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Protective effect of selenium on intussusception-induced ischemia/reperfusion intestinal oxidative injury in rats*

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Aim: To evaluate the protective effect of selenium on intestinal ischemia-reperfusion injury due to intussusception.

Materials and methods: Forty Wistar albino rats were allocated to 4 equal groups (n = 10). After anesthetization, 2 cm of intestinal segment, 20 cm proximal to the ileocecal valve, was removed in the control group (CG). In the intussusception group (ING), intussusception was performed 20 cm proximal to the ileocecal valve by pushing the proximal intestinal segment distally with a stylet, and 2 cm of intestinal segment was removed 4 h later. Intussusception was reduced and a 2 cm segment was removed after another 4 h period in the intussusception-reduction group (IRG). The selenium group (SG) received 0.2 mg kg^{-1} of selenium in 10 mL of 0.9% NaCl solution intraperitoneally 20 min before reduction and segments were removed 4 h thereafter. Intestinal tissues were evaluated for histopathological grades and oxidative injury markers.

Results: Histopathologic evaluations among the 4 groups did not reveal a significant difference ($P > 0.05$). Although catalase activity was decreased in all groups, increased activity was found in SG compared with IRG ($P < 0.05$). GSH-Px and SOD activities were also found to have decreased in ING and IRG ($P < 0.05$). CG and SG had no difference in GSH-Px and SOD activities ($P > 0.05$). Malondialdehyde (MDA) activities did not differ between CG and SG, but the MDA level was increased in ING and IRG when compared with CG ($P < 0.05$).

Conclusion: Although selenium prevents oxidative injury by increasing the antioxidant enzyme activity in experimental intussusception, similar effects on histopathologic findings were not detected.

Key words: Intussusception, selenium, intestine, ischemia-reperfusion, injury, rat, oxidative stress

Sıçanlarda invajinasyona bağlı intestinal iskemi reperfüzyon hasarında selenyumun koruyucu etkisi

Amaç: İnvajinasyona bağlı iskemi-peferfüzyon hasarında selenyumun koruyucu etkisini değerlendirmek üzere deneysel bir çalışma yapılmıştır.

Yöntem ve gereç: Kırk Wistar albino sıçan dört gruba ayrılmıştır (n = 10). Anestezi sonrası, kontrol grubunda (KG) ileoçekal valvden 20 cm proksimaldeki 2 cm'lik intestinal segment çıkarılmıştır. İnvajinasyon grubu (İG) invajinasyonun ileoçekal valvden 20 cm proksimalde bir stile yardımı ile proksimal intestinal segmentin distale itilmesi ile oluşturulmuştur. Dört saat sonrasında 2 cm'lik intestinal segment çıkarılmıştır. İnvajinasyon-redüksiyon grubunda (IRG) invajinasyon redükte edildikten 4 saat sonra 2 cm'lik segment çıkartılmıştır. Selenyum grubu (SG) 10 mL % 0,9'luk NaCl solusyonu içerisinde 0,2 mg kg^{-1} selenium redüksiyondan 20 dakika once intraperitoneal olarak verilerek oluşturulmuş ve 4 saat sonrasında intestinal segment çıkarılmıştır. İntestinal yapılar histopatolojik evrelendirme ve oksidatif hasar belirteçleri bakımından değerlendirilmiştir.

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Bulgular: Gruplardaki histopatolojik değerlendirme istatistiksel bir fark olmadığını göstermektedir (P > 0,05). Katalaz değerleri tüm gruplarda azalmıştır. SG'da katalaz seviyeleri İRG'yle karşılaştırıldığında azalma anlamlı bulunmuştur (P < 0,05). Glutatyon peroksidaz (GSH-Px) ve superoksit dismutaz (SOD) aktiviteleri de İG ve İRG'de istatistiksel olarak anlamlı azalmıştır (P < 0,05). KG ve SG grupları, GSH-Px ve SOD aktiviteleri bakımından istatistiksel anlamlı fark göstermemektedir (P > 0,05). Malonildialdehid (MDA) seviyelerinde KG ve SG grupları arasında istatistiksel anlamlı bir fark bulunamamıştır. MDA seviyeleri İG and İRG gruplarında KG grubuna göre anlamlı olarak artmıştır (P < 0,05).

