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The Effects of Superoxide Dismutase and Catalase in Intestinal Reperfusion Injury: Histopathological and Biochemical Results

İntestinal Reperfüzyon Hasarında Süperoksit Dismutaz ve Katalazın Etkileri: Histopatolojik ve Biokimyasal Sonuçlar

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Özet: Bu deneysel çalışmanın amacı, intestinal iskemi-reperfüzyon (I/R) hasarında süperoksit dismutaz ve katalaz'ın koruyucu etkilerinin araştırılmasıdır. Sonuçlar histopatolojik ve biokimyasal olarak değerlendirildi. Çalışmada 40 adet Sprague-Dawley albino erkek sıçan kullanıldı. Süperior mezenterik arterin 30 dakikalık total oklüzyonunu izleyen 60 dakika süreyle reperfüzyon uygulandı. Sıçanlar 10'ar sıçandan oluşan 4 kümeye ayrıldı: Kontrollerde yalnızca iskemi ve reperfüzyon uygulandı. Diğer üç sağaltım kümesinde, 90.000 U/kg katalaz ya da 15.000 U/kg süperoksit dismutaz ya da her ikisinin bir kombinasyonu reperfüzyondan 5 dakika önce intravenöz olarak uygulandı. Histopatolojik değerlendirmede süperoksit dismutaz ve katalaz kombinasyonunun reperfüzyona bağlı intestinal mukozal hasar yönünden en koruyucu sağaltım olduğu görüldü. I/R'dan önce ve sonra serum kreatin kinaz, laktat dehidrogenaz, aspartat transferaz, alkalen fosfataz ve inorganik fosfat değerleri ölçüldü. Bu değerlerde kümelerin hiçbirinde bir saatlik reperfüzyon periyodu süresince anlamlı bir düzelme olmadı. Kısa süreli reperfüzyon periyodu sonunda histopatolojik ve biokimyasal sonuçlar arasında hiçbir korelasyon olmamasına karşın, süperoksit dismutaz ve katalaz kombinasyonunun histopatolojik olarak intestinal reperfüzyon hasarını belirgin şekilde azaltabildiği kanısına varıldı.

AnahtarSözcükler: İntestinal iskemi, reperfüzyon hasarı, süperoksit dismutaz, katalaz.

Summary: *The aim of this experimental study was the investigation of the preventive effects of superoxide dismutase and catalase in intestinal ischemia-reperfusion (I/R) injury. The results were evaluated both histopathologically and biochemically. Forty male Sprague-Dawley albino rats were used. Total occlusion of the superior mesenteric artery for 30 minutes was performed followed by reperfusion for 60 minutes. The rats were separated into 4 groups of ten rats: In the control group, only ischemia and reperfusion were applied. In the other three treatment groups, 90.000 U/kg catalase or 15.000 U/kg superoxide dismutase or a combination of both were administered intravenously 5 minutes before reperfusion. Histopathological evaluation showed combined superoxide dismutase and catalase to be the most preventive treatment in intestinal mucosal injury due to reperfusion. Serum creatine kinase, lactate dehydrogenase, aspartate transferase, alkaline phosphatase and inorganic phosphate before and after I/R were measured. There was no significant difference in these values during the one-hour-reperfusion period in any of the groups. It is concluded that although there is no correlation between the histopathological and biochemical results at the end of the short-term reperfusion period, combined superoxide dismutase and catalase may significantly reduce the intestinal reperfusion injury histopathologically.*

Key Words: *Intestinal ischemia, reperfusion injury, superoxide dismutase, catalase.*

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Ischemic injury to the intestinal mucosa occurs when the tissue is deprived of oxygen and other nutrients necessary to maintain cellular metabolism and integrity (1). Whatever the cause, intestinal ischemia causes a spectrum of injury ranging from completely reversible functional alterations to transmural necrosis of portions or all of the bowel. Reperfusion of the ischemic tissue also paradoxically causes a series of events that leads to tissue injury. Characteristic mucosal lesions are also demonstrated to occur after reperfusion (2, 3, 4).

This experimental study was designed to evaluate the preventive effects of superoxide dismutase (SOD), an enzyme that provides the dismutation of superoxide anion radical (O_2^-), and catalase (CAT), an enzyme that catalyzes the disproportionation of hydrogen peroxide (H_2O_2) to H_2O and O_2, in reactive oxygen metabolites induced intestinal ischemia/reperfusion (I/R) injury.

