetal and postnatal small bowel intestine and that Epo has a role in growth and development of the gastrointestinal tract.[4] Early reports suggested that Epo treatment decreased incidence of necrotizing enterocolitis and increased wound healing and resistance to formation of intestinal anastomoses.[4,5]

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Pentoxifylline (Ptx) is a methyl–xanthine derivative that has been used for its regulator effects on the blood flow for the treatment of peripheral vascular disease, cerebrovascular disease, and a number of other conditions involving a defective regional microcirculation.[6] It has also been shown that it has beneficial effects in patients with various ischaemia–reperfusion injury.[7–10]

In this experimental study, we investigated the effect of Epo and Ptx on the oxidant and antioxidant system in the experimental short bowel syndrome.

MATERIAL AND METHODS

Thirty-two healthy male Spraque-Dawley rats aged between 3 and 6 months and weighing between 110 and 198 g were used in this study. Rats were kept under standardized conditions for food, water, light and temperature. The approval of Yuzuncu Yil University, School of Medicine Animal Ethics Committee was obtained.

After an overnight fast, the rats were anaesthetized using intramuscular injection of 50 mg kg^{-1} ketamine hydrochloride. A mid-line laparatomy was performed under sterile conditions. The small bowel and colon were exteriorized. About 75% of the small bowel was resected from the 8 cm distal to Treitz's ligament to the 8 cm proximal to the ileocaecal valve. The rest of the small bowel was then anastomosed end to end. The bowel was replaced in the abdominal cavity and the incision was closed with interrupted 3/0 silk sutures.

Experimental design

The rats were divided into four groups:

Group E: These animals underwent the 75% small bowel resection operation and were treated with 500 IU kg^{-1} s.c. One dose only of Epo was injected on Mondays, Wednesdays and Fridays every week.
Group P: These animals underwent the 75% small bowel resection operation and were treated with 50 mg kg^{-1} day^{-1} s.c. One dose of Ptx was administered daily for the 28-day period.
Group E + P: These animals underwent the 75% small bowel resection operation and were treated with 50 mg kg^{-1} day^{-1} s.c. of Ptx for 28 days in single doses plus 500 IU kg^{-1} s.c. Epo for 28 days (one dose of Epo was injected only on Mondays, Wednesdays and Fridays each week).
Group C: These animals underwent the 75% small bowel resection and were not given any drug treatment.

Biochemical analysis

The ileum samples taken around the anastomosed area were washed in saline in an ice bath and homogenized in the ratio 1:10 (w:v) with ice-cold 150 mM KCl for MDA, GSH-Px, SOD and protein determination. The MDA and protein levels of homogenates were measured immediately, the rest of the homogenates were stored at −70°C until tissue GSH-Px and SOD assays were performed.

Lipid peroxidation was determined by the method of thiobarbituric acid (TBA) reactivity.[11] MDA, an end-product of fatty acid peroxidation, reacts with TBA to form a coloured complex that has maximum absorbance at 532 nm. For this purpose, the homogenate was centrifuged for 15 min at 1800 *g*. Of each supernatant, 1 ml was transferred to another tube with the addition of 0.075 ml 0.1 mol l^{-1} EDTA and 0.25 ml 1% TBA in 0.5 mol l^{-1} NaOH. The tubes were mixed and kept in a boiling water bath for 15 min. The absorbance was read at 532 nm after the tubes had cooled to room temperature. Butylated hydroxytoluene (BHT), an antioxidant, was added to prevent MDA formation during the assay. The addition of BHT to standard MDA did not affect the colour development with TBA. MDA values (μmol) were calculated from the absorbance coefficient of MDA-TBA complex at 532 nm, (1.56×10^5 cm^{-1} mol^{-1}) and were expressed as μmol g^{-1} protein.