Sonuç: Selenyum invajinasyonda oluşan oksidatif hasarı antioksidan enzim aktivitesini arttırarak önlesede, benzer etkiler histopatolojik bulgularda izlenmemiştir.

Anahtar sözcükler: İnvajinasyon, selenium, barsak, iskemi-reperfüzyon, hasar, sıçan, oksidatif stress

Introduction

Intussusception (IN) is the most common cause of acute small bowel obstruction in infants and preschool children (1). The invagination of one part of the intestine to another causes intense local edema in the intussusceptum and produces venous compression, congestion, and stasis (2). If this process continues unabated, bowel congestion and pressure increase and ultimately produce ischemic changes that may lead to bowel necrosis (1). The goal of the treatment consists of either radiological or operative reduction without bowel resection (2). Both ischemic and reperfusion injury may occur during the clinical course of IN.

Ischemia and consecutive perfusion cause oxidative stress that is characterized by an imbalance between reactive oxygen species (ROS) and the antioxidative defense system (3). The reperfusion of ischemic tissue has been shown to worsen acute ischemic injuries by releasing ROS (4). To control the flux of free radicals, aerobic cells have developed their own defense system, the antioxidant system superoxide dismutase (Cu-Zn SOD), the first line of defense in the dismutation of superoxide radicals into hydrogen peroxide (H_2O_2). H_2O_2 can be transformed into H_2O and O_2 by the enzyme glutathione peroxidase (GSH-Px). GSH-Px reduces lipidic or nonlipidic hydroperoxides as well as H_2O_2 while oxidizing glutathione (5). Ischemia-reperfusion (I/R) injury to the intestine results in local production of ROS and plays a crucial role in gut epithelial damage (4). Although many studies have been performed to evaluate intestinal I/R injury, I/R injury after reduction of IN has not been previously evaluated.

Selenium (Se) is a trace element distributed in small amounts in the soil and food, and it has remarkable variability in regional distribution and bioavailability (6). Its major function is as a cofactor for GSH-Px, which catalyzes the reduction of hydrogen peroxide by GSH. It is therefore essential in removing free oxygen radicals from the body and preventing oxidative stress (7). It has been shown that selenium has beneficial effects in preventing I/R injury in several organs, such as the intestines, heart, lungs, and kidneys (8,9). The aim of this study was to evaluate the protective effect of selenium on intestinal injury due to IN. A new experimental model of IN that is eligible for I/R studies is proposed.

Materials and methods

The experiments were performed in adherence to the Declaration of Helsinki and by the approval of the Ethics Committee of Kırıkkale University. Forty Wistar albino rats of both sexes, 3 months old, weighing 300 ± 20 g, were included in the study. The animals were obtained from Gülhane Military Medical Academy, Animal Research Laboratory, Department of Laboratory Animals, fed with laboratory chow (Bil-Yem Yem San. Tic. A.Ş., Balıkesir, Turkey) and tap water ad libitum during the experiment, and kept under artificial lighting 12 h daily, at 20-22 °C. The rats were randomized into 4 groups. After fasting overnight, the rats were anesthetized with intramuscular ketamine hydrochloride (80 mg kg^{-1}, Ketalar, Eczacıbaşı, İstanbul, Turkey). The first experimental group served as the control group (CG) (n = 10), and 2 cm of intestinal segment, 20 cm proximal to the ileocecal valve, was removed after a 10 mL intraperitoneal 0.9% NaCl infusion. In the IN group (ING) (n = 10), IN was induced 20 cm proximal to the ileocecal valve by pushing the proximal intestinal segment distally with