Materials and Methods

The effects of SOD and CAT in intestinal reperfusion injury were evaluated both histopathologically and biochemically. Histopathologically, jejunum and ileum were assessed separately to evaluate which part of the small bowel was more suspectible to the I/R injury. Serum creatine kinase (CK), lactate dehydrogenase (LDH), aspartate transferase (AST), alkaline phosphatase (ALP) and inorganic phosphate (IP) results were assessed as biochemical parameters.

Experimental Protocol: Forty male, Sprague-Dawley albino rats whose weights ranged from 220gm to 450gm were used. Rats were separated into 4 groups (one control and three treatment groups) each with 10 rats. After an overnight fast intramuscular anesthesia with 20mg/kg Ketamine was administered to the rats. Through a midcervical incision a catheter (Angiocath 24G 3/4 IN) was inserted into the internal jugular vein. One hundred IU/kg heparin was administered and 0.5 ml blood was drawn from each subject. The abdomen was opened through a midabdominal incision and the following procedures were applied to each subject in the four groups:

In the control group (group C) the superior mesenteric artery was clamped and intestinal ischemia for 30 minutes was applied. Only isotonic NaCl solution was infused. Following ischemia the clamp was opened and reperfusion was allowed for 60 minutes. At the end of reperfusion 0.5 ml blood samples were drawn and full thickness intestinal biopsies from proximal jejunum and distal ileum were taken. Catalase 90.000 U/kg (Sigma, catalase, Mouse liver, 5400 U/mg protein, C-8531) in the second group (group CAT), superoxide dismutase 15.000 U/kg (Sigma, superoxide dismutase, Bovin erytrocytes, 5100 U/mg solid, S-2515) in the third group (group SOD), and a combination of both was used in the forth group (group SC). These were administered intravenously 5 minutes before reperfusion.

Histopathological Grading: Full thickness intestinal pieces taken from proximal jejunum and distal ileum were fixed in 10% formalin and subsequently stained with hematoxylin-eosin. Morphologic changes were evaluated by a specific pathologist in a blinded fashion with light microscopy. Results were evaluated according to the scale below:

Grade 0: Normal mucosa (Figure 1).
Grade 1: Subepithelial space at villous tip; capillary congestion.
Grade 2: Subepithalial space enlargement with patchy separation of mucosa from lamina propria; oedema; extravasated erythrocytes (Figure 2).
Grade 3: Slight exfoliation at villous tip, acute inflammation; focal, demarcated necrotic areas.
Grade 4: Denuded and haemorrhage at apical villi; oedema; chronic inflammation.
Grade 5: Significant loss at all villi with common necrosis.

Biochemical Assay: Serum CK, LDH, AST, ALP and IP levels were determined on the same day. Biochemical examinations were executed using the IFCC approved, 37°C, single reactive n-acetyl cystein activated CK method for CK (u/l); with SEC approved 37°C, single reactive piruvate-lactate conversion method for LDH (u/l); with IFCC approved 37°C single reactive without pyridoxal-5-phosphate AST method for AST (u/l); with IFCC approved 30°C AMP-buffered method for ALP (u/l) and using phosphomolibdate method at 340 nm. for IP (mg/dl).

Statistical Analysis: Histopathological values were expressed as means with standard deviations in parentheses and biochemical values were expressed as means on Table. Mann-Whitney-U and Wilcoxonís paired signed rank tests were used for the statistical analysis. P values of <0.05 were considered significant.

Results

Histopathological Results: The jejunum and ileum in each group responded in a similar manner ($p>0.05$) to the injury and treatment (4.4±0.66 vs 3.6(1.02 for group C, 1.9±1.37 vs 3.6(1.11 for group CAT, 1.9±1.58 vs 1.7(1.10 for group SOD and 1.0±0.44 vs 1.3±0.90 for group SC) (Figure 3). When the injury scores for jejunum were compared between the groups all three treatment groups were found to respond well ($p<0.05$) to the treatment (1.9±1.37, and 1.9±1.58, and 1.0±0.44 vs 4.4±0.66) and the SC group responded significantly better ($p<0.05$) than that of other treatment groups (1.0± 0.44 vs 1.9±1.37, and 1.9±1.58). When the injury scores for ileum were compared between the groups all the treatment groups expect CAT (3.6±1.02 vs 3.6±1.11) ($p>0.05$) were found to respond well to the treatment (1.7±1.10, and 1.3±0.90 vs 3.6±1.02) ($p<0.05$). Although the SC group was seen to be the best treatment group in both jejunal and ileal injury scores, the results were not significantly different ($p>0.05$) in ileal injury scores than that of SOD group (1.3±0.90 vs 1.7±1.10).