SOD and GSH-Px activities were estimated on homogenates by use of commercially available kits (Randox Lab., Ireland). SOD estimation was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with 2-(4-iodophenyl)-3(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. The SOD activity is then measured by considering the degree of inhibition of this reaction, which is a technique used by many investigators.[12,13] GSH-Px estimation was based on the following principle: GSH-Px catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH the oxidized glutathione is immediately converted into the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured. The technique is based on the method of Paglia and Valentina.[14]

The total protein content of the homogenates was determined by the method of Lowry *et al.*[15]

Histopathological evaluation

The tissue specimens were fixed in 10% formalin. Samples of intestine were sectioned and stained with haematoxylin and eosin and submitted for histopathological evaluation by pathologists. The average mucosal length, villous length, crypts depth and muscular thickness were measured using an ocular micrometer.

All data were expressed as mean ± SE values. Kolmogrov–Smirnov goodness of fit test was used to control whether the distribution of parameters was normal or not. Then groups of data were compared with an analysis of variance (one-way ANOVA) followed by least significant difference (LSD) multiple comparison tests.

RESULTS

The levels of MDA, SOD and GSH-Px in all groups are given in Table 1. As seen from the table, the levels of MDA were significantly decreased in the P and E + P groups ($p < 0.05$) when compared with the C group, but there was no significant difference between groups E and C ($p > 0.05$). Also, when compared to group C, the levels of GSH-Px were significantly decreased in the P and E + P groups ($p < 0.05$), but there was no significant difference between groups E and C ($p > 0.05$). The levels of SOD were not significantly different in the E, P, E + P groups as compared to group C ($p > 0.05$).

The histopathological characteristics of all groups are given Table 2 and Figures 1–4. The mean villous length was not significantly different in the E, P, E + P groups as compared to group C ($p > 0.05$). But, the mean depth of the mucosal crypts was significantly different in the E, P, and E + P groups ($p < 0.001$, $p < 0.001$ and $p < 0.01$, respectively) as compared to group C. The mean muscular thickness was not significantly different in the E, P, E + P groups as compared to group C ($p > 0.05$). The mean mucosal length was significantly different between groups P and C ($p < 0.01$), but was not significantly different in the groups E and E + P as compared to group C ($p > 0.05$). Briefly, the crypt depth increased in all groups (E, P, E + P) as compared to group C, while the mucosal length increased only in the group P. The villous length and muscular thickness were not different in any group (E, P, E + P) compared to group C.

DISCUSSION

The importance of free radical-mediated fatty acid oxidation has received much attention. The generation of active oxygen species may lead to lipid peroxidation and formation of reactive products, which may be involved in severe damage of cell molecules and

Table 1. MDA, SOD and GSH-Px values for groups E, P, E + P and C

Parameters	E $X \pm SE$	P $X \pm SE$	E + P $X \pm SE$	C $X \pm SE$
n	8	8	8	8
MDA (μmol g^{-1} protein)	13.7 ± 2.1	9.2 ± 1.1*	9.4 ± 1.2*	16.5 ± 3.6
SOD (U g^{-1} protein)	1312.3 ± 199.8	855.7 ± 186.5	577.5 ± 59.0	950.9 ± 136.3
GSH-Px (U g^{-1} protein)	734.8 ± 74.5	483.5 ± 36.6*	453.2 ± 31.9*	708.7 ± 152.7

All groups were compared with the control group.
*$p < 0.05$.

Table 2. Villous length, crypt depth, muscular thickness, and mucosal length for groups E, P, E + P, and C

Groups	Villous length (μm) $X \pm SE$	Crypt depth (μm) $X \pm SE$	Muscular thickness (μm) $X \pm SE$	Mucosal length (μm) $X \pm SE$
C	694.7 ± 21.0	207.7 ± 11.9	177.7 ± 8.4	904.5 ± 22.8
E	713.1 ± 18.0	298.2 ± 12.7†	173.2 ± 9.2	1002.2 ± 24.9
P	741.5 ± 33.1	317.0 ± 16.3†	209.8 ± 20.8	1093.0 ± 52.2*
E + P	624.1 ± 21.0	274.3 ± 11.3*	177.7 ± 8.4	914.0 ± 45.1

All groups were compared with the control group.
*$p < 0.01$; †$p < 0.001$.

