Pharmacological Research, Vol. 46, No. 2, 2002
doi:10.1016/S1043-6618(02)00076-2, available online at http://www.idealibrary.com on IDEAL®

SELENIUM PRETREATMENT PREVENTS BACTERIAL TRANSLOCATION IN RAT INTESTINAL ISCHEMIA/REPERFUSION MODEL

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Accepted 24 April 2002

Protective role of selenium against free radical damage was first demonstrated in the heart and this effect was further questioned in other systems. In the present study, the effects of exogenously administered selenium on intestinal fine morphology, lipid peroxidation, and bacterial translocation (BT) in experimental intestinal ischemia/reperfusion (I/R) model were examined. Thirty-two male Wistar rats weighing 250–300 g were randomized into four groups. Sham group ($n = 8$) underwent laparotomy only. In the I/R group ($n = 8$), laparotomy was performed and the superior mesenteric artery was occluded using an atraumatic microvascular clamp for 30 min. In corresponding selenium-treated groups ($n = 8$ each), sodium selenate was given 0.2 mg kg^{-1} day^{-1} intraperitoneally (i.p.) for 3 consecutive days, prior to surgery for either laparotomy only or with I/R. Twenty-four hours later, tissue samples from liver, spleen, and mesenteric lymph nodes were obtained under sterile conditions for microbiological analysis and further evaluation of I/R-induced intestinal injury. Ileum samples were fixed in 10% formaldehyde for histopathological evaluation. In the I/R group, the incidence of bacteria-isolated mesenteric lymph nodes, spleen, and liver was significantly higher than other groups ($P < 0.05$). Selenium supplementation prevented I/R-induced BT and significantly reduced the I/R-induced intestinal injury ($P < 0.05$). Tissue MDA levels from the ileum specimens of selenium-treated rats were significantly lower than that of the I/R group ($P < 0.05$). Our results provide evidence that the relationship between BT and lipid peroxidation in intestinal tissue is crucial. Selenium pretreatment reduces lipid peroxidation which contributes to the maintenance of intestinal mucosal integrity.

KEY WORDS: selenium, bacterial translocation, intestinal injury, ischemia/reperfusion, lipid peroxidation.

INTRODUCTION

Ischemia and consecutive reperfusion causes oxidative stress, which is characterized by an imbalance between reactive oxygen species (ROS) and the antioxidative defense system [1]. Reperfusion of ischemic tissue, although necessary for reparative mechanisms, has been shown to worsen acute ischemic injury via the release of ROS. ROS have been implicated in the pathogenesis of the structural and functional alterations to the tissues that are associated with a variety of pathological processes, including mesenteric ischemia/reperfusion (I/R) injury [1–3]. I/R injury to the small intestine cause local production of the ROS which are known to play an important role in gut epithelial damage. This event may facilitate bacterial translocation (BT) and release of endotoxins due to alteration in gut barrier function [4, 5].

BT is the passage of viable indigenous bacteria to sterile body sites, such as the mesenteric lymph nodes (MLNs), spleen, liver and/or the blood stream after the damage of mucosal barrier by numerous factors [6, 7]. Bacterial overgrowth or alteration of the mucosal barrier integrity promotes BT from the intestinal tract [8]. The underlying pathophysiological mechanism for mucosal barrier damage has been attributed to I/R phenomena in numerous clinical conditions like systemic inflammatory response syndrome (SIRS), sepsis, and multiorgan failure (MOF) states [9].

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Antioxidant therapies used to overcome I/R-related phenomena include the use of *N*-acetylcysteine, Vitamin E, Vitamin C, superoxide dismutase, catalase, and allopurinol [12]. Selenium, an essential trace element involved in many physiological functions, is known to have an antioxidant effect as well as being an immune system modulator [10]. It is a major constituent of the enzyme glutathione peroxidase which plays an important role in protecting the cells from oxidative stress [11, 12]. It has been shown that selenium has beneficial effects in preventing I/R injury in the heart [13], lung [14], and kidney [15]. These facts led us to investigate the effects of selenium pretreatment in the intestinal I/R model experimentally. The aim of this study is, therefore, to determine the effects of sodium selenate on the intestinal mucosal morphology, lipid peroxidation, and bacterial translocation.