a stylet. Four hours later, 2 cm of intestinal segment was removed. After obtaining IN by a similar method, the IN was reduced and 2 cm of intestinal segment was removed after another 4 h period. This group was assessed as the IN-reduction group (IRG) (n = 10). Another group of rats (n = 10) underwent laparotomy to have IN and a reduction, similar to IRG. In this group (selenium group - SG), 0.2 mg kg^{-1} of selenium (sodium selenate, Aldrich, USA) in 10 mL of 0.9% NaCl solution was infused intraperitoneally 20 min before reduction. The intestinal segments were removed 4 h after reduction. Specimens were evaluated for histopathological grades and oxidative injury markers (catalase (CAT), superoxide dismutase (SOD), GSH-Px, and MDA).

Preperation of homogenate

The tissues were homogenized in 150 mmol/L of ice-cold KPO4 buffer to make a 10% homogenate, using a glass Teflon homogenizer. The homogenates were centrifuged at 12,500× *g* for 30 min at 4 °C, and then the supernatant fractions were obtained. Enzyme analyses of intestinal tissue were performed on the supernatant fractions of the tissue homogenates.

Protein measurement

The protein content of the homogenate was determined using Lowry's method (10). The absorbance of the samples was measured spectrophotometrically (UV-1601, Shimadzu) at 750 nm.

Histopathological evaluations

Intestinal samples were inflated and fixed with 10% formalin, and then all segments were embedded in paraffin. Tissues were sectioned in 4-5 μm pieces and stained with routine hematoxylin and eosin stain. The specimens were examined under a light microscope (Leica Microsystems, Germany) by a pathologist who was blind to the study. Histopathologic findings were graded according to the Chiu scoring system (11) (Table 1).

Determination of CAT activity

Aebi's method was used for the estimation of catalase in supernatants (12). The reaction mixture contained 50 mM of potassium phosphate buffer (pH 7.0), 30 mM of H_2O_2, and an appropriate amount of sample. Every sample was run with appropriate blanks lacking H_2O_2. The decomposition of H_2O_2 was followed directly by the decrease in absorbance at 240 nm (UV-1601, Shimadzu) for 30 s. The difference in absorbance per unit time is a measurement of the catalase activity. The enzyme activity was expressed in mg mg^{-1} protein.

Determination of SOD activity

Total SOD activity was determined according to the method of Sun et al. (13). The principle of the method is based on the inhibition of nitro blue tetrazolium (NBT) reduction by the xanthine–xanthine oxidase system as a superoxide generator. The activity was assessed in the ethanol phase of the supernatant after 1 mL of ethanol-chloroform mixture (3:1, v/v) was added to the same volume of sample and centrifuged at 4000× *g* for 30 min at 4 °C. The production of formazan was determined at 560 nm (UV-1601, Shimadzu). One unit of SOD was defined as the amount of enzyme causing 50% inhibition in

Table 1. Criteria for histopathologic grading according to the Chiu scoring system.

Grade	Histopathologic finding
0	Normal mucosal villi
I	Development of a subepithelial space, usually at the tip of the villus, with capillary congestion
II	Extension of the subepithelial space with moderate lifting of the epithelial layer
III	Massive epithelial lifting down the sides of villi
IV	Denuded villi with lamina propria, dilated capillaries exposed, increased cellularity of the lamina propria
V	Digestion and disintegration of the lamina propria; hemorrhage and ulceration

the NBT reduction rate. The SOD activity was expressed in U g^{-1} protein.

Determination of GSH-Px activity

GSH-Px activity was determined using the method of Flohe and Gunzler (14). The enzyme reaction in the tube containing 50 mM of potassium phosphate buffer, 0.2 M of EDTA, 2 mM of NADPH, 0.1 M of reduced glutathione (GSH), 0.4 M of sodium azide, 10 U mL^{-1} of glutathione reductase, and an appropriate amount of sample was initiated by the addition of 10 mM of H_2O_2. The decrease in absorbance due to NADPH oxidation was measured spectrophotometrically at 340 nm (UV-1601, Shimadzu) and the activity was expressed in U g^{-1} protein.