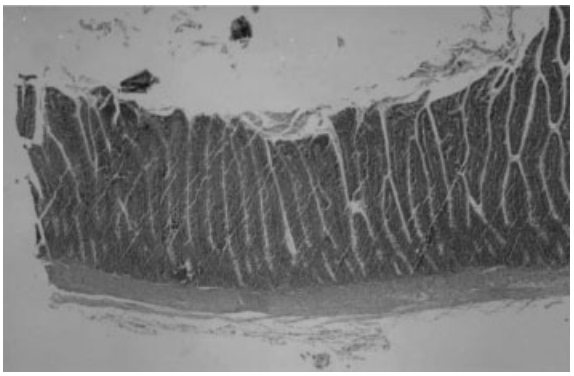

Figure 1. Histopathological changes (the crypt depth was increased) in group E (haematoxylin–eosin stain, orginal magnification × 10)

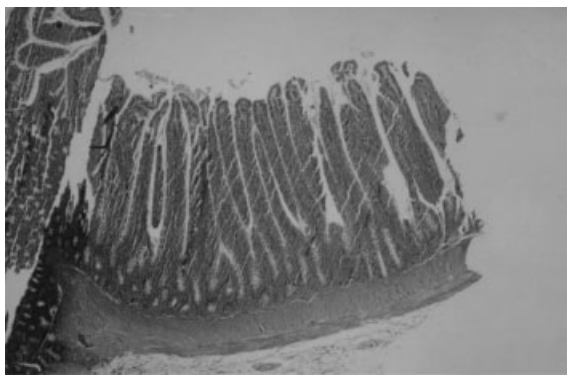

Figure 2. Histopathological changes (the crypt depth and mucosal length were increased) in group P (haematoxylin–eosin stain, orginal magnification × 10)

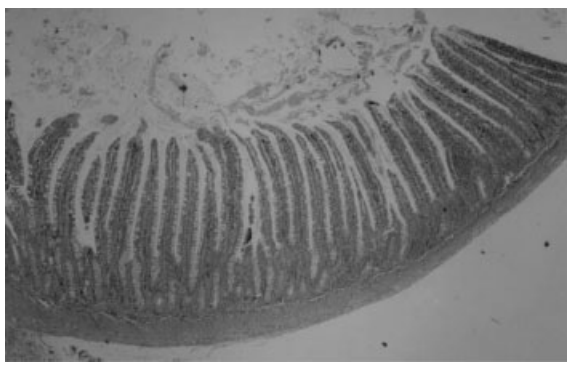

Figure 3. Histopathological changes (the crypt depth was increased) in group E + P (haematoxylin–eosin stain, orginal magnification × 10)

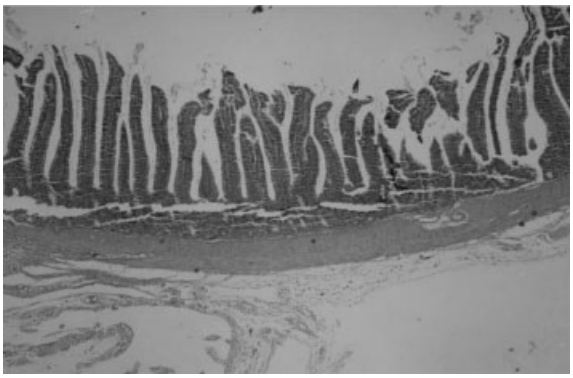

Figure 4. Histopathological view in group C (haematoxylin–eosin stain, orginal magnification × 10)

structures. Free radicals and peroxides are involved in the pathogenesis of ageing and in various diseases such as arteriosclerosis, diabetes mellitus, inflammatory diseases, and cancer.[16–21] Specific quantitation of MDA is useful for revealing the extent of lipid damage as a result of oxidative stress in a variety of lipid systems, such as plasma, organs and cell membranes.[22,23]

