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Oxidative Stress and Lipid Peroxidation in the Ischemic Small Intestine: Pathological and Biochemical Evaluation in a Rat model of Superior Mesenteric Ischemia

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The purpose of our study was to evaluate the role of oxidative stress and lipid peroxidation in acute mesenteric ischemia. Thirty male Wistar albino rats weighing 240–260 g were randomized into control (no operation), sham (operation without ischemia), and ischemia groups. To induce ischemia, the superior mesenteric artery was sutured. Total antioxidant and oxidant capacity and lipid peroxidase activity were measured in blood samples collected at 0 min, 60 min, and 240 min, and the pathology of ileum segments resected at 240 min was evaluated. Total oxidant status did not differ among the groups. Total antioxidant status increased significantly with time in the ischemia group compared to the control and sham groups ($P < 0.001$). Although basal arylesterase activity was lower in the ischemia group than controls ($P < 0.05$), post-ischemia values were similar among the groups. Similarly, basal and stimulated paraoxonase activity in blood samples did not differ among the groups. In conclusion, oxidative stress and lipid peroxidation have no significant role in the pathophysiology of acute mesenteric ischemia.

Key words: Mesenteric ischemia – Oxidative stress – Lipid peroxidation

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Oxygen is both vital and damaging, as it is involved in the pathogenesis of many types of tissue injury.[1,2] Oxidative stress, which is an imbalance between reactive oxygen species and antioxidant defense systems, can be caused by several factors such as ischemia, heavy exercise, and inflammation.[3,4] Oxidative stress has been linked to some neurodegenerative and cardiovascular diseases, preeclampsia, gene mutations, and aging.[5–9]

The role of oxidative stress and oxygen-derived free radicals in the pathophysiology of ischemic diseases was postulated first in the mid-1950s.[10] After the development of advanced biochemical techniques to measure oxidant and antioxidant capacities in tissues, the relationship between ischemia and oxidative stress has been studied extensively.[10]

Biochemical indicators of oxidative stress include total oxidant and antioxidant status and the activity of antioxidant enzymes such as serum dismutase, glutathione peroxidase, and stimulated paraoxonase. Specifically, paraoxonase, an ester hydrolase antioxidant with both arylesterase and paraoxonase activities, acts as an enzymatic defense against lipid hydroperoxides.[11,12] Serum paraoxonase and arylesterase activities recently have been found to be reduced in gastrointestinal diseases such as chronic liver disease.[13,14]

Oxidative stress has been proposed as an underlying mechanism of many chronic diseases, and reactive oxygen species are known to play a critical role in gut epithelial cell damage induced by ischemia.[15] Intestinal ischemia includes a variety of potentially life-threatening conditions such as ischemic colitis and acute or chronic mesenteric ischemia.[16] To determine the role of oxidative stress and lipid peroxidation in the pathophysiology of intestinal ischemia could lead to new approaches to treat and prevent these clinical conditions.

In this experimental animal study, we aimed to evaluate the effect of arterial ischemia on both oxidant/antioxidant capacities and lipid peroxidation in a rat model of superior mesenteric ischemia.

Materials and Methods

Study design

This was a prospective, randomized, controlled, experimental animal study. This study was approved by the Ankara Hospital Ethics Committee for Animal Experiments, and all animal experiments were carried out in accordance with Guide for the Care and Use of Laboratory Animals issued by the US National Research Council Committee[17] and local requirements.

Animals and experimental procedure

Thirty male Wistar albino rats weighing 240–260 g were used in this study. Rats were maintained under standard conditions of room temperature and humidity for two days before the experimental procedure, with access to water and standard feed *ad libitum*.

Femoral veins were cannulated and infused with 4 mL/kg/h saline solution under general anesthesia (50 mg/kg ketamine hydrochloride (Ketalar, Parke-Davis, İstanbul, Turkey) and 5 mg/kg xylazine hydrochloride (Rompun, Bayer Türk Kimya San. Ltd., İstanbul, Turkey), intramuscular. Heart rate and arterial oxygen saturation were monitored during the procedure.

Animals were randomly assigned to one of three study groups, each containing 10 rats:

Control:
Five mL blood collected at 0 min and 60 min.