MATERIALS AND METHODS

The experiments were performed in adherence to the National Institutes of Health Guidelines on the Care and Use of Laboratory Animals, and by approval of the Ethics Committee of Mersin University School of Medicine. Thirty-two male Wistar rats (250–300 g) were used in the present study. As a standard protocol, all the rats were housed in a quiet nonstressful environment for 1 week prior to study.

Experimental protocol

Male Wistar rats weighing 250–300 g were randomized into four experimental groups. After fasting overnight, all animals (including the control group) were anesthetized with intramuscular ketamine (80 mg kg^{-1}) and xylazine (7 mg kg^{-1}) and were placed in supine position on a heating pad. The body temperature was kept at 36–37 °C. The first experimental group served as the Sham group ($n = 8$) and the rats underwent laparotomy only. In the second group (I/R group, $n = 8$), laparotomy was performed and the superior mesenteric artery (SMA) was exposed and occluded by an atraumatic fine microvascular clamp for 30 min. Another group of eight rats (Sham + Se group) pretreated with selenium (sodium selenate, Aldrich, USA; 0.2 mg kg^{-1} day^{-1} i.p.) for 3 consecutive days before rats underwent laparotomy only. In the fourth group (I/R + Se group, $n = 8$), rats pretreated with selenium (using the same dose and duration as described earlier) underwent I/R using the microvascular clamp. The abdominal wall was closed with 3/0 silk suture in all.

Twenty-four hours later, rats were anaesthetized with intramuscular ketamine (50 mg kg^{-1}) and xylazine (7 mg kg^{-1}). Tissue samples were collected to evaluate the I/R-induced intestinal injury and BT. Under aseptic conditions, a midline laparotomy was performed and liver, spleen, and MLN specimens were removed. Finally, segments of ileum were removed for lipid peroxidation and histopathological evaluation.

Microbiological analysis

Microbiological analysis was performed as described previously [16]. A 1 ml sample of blood from each animal was immediately placed into Bactec Peds Plus/F blood culture medium (Becton Dickinson Microbiology Systems, Maryland, USA) and was incubated at 37 °C for 7 days under aerobic conditions in Bactec 9240 system. Broths were incubated at 35 °C until turbid, and the turbidity was adjusted to match that of a 0.5 McFarland standard (10^8 CFU ml^{-1}). By using normal saline, a 1:100 dilution of the suspension was made to give an adjusted concentration of 10^6 CFU ml^{-1}. Subsequent subcultures were performed on blood agar, eosin–methylene blue (EMB) agar, and chocolate agar [16]. All samples were stained by acridine orange and gram technique. The liver, spleen, and MLN specimens were placed into 2 ml brain hearth infusion (BHI) broth after being weighed and homogenized. These samples were also placed on blood agar and EMB agar. All cultures were incubated under aerobic and anaerobic conditions and examined at 24 and 48 h for presence of growth. The identification of bacterial species was performed using standard microbiological methods. Colonization was expressed as the number of colony-forming units per gram of tissue homogenate (CFU g^{-1}).

Thiobarbituric acid assay for intestinal MDA concentrations. The segments of ileum was removed and homogenized with cold 1.15% KCl to make a 10% homogenate at the end of experiment. An assay for tissue MDA concentration as an index of lipid peroxidation was performed according to the method of Hiroshi *et al.* [17]. The principle of the method depends on measurement of the pink color produced by the interaction of barbituric acid with MDA. The reaction mixture (0.5 ml homogenate, 3 ml 1% phosphoric acid, and 1 ml 0.6% thiobarbituric acid solution) was heated for 45 min in a boiling water bath. After cooling, 4 ml of *n*-butanol was added and mixed vigorously. The butanol phase was separated by centrifugation and the absorbance was measured at 530 nm. Values were expressed as nanomole per gram wet tissue weight.