Determination of MDA levels

The tissue MDA level was determined using the method of Draper and Hadley (15). MDA, an end product of fatty acid peroxidation, reacts with thiobarbituric acid (TBA) to form a colored complex. The principle of the method depends on the spectrophotometric measurement of the color generated by the reaction of TBA with MDA, with a maximum absorption at 532 nm. For this purpose, 2.5 mL of 100 g L^{-1} trichloroacetic acid (TCA) solution was added to 0.5 mL of homogenate in each tube, and the tubes were placed in a boiling water bath for 15 min. After cooling in tap water, the tubes were centrifuged at 1000× *g* for 10 min, and 2 mL of supernatant was added to 1 mL of 6.7 g L^{-1} TBA solution in a test tube and placed in a boiling water bath for 15 min. The solution was then cooled in tap water and its absorbance was measured with a spectrophotometer (UV-1601, Shimadzu) at 532 nm. The results were expressed in nmol g^{-1} protein by reference to a standard curve prepared with an MDA standard solution (1-15 μmol L^{-1}).

Since the data obtained from the experiments did not show normal distribution, nonparametric tests, the Kruskal-Wallis test and the Mann-Whitney *U* test, were used in the statistical analyses (SPSS 15.0). P-values lower than 0.05 were considered significant.

Results

The histopathologic grades encountered in the 4 groups are listed in Table 2. The normal histopathological appearance of intestine in CG and grade 3 lesion in IRG is illustrated in Figure 1. When histopathologic grades were compared, higher grades of ischemia were detected in ING and IRG when compared with CG ($P > 0.01$ and $P > 0.05$, respectively). Although the histopathologic grades in SG were higher than in CG ($P > 0.001$), there was no statistical difference between SG and the other groups ($P > 0.05$). Moreover, the histopathological grades did not reveal a significant difference between ING and IRG ($P > 0.05$).

The activity of CAT, SOD, GSH-Px, and MDA in the 4 groups is listed in Figure 2. Catalase activity was decreased in ING, IRG, and SG when compared with CG ($P < 0.05$). However, CAT activity was decreased in ING with respect to IRG ($P < 0.05$), and no difference was detected between SG and ING ($P > 0.05$). In SG, CAT activity was also increased when compared with IRG ($P < 0.05$).

SOD activity was decreased in ING and IRG ($P < 0.05$). There was no difference between CG and SG ($P < 0.05$). When compared with ING and IRG, SOD activity was found to have increased in SG ($P < 0.05$).

When GSH-Px activities were evaluated, decreased activity was detected in ING and IRG ($P < 0.05$). However, there was no difference between CG and SG ($P > 0.05$). GSH-Px activity was significantly increased in SG when compared with IRG ($P < 0.05$).

Table 2. Distribution of histopathological grades in the study groups.

Groups	Grade 0	Grade I	Grade II	Grade III	Grade IV	Grade V
CG (n = 10)	9	1	-	-	-	-
ING (n = 10)	2	5	1	1	-	1
IRG (n = 10)	3	2	2	1	2	-
SG (n = 10)	1	4	1	1	1	2