Sham:
Following collection of 5-mL blood at 0 min, the abdominal area was shaved and sanitized with 10% povidone iodine (Poviodine 10%, Diagnokim, İstanbul, Turkey), and a linear midline incision was cut for laparotomy. After reaching the peritoneal region, the abdominal and peritoneal walls were sutured with 2/0 silk. Five milliliters of blood were collected at 60 min following surgical closure.

Ischemia:
Following collection of 5-mL blood at 0 min, sanitization, and opening of the abdomen as above, the superior mesenteric artery was sutured with 3/0 silk (Fig. 1). The abdominal and peritoneal walls were sutured with 2/0 silk. Five milliliters of blood were collected at 60 min following surgical closure.

All rats were sacrificed with high dose ketamine hydrochloride after 240 min. Following relaparatomy, a 5-cm sample segment of the ileum (in the ischemia group, an injured segment was selected) was, placed in 10% neutral formalin, and sent for pathologic evaluation (Fig. 2). Five milliliters of blood were collected.

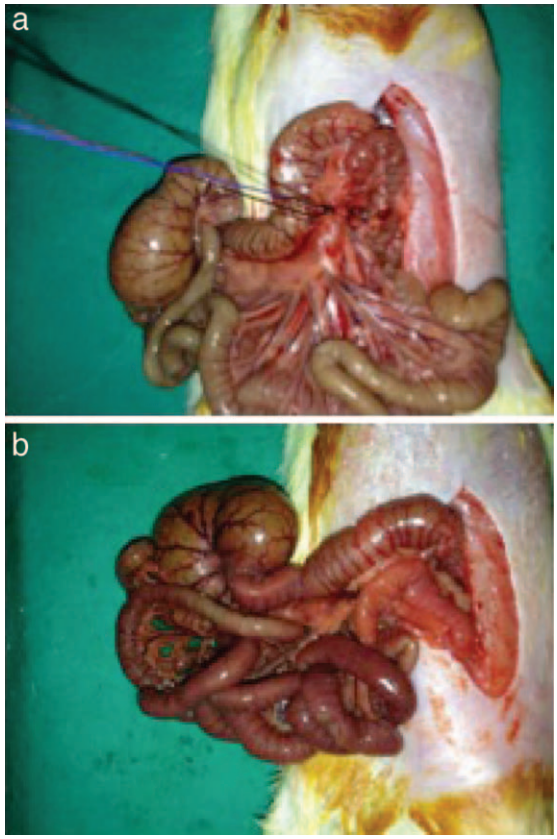

Fig. 1 (a, b) Images of experimental intervention. After an abdominal linear midline incision, the superior mesenteric artery was identified and sutured (a). The abdominal and peritoneal walls were then closed surgically. Four hours (240 min) after closure, a relaparatomy was performed; and a 5-cm sample segment with appearance of ischemia (*arrow* in b) was resected from the ileum for pathologic evaluation.