Histopathological analysis

Intestinal sections were stained with hematoxylin–eosin and mucosal injury, inflammation, and hyperemia/hemorrhage were assessed and graded in a blinded manner by a pathologist using the histological injury scale previously defined by Chiu *et al.* [18]. Briefly, mucosal damage was graded from 0 to 5 according to the following criteria: grade 0, normal mucosal villi; grade 1, development of subepithelial Gruenhagen's space at the apex of the villus, often with capillary congestion; grade 2, extension of the subepithelial space with moderate lifting of the epithelial layer from the lamina propria; grade 3, massive epithelial lifting down the sides of villi, possibly with a few denuded tips; grade 4, denuded villi with lamina propria and dilated capillaries exposed, possibly with increased cellularity of lamina propria; and grade 5, digestion and disintegration of the lamina propria, hemorrhage, and ulceration.

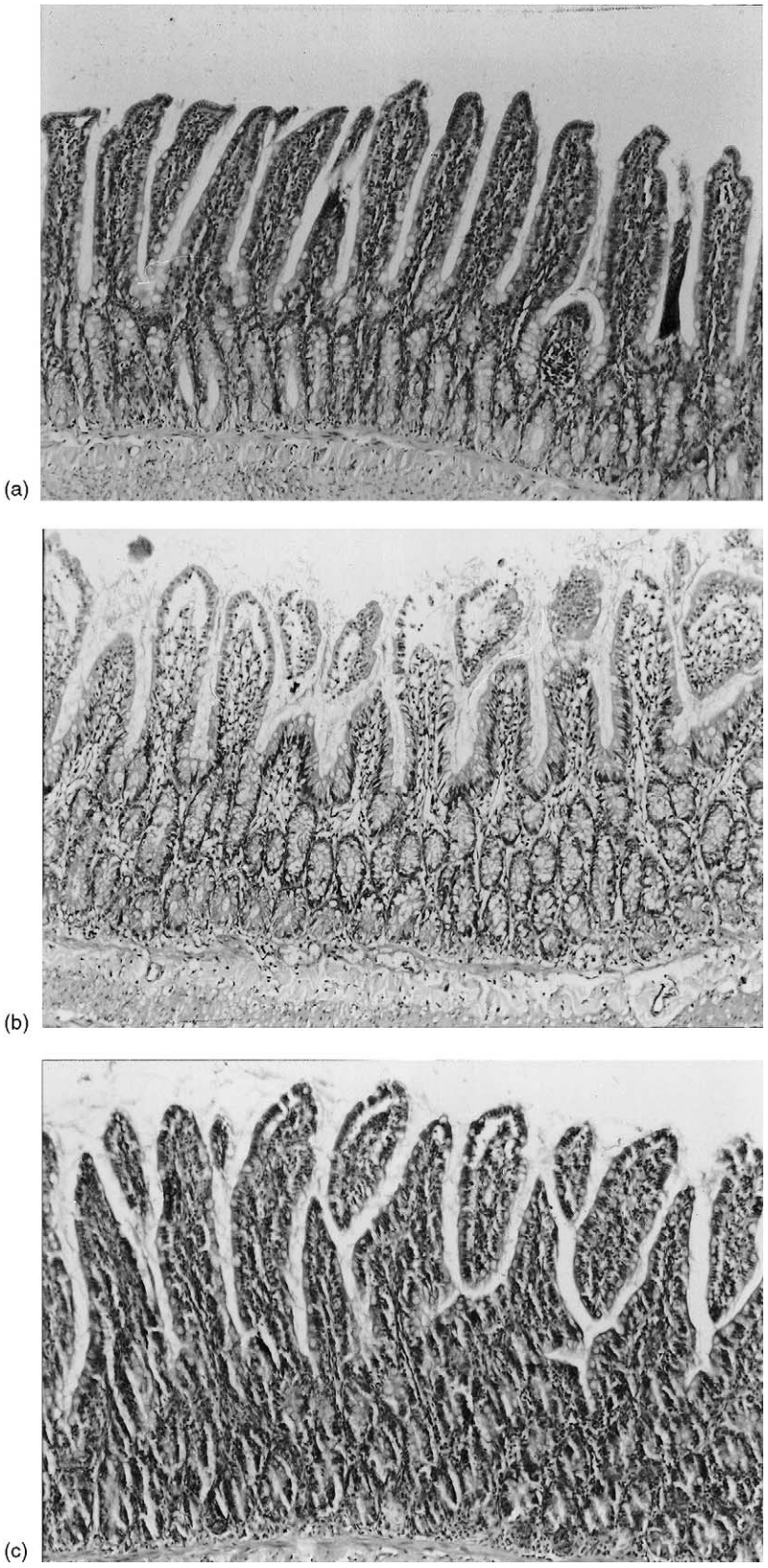

Fig. 1. Photomicrographs of small intestine segments (H&E, ×200). (a) Control group showing normal histology, (b) I/R group showing massive epithelial lifting down the sides of villi with a few denuded tips, and (c) selenium pretreatment before I/R results in a decrease in epithelial injury.