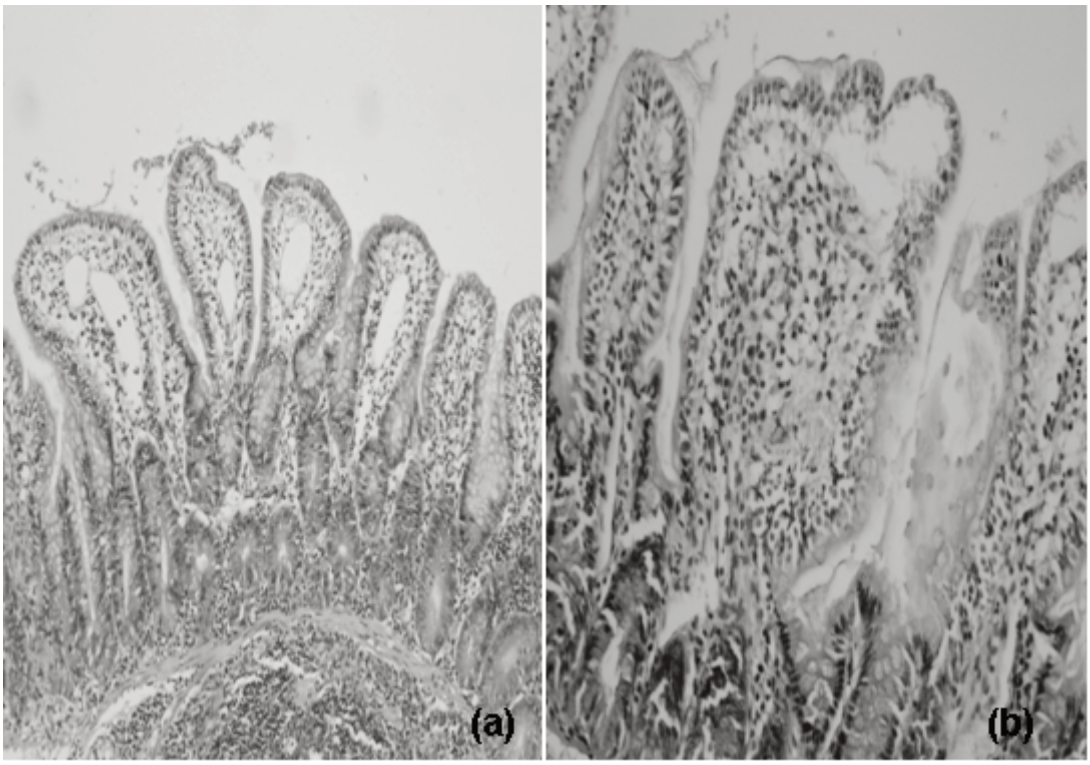

Figure 1. The normal histopathological appearance of intestine in CG (a) and grade 3 lesion in IRG (b) (H&E, ×100).

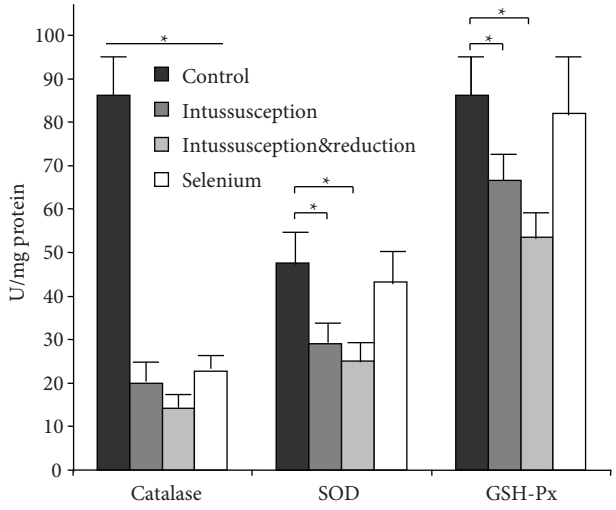

Figure 2. The values of oxidative stress markers in groups (bars represent means and standard errors of means).

Although MDA levels showed no difference in SG, they were significantly increased in ING and IRG when compared with CG ($P < 0.05$).

Discussion

In this experimental study, we aimed to evaluate the protective effect of selenium on intestinal ischemic injury due to IN. Although intestinal injury after ischemia has been investigated by several authors, intestinal I/R injury due to IN has not been evaluated previously. The reason for this lack of information might be related to the experimental model of IN.