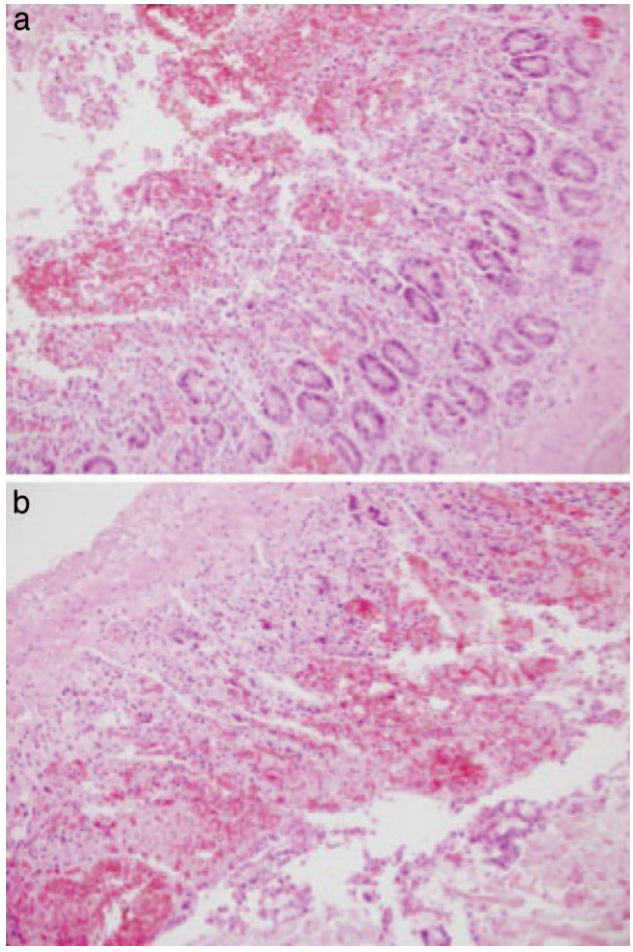

Fig. 2 (a, b) Images of pathologic specimens showed mucosal damage exuding of lamina propria (a; Grade 5) and villi that displayed hemorrhage or were denuded (b; Grade 6). Hematoxylin and eosin, ×200.

Pathologic evaluation

Ileum specimens were fixed in 10% neutral formalin, embedded in paraffin and sectioned into 5-μm slices. Sections were stained with hematoxylin and eosin, and histologic architecture was graded on a 6-tiered scale defined by Chiu's classification[18]: 0, normal villi; 1, villi with tip distortion; 2, Guggenheim's spaces; 3, villi with patchy disruption of the epithelial cells; 4, villi with exposed but intact lamina propria with epithelial sloughing; 5, exuding lamina propria; 6, hemorrhaged or denuded villi.

Biochemical analysis

Blood samples were centrifuged at 3000 rpm for 10 min and sent to Atatürk Research and Training Hospital Biochemistry Laboratory. The following analyses were performed: total antioxidant status, total oxidant status, and paraoxonase and arylesterase activity.

Measurement of total antioxidant status

Total antioxidant status was determined using a fully automated colorimetric method developed by Erel,[19] which measures total antioxidant capacity against strong free radicals. The principle of this method is that the e^{2+}-*o*-dianisidine complex reacts with hydrogen peroxide in a Fenton type reaction to produce hydroxyl radical, which is reduced at low pH to react with the colorless *o*-dianisidine molecule to produce the bright yellowish-brown dianisyl radical. Dianisyl radicals participate in further

Table 1 Oxidative and antioxidative parameters in all subjects at different times after ischemia

	Time of measurement following ischemia			*P* values		
	0 min	60 min	240 min	0 vs. 60 min	0 vs. 240 min	60 vs. 240 min
Total antioxidant status (mmoL Trolox eq./L)	0.65±0.25	0.48±0.23	0.73±0.49	0.007	0.377	0.021
Total oxidant status (μmol H_2O_2 eq/L)	27.40±16.48	34.88±18.88	19.14±18.71	0.162	0.052	0.001
Paraoxonase (U/L)	77.13±88.90	78.12±89.89	118.04±264.02	0.510	0.588	0.781
Stimulated paraoxonase	23.14±17.44	28.82±22.42	26.51±24.08	<0.001	0.118	0.027
Arylesterase	42.18±37.98	36.86±34.93	38.46±31.22	0.068	0.188	0.472

oxidation reactions, increasing color formation. Antioxidants in test samples stop coloration by inhibiting these oxidation reactions to a degree proportional to their concentration, which is measured spectrophotometrically using an automatic analyzer. The assay results are calibrated against a Trolox standard curve, yielding data expressed as millimolar Trolox equivalent per liter (mmoL Trolox eq/L).

Measurement of total oxidant status

Total oxidant status was determined by a fully automatic colorimetric method developed by Erel.[20] In this method, oxidants in the sample oxidize ferrous ion-o-dianisidine complex to ferric ion; glycerol speeds the reaction. Ferric ions in the acidic media then form a colored complex with xylenol orange. Color intensity, which is proportional to the amount of oxidants, is measured spectrophotometrically. The assay results are calibrated against a hydrogen peroxide (H_2O_2) standard curve, yielding data expressed as micromolar H_2O_2 equivalents per liter (μmol H_2O_2 eq/L).