Biochemical Results: Serum CK, LDH, AST, ALP and IP levels for each group were determined, separately (Table I). Postreperfusion levels were significantly higher ($p<0.05$) than preischemic levels for CK, LDH, AST and IP in all the groups and there were no difference between the groups ($p>0.05$). Postreperfusion levels were significantly lower ($p<0.05$) than preischemic levels for ALP in all the groups and there were no difference between the groups ($p>0.05$). Postreperfusion results in all treatment groups showed no improvement when they were compared to the control group ($p>0.05$).

Table I. Biochemical results of the groups in means.

Groups	C		CAT		SOD		SOD+CAT	
	Preisc.	Postre .	Preisc.	Postre.	Preisc.	Postre.	Preisc.	Postre.
CK (u/l)	1912	4933	1941	4960	1915	5204	1985	5018
LDH (u/l)	1066	4546	1061	4428	1057	4691	1025	4641
AST (u/l)	229	395	219	396	213	426	217	382
ALP (u/l)	243	110	231	119	225	115	220	110
IP (mg/dl)	8.1	9.3	8.1	9.1	8.0	9.0	8.2	9.2

C=Control; SOD=Superoxide dismutase; CAT=Catalase; SOD+CAT= Superoxide dismutase + Catalase; CK=Creatinine kinase; LDH=Lactate dehydrogenase; AST=Aspartate transferase; ALT=Alkaline phosphatase; IP=Inorganic phosphate; Preisc. = Preischemic; Postre. = Postreperfusion