Wang et al. reported that the nitric oxide synthase/nitric oxide pathway mediates IN pathogenesis in rats (16). They obtained IN in 40% of rats after infusion of 10 mg kg^{-1} of lipopolysaccharide intraperitoneally. Türkyılmaz et al. obtained IN in 30% of rats with a similar method (17), but this method is not suitable for I/R studies. It acts via nitric oxide activity, which may damage intestinal integrity. Another method, defined by Spiro et al., consists of the use of antibiotics to obtain mesenteric lymphadenopathy (18). This method is limited by low IN rates (only 38%-54% of all rats developed IN). In our experimental model, we invaginated the proximal

intestine distally with a stylet and obtained a mechanical obstruction similar to that which occurs in IN. All of the rats developed IN, and intestinal injury was achieved with varying histopathological grades. We suggest that this method can be assessed as a new model for IN and is suitable for IN-related I/R studies. However, the clinical relevance of this model is questionable and can be considered the most important limitation of our study.

Selenium is an essential trace element involved in many physiological functions. It is known to have an antioxidant effect as well as being an immune system modulator (1). Antioxidant pretreatment of selenium preserved intestinal integrity by a mechanism of blockage of lipid peroxidation after I/R. This antioxidant effect of selenium consists of several mechanisms. In particular, it is a cofactor of GSH-Px. Additionally, selenium diminishes the activity of nuclear factor kappa-B, which plays a crucial role in regulating the mediators of multiple organ dysfunction associated with I/R injury (4). Öztürk et al. reported that selenium pretreatment attenuates postischemic intestinal injury and prevents I/R-induced bacterial translocation (4). For instance, we investigated biochemical markers in accordance with histopathologic features.

CAT, SOD, and GSH-Px are antioxidant defense system enzymes (18). In I/R injury, these enzymes are usually decreased in biochemical evaluations (3). The SOD enzyme catalyzes dismutation of the superoxide anion into hydrogen peroxide, whereas GSH-Px and catalase detoxify hydrogen peroxide and convert lipid peroxides into nontoxic alcohols (19). These antioxidants are also essential for inhibition of inflammation related to the function of neutrophils and arachidonic metabolism (19). Reactive oxygen species (ROS) are produced during normal anaerobic metabolism and their production significantly increases during inflammation (19). The rise in ROS could be due to a decrease in the activity of ROS-metabolizing enzymes such as SOD, CAT, and GSH-Px (19). According to our results, IN and reduction cause severe I/R injury and alter the activity of antioxidant enzymes. Since selenium is a co-factor of the GSH-Px mechanism, increased enzyme activity following selenium administration is an expected finding. Our results revealed that GSH-Px activity is increased in SG when compared with IRG. On the other hand, the mechanism of increased catalase and SOD activities after selenium infusion should be related to the blockage of lipid peroxidation. Briefly, these findings indicate that selenium administration increases CAT, SOD, and GSH-Px activities and prevents antioxidant damage to intestines.

Malondialdehyde is a product of lipid peroxidation and is used as an indicator of oxidative damage induced by I/R (20). It has been shown that I/R causes a significant increase in MDA activity (20). We found increased MDA levels in ING and IRG. However, MDA levels in SG were not as high as in ING and IRG. We conclude that selenium reduces lipid peroxidation and production of MDA after intestinal injury.

When the effect of selenium on intestinal histopathology was evaluated, our results were different from those of other studies. Gupta et al. found that selenium prevents cellular injury in neurons as well as oxidative damage due to I/R (21). It has been shown that histopathologic findings confirm the preventive effect of selenium on intestinal I/R injury when used before generating ischemia (4). In this study, we used the selenium before reperfusion, not before ischemia, but we did not encounter such a protective effect on the histopathologic findings in intestinal mucosa. We supposed that the mechanism of ischemia is the major arbiter for histopathologic findings. Since we performed intestinal I/R injury by mechanical obstruction, not by clamping the mesenteric artery, we had higher grades of histopathologic damage in both the groups that received selenium and those that did not. It can be suggested that selenium pretreatment may be more effective on histopathologic findings if used before ischemia.

In conclusion, the experimental model proposed above is suitable for I/R studies in IN. Although selenium prevents oxidative injury by increasing CAT, SOD, and GSH-Px activities in experimental IN, similar effects were not detected in histopathologic findings. Our results suggest that additional studies are needed to evaluate the effect of selenium as a therapeutic agent in I/R injury due to IN.

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