Measurement of paraoxonase, stimulated paraoxanase, and arylesterase activities

Paraoxonase 1 (PON1) and arylesterase activities were measured using commercial Rel assay kits.[21] PON1 activity was determined at baseline and in the presence of NaCl (stimulated paraoxonase) via absorbance at 412 nm, resulting from formation of 4-nitrophenol. Enzymatic activity was calculated using the absorptivity coefficient of 18290 $M^{-1}cm^{-1}$. Phenylacetate was used as the substrate for the arylesterase activity assay, and its molar absorptivity coefficient, 1310 $M^{-1}cm^{-1}$, was used to calculate enzymatic activity in U/L.

Statistical analysis

Study data were summarized using descriptive statistics (means ± standard deviation for quantitative data; frequencies and percentage for qualitative data). Normality of the data distribution was tested using the Kolmogorov–Smirnov test. Multiple in-group comparisons employed the Friedman repeated measures test and between-group comparisons used the Kruskal-Wallis test. For comparisons of two groups, the Mann-Whitney *U* test was performed.

A value of $P < 0.01$ was considered to indicate statistical significance. Statistical analysis was executed using commercially available software (Statistical Package for Social Sciences, version 17.0, SPSS Inc, Chicago, Illinois, USA).

Results

Oxidant and antioxidant capacity

The mean total antioxidant capacity for all 30 rats at 60 min post-procedure was significantly higher than that at 0 min ($P = 0.007$) and the value at 240 min was significantly higher than at 60 min ($P = 0.021$). Total oxidant capacity was significantly lower at 240 min than at 60 min ($P < 0.001$). Stimulated paraoxonase activity increased significantly at 60 min (for 0 min versus 60 min, $P < 0.001$); however, it then decreased at 240 min (for 60 min versus 240 min, $P < 0.027$). Paraoxonase and arylesterase activities did not change significantly with time (Table 1).

Total antioxidant capacity was significantly lower in sham-treated rats than both controls and those with intestinal ischemia at 0 min ($P < 0.05$ for both). Although there were no differences among groups at 60 min, total antioxidant capacity was lower in sham-treated and ischemic rats than controls at 240 min ($P < 0.001$ for both). While arylesterase activity was significantly lower in ischemic rats than sham-

Table 2 Oxidative and antioxidative parameters between study groups at different times after ischemia

	Study groups			*P* values			
Time of measurement following ischemia	Group 1 (control)	Group 2 (sham)	Group 3 (ischemia)	Overall comparison	Groups 1 vs. 2	Groups 1 vs. 3	Groups 2 vs. 3
Total antioxidant status							
0 min	0.78±0.23	0.46±0.24	0.70±0.16	<0.05	0.002	0.396	0.015
60 min	0.58±0.23	0.44±0.15	0.42±0.28	0.212			
240 min	0.58±0.18	0.42±0.24	1.19±0.56	<0.05	0.331	0.001	<0.001
Total oxidant status							
0 min	35.06±19.98	20.31±14.49	26.82±12.02	0.133			
60 min	26.37±12.69	33.71±18.59	44.56±21.37	0.063			
240 min	17.93±14.04	10.51±7.85	28.98±26.04	0.126			
Paraoxonase							
0 min	92.59±109.65	83.36±83.92	55.44±74.66	0.576			
60 min	90.48±104.69	87.89±91.97	56.01±76.19	0.34			
240 min	90.49±102.92	205.25±442.29	58.38±71.8	0.801			
Stimulated paraoxonase							
0 min	30.45±22.55	22.01±14.72	16.98±12.36	0.179			
60 min	32.96±28.79	29.65±20.79	23.86±17.68	0.504			
240 min	28.75±30.09	26.35±22.27	24.45±21.36	0.94			
Arylesterase							
0 min	57.17±46.28	42.86±35.38	26.53±27.07	<0.05	0.151	0.019	0.140
60 min	47.48±43.05	37±30.29	26.13±30.12	0.217			
240 min	47.76±37.68	36.29±26.43	31.36±29.45	0.481			

treated and control rats at 0 min ($P < 0.05$), it did not differ among the groups at 60 min and 240 min. Total oxidant capacity and basal and stimulated paraoxonase activity did not significantly differ among the groups throughout the study (Table 2).