Statistical analysis

All scores are given as mean ± SD. Statistical evaluation for proportional comparisons for cultures of tissues was made using the 'Chi-square test with Yates correction'. Comparisons for quantitative culture and ileal MDA levels were analyzed using ANOVA followed by Student–Newman–Keuls test. Comparisons for intestinal injury score was analyzed using Kruskall–Wallis variance analysis. *P*-values less than 0.05 were considered statistically significant.

RESULTS

All animals survived the experimental protocol. I/R caused severe BT in the I/R group (Table I) and the incidence of bacteria isolated from MLNs, spleen, and liver was significantly higher than the Sham-operated, Sham + Se, and selenium-treated I/R group ($P < 0.05$). The predominating bacteria was *Escherichia coli*, yet *Citrobacter freundii*, *Proteus vulgaris*, and *Acinetobacter baumanii* were also encountered (data not shown). Selenium supplementation prevented I/R-induced BT. Animals from the Sham-operated group demonstrated no bacterial colonization in the harvested tissues. Intestinal sections were assessed for tissue damage by histological examination. Figure 1(a) shows a representative photomicrograph of an intestinal section from a sham-operated animal. There was no evidence of epithelial disruption and the villi were intact. Figure 1(b) obtained from a representative rat subjected to intestinal I/R, demonstrates the presence of moderate morphological damage indicated by the appearance of massive epithelial lifting down the sides of villi with a few denuded tips. Rats in the I/R group had an higher histological score compared to Sham-operated and selenium-pretreated groups (Fig. 2, Table II). Selenium pretreatment significantly reduced the I/R-induced intestinal injury [Fig. 1(c)].

As shown in Figure 3, MDA levels were also significantly increased in the I/R group in comparison to Sham-operated and selenium-pretreated groups ($P < 0.05$).

Table I
Incidence of bacterial translocation in tissue specimens

Groups	*MLNs*		*Spleen*		*Liver*	
	Incidence	*CFU g*$^{-1}$	*Incidence*	*CFU g*$^{-1}$	*Incidence*	*CFU g*$^{-1}$
Sham ($n = 8$)	0/8 (0%)	–	0/8 (0%)	–	0/8 (0%)	–
I/R ($n = 8$)	7/8 (87.5%)*	622.8 ± 403.7*	5/8 (62.5%)*	671.9 ± 576.8*	4/8 (50%)*	127.9 ± 146.3*
Sham + Se ($n = 8$)	1/8 (12.5%)	89.2 ± 252.1	0/8 (0%)	–	0/8 (0%)	–
I/R + Se ($n = 8$)	3/8 (37.5%)	165.9 ± 254.1	2/8 (25%)	285.9 ± 534.9	1/8 (12.5%)	41.2 ± 116.3

MLNs: mesenteric lymph nodes; CFU g^{-1}: mean ± SD of colony-forming units per grams of tissue. These values reflect just the samples that contained translocating bacteria. * $P < 0.05$, significantly different from the other groups.