Ptx increases the flexibility of red and white blood cells, reduces the blood viscosity by decreasing plasma fibrinogen concentrations, and decreases the platelet aggregation and thrombus formation.[24] It is well known that free oxygen radicals after ischaemia and reperfusion play a role in gastrointestinal pathologies.[25] Various studies have verified that free oxygen radicals cause tissue damage in intestinal ischaemia.[26,27] It has been shown that during reperfusion oxygen radicals contribute significantly to the damage and are generated in high concentrations.[28] These oxygen radicals are generated by the hypoxanthine–xanthine oxidase system (the conversion of xanthine dehydrogenase to xanthine oxidase and the increase of hypoxanthine concentrations in the tissue and plasma). Oxygen radicals react directly with polyunsaturated fatty acids, leading to lipid peroxidation within the cell membranes. Indirectly, the radicals trigger the accumulation of neutrophils within the affected tissue initiating inflammatory processes that lead to severe mucosal lesions, impaired intestinal function and an enhanced absorption of bacteria and endotoxin. Similarly, phospholipase A_2 activity also leads to severe mucosal lesions. Phospholipase A_2 is an hydrolytic enzyme capable of increasing the formation of cytotoxic lysophospholipids within the tissue. Enhanced activity of phospholipase A_2 stimulates the production of prostaglandin and leukotrienes.[29] On the other hand, in this study histopathological characteristics such as crypt depth and mucosal length were found to be higher in group P compared to the

control group. These histhopathological results indicate that administration of Ptx may have a useful protective effect as defined by histological measurements during the recovery period of short bowel syndrome. Also, the low MDA levels in the P and E + P groups may be evidence that Ptx inhibits the lipid peroxidation by several mechanisms and these mechanisms protect the tissue from free radical damage.

Until now most studies have looked into the antioxidant system of intestinal ischaemia,[8,30,31] but no comparative study of these antioxidants in short bowel syndrome has been reported. We analysed the antioxidant system in the treatment of short bowel syndrome. It is known that oxygen radical scavenging enzymes can respond to conditions of increased oxidative stress with compensatory increases in activity.[32,33] However, as seen in the present experimental study, there is an imbalance between the MDA level and antioxidant enzyme levels in the groups P and E + P. In groups P and E + P, a decrease in both GSH-Px enzyme and MDA levels are interesting. Epo and Ptx have different mechanisms of action. Mammals respond to oxygen deficiency in many different ways.[34] One strategy for survival of the individual cells under hypoxic conditions is the induction of glycolytic enzymes, facilitating ATP production by glycolysis rather than mitochondrial oxidative phosphorylation. In response to the systemic oxygen deficiency due to anaemia or decreased environmental oxygen concentration, Epo production is stimulated. Epo is a glycoprotein that stimulates differentiation and proliferation of erythroid precursor cells, and hypoxic induction of Epo production increases red blood cells, leading to a better oxygen supply to tissues.[35,36] The action of Epo is mediated by binding to a specific receptor that belongs to a new family of cytokine receptors that have no tyrosine kinase domain.[37] Epo for regulating erythropoiesis is mainly produced by the kidney in adults and by the liver during fetal stages.[35,36] Ptx does not have any direct effect on the vascular tone, however it may alter the synthesis of various inflammatory mediators that affect the vasomotor tonus as vasodilators or vasoconstrictors.[38,39] Ptx also restores the normal relationship between flow and vascular diameter via improving flow dynamics between erythrocytes, granulocytes, and vascular endothelia within the microcirculation.[40] Recent reports suggest that Ptx can enhance the chemotactic response of neutrophils, but may inhibit phagocytosis and superoxide production by neutrophils and monocytes.[41] There is little information however, regarding its antioxidant activity. When neutrophils are activated by various stimulants, myeloperoxidase is released with other tissue-damaging substances from the cells.[42] Myeloperoxidase plays a fundamental role in oxidant production by neutrophils. Neutrophils are considered to be major effector cells in the tissue damage that occurs in inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, cancer and reperfusion injury.[43,44] The present results may be evidence that Ptx has an independent antioxidant effect from antioxidant enzymes. We have not found any previous experimental study on the short bowel syndrome that reported the effects of Ptx and Epo treatment on the oxidant and antioxidant status.

In conclusion, our study indicates that Ptx seems to be more effective than Epo in reducing lipid peroxidation. Ptx may prevent the development of lesions by inhibiting the production of reactive oxygen species. Further studies however, are necessary to explain the importance of antioxidant enzymes and Ptx in the therapy of short bowel syndrome.

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