Pathologic findings

The pathology of specimens obtained from the control and sham groups was normal. In rats whose mesenteric arteries were occluded, mucosal damage included exuding lamina propria (Grade 5, n = 5) and hemorrhages and denuded villi (Grade 6, n = 5).

Discussion

In this experimental animal study, we evaluated the effect of arterial ischemia on both oxidant/antioxidant and lipid peroxidation parameters in a rat model of superior mesenteric ischemia and showed that oxidative stress and lipid peroxidation have no primary role in the ileal tissue damage induced by mesenteric ischemia.

Intestinal ischemia is caused by reduced intestinal blood flow. Although the exact mechanisms underlying intestinal ischemic injury have not been reported, it has been suggested that polymorphonuclear neutrophils, pro-inflammatory cytokines, and mediators of oxidative stress play important roles.[15]

Oxidative stress and lipid peroxidation have been shown to contribute to the pathogenesis of intestinal ischemic diseases.[4] However, few studies have evaluated oxidative and nonoxidative factors along with lipid peroxidation indicators. A recent study by Unal *et al*[22] revealed that experimental ischemic colitis causes a significant decrease in paraoxonase and arylesterase activities and an increase in malonyldialdehyde, an oxidative stress marker. The investigators of this study thus concluded that in addition to oxidative stress, lipid peroxidation plays a role in the pathophysiology of ischemic colitis.

Mesenteric ischemia, although rare in clinical practice, is associated with substantial morbidity, including bacterial translocation, multiple organ failure, and mortality. In the present study we hypothesized that both oxidative stress and lipid peroxidation contribute to the pathophysiology of acute mesenteric ischemia. To test this hypothesis, we compared total oxidant and antioxidant capacity and paraoxonase and arylesterase activity among blood samples from control, sham-treated, and ischemic (via mesenteric artery occlusion) rats. We also

pathologically evaluated ileum specimens according to Chiu's classification.[18] Since we aimed to evaluate the relationship between acute ischemia and oxidative stress/lipid peroxidation, we collected blood samples three times during the 240 min (4 h) post-ischemia. On pathologic evaluation, ischemic mucosal damage including exuded lamina propria, hemorrhagic, and denuded villi, was recorded in rats with occluded superior mesenteric arteries.

We observed no differences in total oxidant capacity among the control, sham and ischemia groups. Total antioxidant capacity, however, increased significantly with time following mesenteric occlusion compared to the control and sham groups. Based on these results, we speculate that although oxidant activity does not change immediately following ischemia, antioxidant capacity deteriorates significantly.

Although basal arylesterase activity was lower in ischemic than control rats, postprocedure values were similar. Similarly, basal and stimulated paraoxonase levels in blood samples did not differ among control, sham, and ischemia groups during the study. These findings suggest that lipid peroxidation has no role in the acute phase of mesenteric ischemia.

Antioxidant supplementation has been used successfully to treat some conditions related to oxidative stress, such as chronic pancreatitis.[23] However, whether antioxidant approaches would be useful to treat and prevent acute conditions such as mesenteric ischemia remains unclear; further studies should examine the role of oxidative stress in ischemic intestinal damage in humans and whether antioxidants reduce damage.

The main limitation of the present study was its use of animal subjects, limiting conclusions regarding pathophysiology of intestinal ischemia in humans. Furthermore, for technical reasons we could not determine oxidant/antioxidant and lipid oxidation parameters in tissue samples. However, ours is the first study to evaluate the role of both oxidative stress and lipid peroxidation in a small intestine ischemia model.

In conclusion, total antioxidant response surprisingly increases in ischemic intestinal tissue after acute mesenteric occlusion in a rat model. In contrast, total oxidant capacity and lipid peroxidation seem not to be related to ischemia. Therefore, oxidative stress and lipid peroxidation appear to play no significant role in the pathophysiology of acute mesenteric ischemia. Further larger scale and clinical studies are needed to support these results and clarify the underlying mechanisms of acute mesenteric ischemia, a potentially life-threatening condition.

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