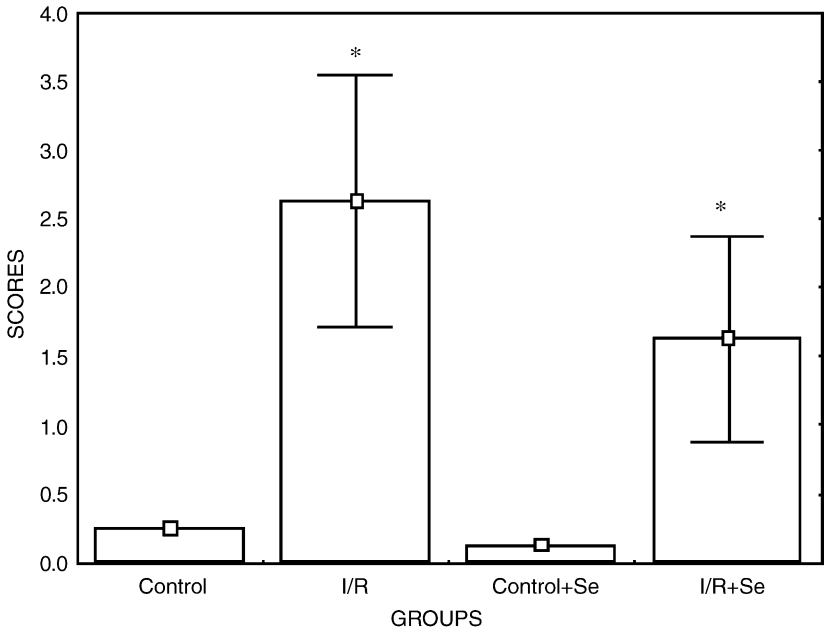

Fig. 2. Histopathological scores of the bowel specimens obtained from the study groups. Results are presented as mean ± SD. (∗) I/R group shows significantly higher injury scores when compared to Sham-operated and pretreated experimental groups (* $P < 0.05$) (Kruskall–Wallis variance analysis).

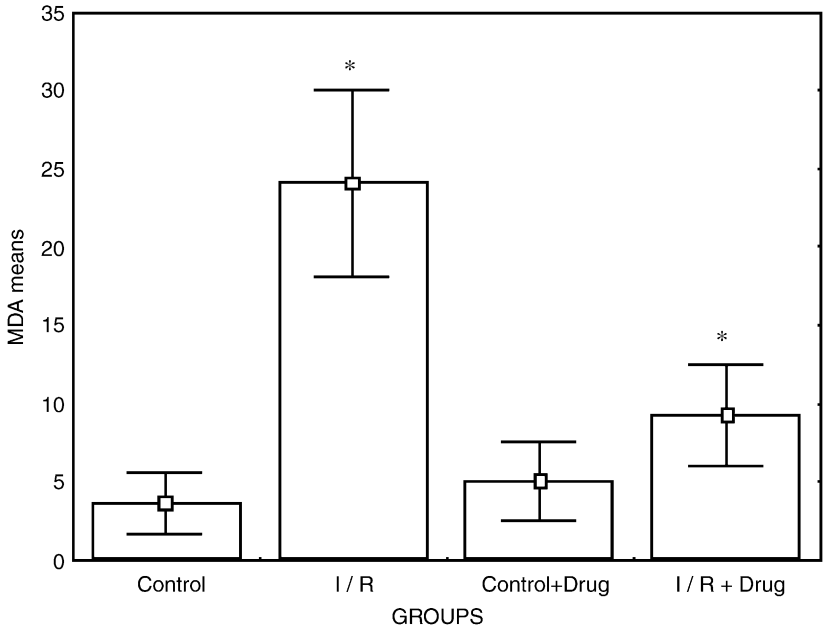

Fig. 3. Tissue MDA levels in all groups. Data expressed as mean ± SD nmol g^{-1} tissue. $^{*}P < 0.05$; significantly different from the other groups.

Table II
Descriptive statistics of ileal histopathology; scores of the study groups at the end of the experiment

Groups	*n*	*Mean* ± SD	*Median*	*Minimum*	*Maximum*
Sham	8	0.25 ± 0.46	0^{a}	0	1
I/R	8	2.63 ± 0.92	3^{b}	1	4
Sham + Se	8	0.13 ± 0.35	0^{a}	0	1
I/R + Se	8	1.63 ± 0.74	1.5^{c}	1	3

Comparison of the I/R group with Sham-operated and pretreated experimental groups yielded statistically significant difference ($P < 0.05$) (captions as mentioned a, b, c above the median show statistical difference).

DISCUSSION

In the present study, we demonstrated that selenium pretreatment attenuates postischemic ileal injury and prevents I/R-induced BT. Selenium pretreatment reduces lipid peroxidation which contributes to the maintenance of intestinal mucosal integrity. Our results suggest a relationship between BT and lipid peroxidation in intestinal tissue, since selenium pretreatment protected intestinal barrier and prevented BT which might be due to the inhibition of lipid peroxidation in the I/R group.

I/R injury to the small intestine causes local production of ROS and cytokines that induce endothelial responses which attract circulating neutrophils into the area of local injured tissue [5, 19]. This involves complex interaction between intestinal wall integrity and BT. As an antioxidant, iron chelator desferoxamine has been used to limit organ-specific oxidant-mediated lipid peroxidation in postischemic injury in immature intestine by Lelli *et al.* [20]. Despite the fact that beneficial effects of selenium were shown in preventing I/R injury in the heart [13], lung [14], and kidney [15], we could not find any study on its effect on I/R injury to the small intestine.

There is an increasing body of evidence that overproduction of nitric oxide (NO), as one of the ROS, may damage the intestinal integrity leading to failure of the gut barrier function [21], hence play a regulatory role in BT [22, 23]. The most thermodynamically favorable reaction of NO is with the superoxide radical to form peroxynitrite, a potent oxidant [24], which inhibits cellular respiration to trigger apoptosis in enterocytes [22, 25]. Shedding of apoptotic enterocytes results in space formation between the cells through which BT can occur.

Our results clearly showed that selenium pretreatment protected the small intestine from I/R injury The microbiological data also showed that selenium supplementation reduced BT. In concordance with the findings of Yoon *et al.* [26], a possible explanation is that selenium supplementation reduces apoptosis by regulating gene expression related to the apoptotic pathway. We were able to demonstrate that I/R increased lipid peroxidation and intestinal mucosal injury, which were protected by selenium pretreatment. Other proposed mechanisms of intestinal barrier failure during I/R include massive NO production, peroxynitrite formation, and activation of oxidant enzymes [27]. Albrecht *et al.* [28] suggested that selenium might directly neutralize peroxynitrite. In the present study, antioxidant selenium pretreatment preserved the intestinal integrity by a mechanism which was probably initiated by the blockage of lipid peroxidation after I/R. Our results indicate that selenium which is the cofactor of one of the antioxidant defense mechanisms (GPx) do modulate some of the functional and structural sequels of ischemia and reperfusion in the rat ileum.

As a transcriptional regulatory protein, nuclear factor-kappa B (NF-κB) plays a central role in regulating

mediators involved in multiple organ dysfunction associated with I/R injury [29]. Excessive activation of NF-κB results in an exuberant inflammatory injury to the organs and it has been shown that antioxidant treatment suppresses NF-κB activation [30]. Similarly, selenium pretreatment may have created a defence against ischemia by diminishing the activity of NF-κB in I/R. In addition to these studies, Banan *et al.* [31] recently reported that iNOS upregulation mediates oxidant-induced disruption of F-actin and barrier of intestinal cytoskeletal structure and Qu *et al.* [32] showed that NF-κB regulates the expression of iNOS in rat small intestine. Furthermore, the changes in NF-κB expression might play a role in iNOS suppression and in the preservation of ileal histology in the present study. Additionally, the formation of NO and peroxynitrite cause the breakage of DNA strands and activation of poly(ADP-ribose) synthetase (PARS) enzyme in I/R and septic states. In previous studies, we were able to demonstrate that PARS inhibition prevented intestinal injury and BT in LPS-induced sepsis [33–35]. Considering the above-mentioned mechanisms, it seems plausible to propose that the inhibitory effect of selenium supplementation in the intestine may be related with the iNOS in the present study. Our study has some limitations since we did not obtain direct biochemical changes related to antioxidant enzymes and iNOS expression. Therefore, we do not have any data confirming that selenium could upregulate the antioxidant enzymes such as GPx.

In summary, selenium pretreatment abrogated postischemic ileal injury and prevented I/R-induced BT. This study shows that intestinal selenium pretreatment helps to block the cascade of events that causes BT and septic states by inhibiting lipid peroxidation and BT. The results presented here support the view that selenium as an antioxidant warrants additional evaluation as a therapeutic agent for the treatment of a variety of conditions associated with tissue ischemia.

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