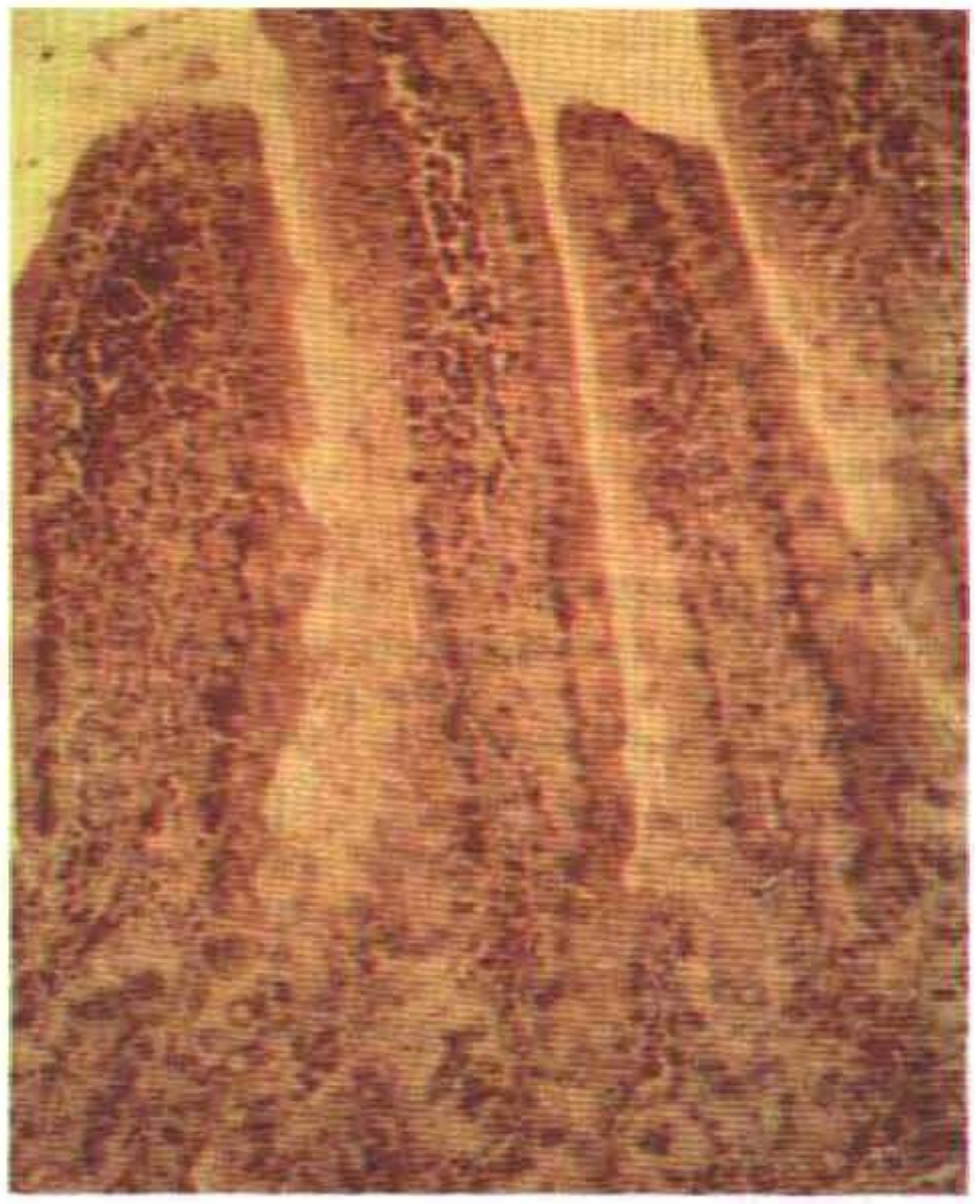

Figure 1. Normal morphological appearance of rat intestine (Grade 0).

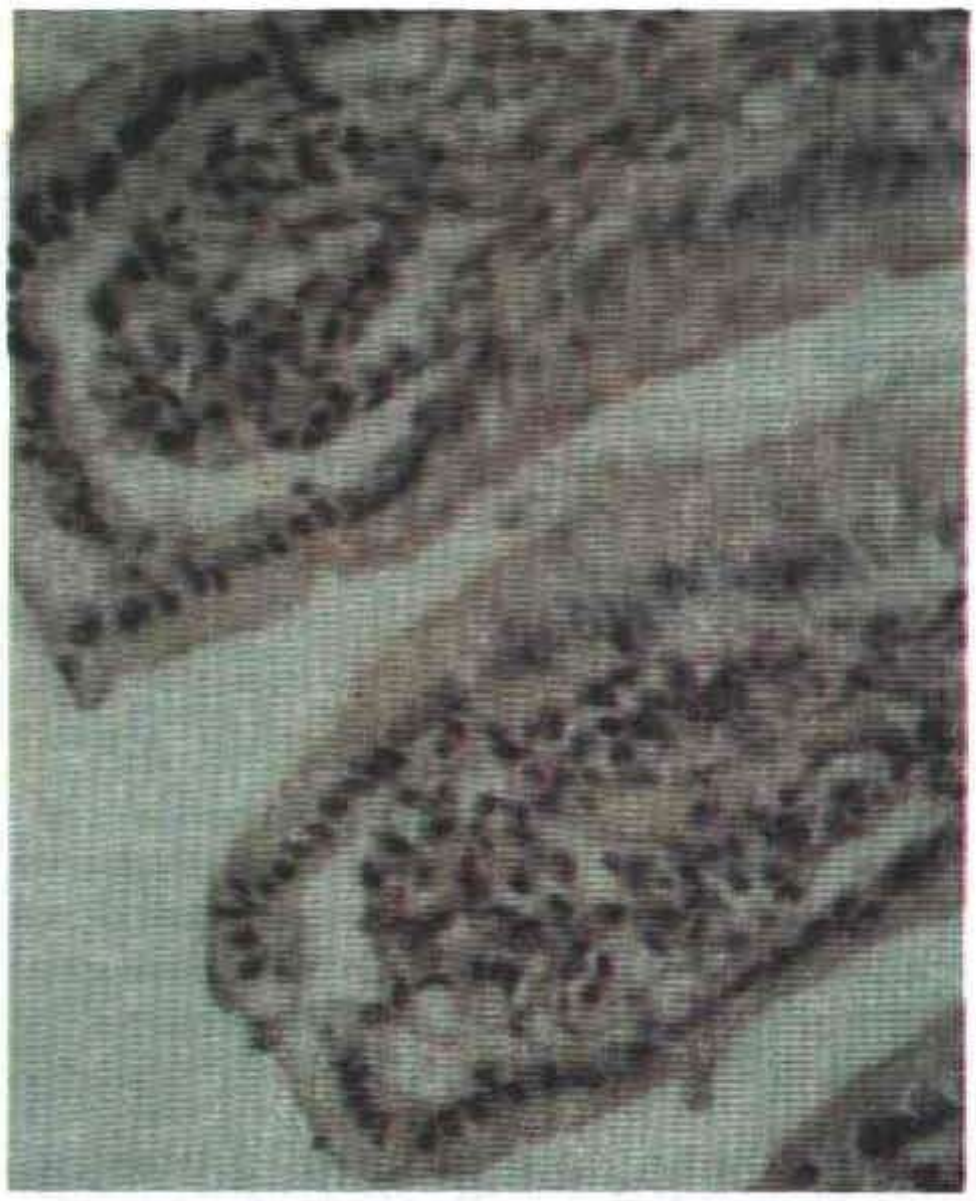

Figure 2. Grade 2 morphological changes in rat intestine.

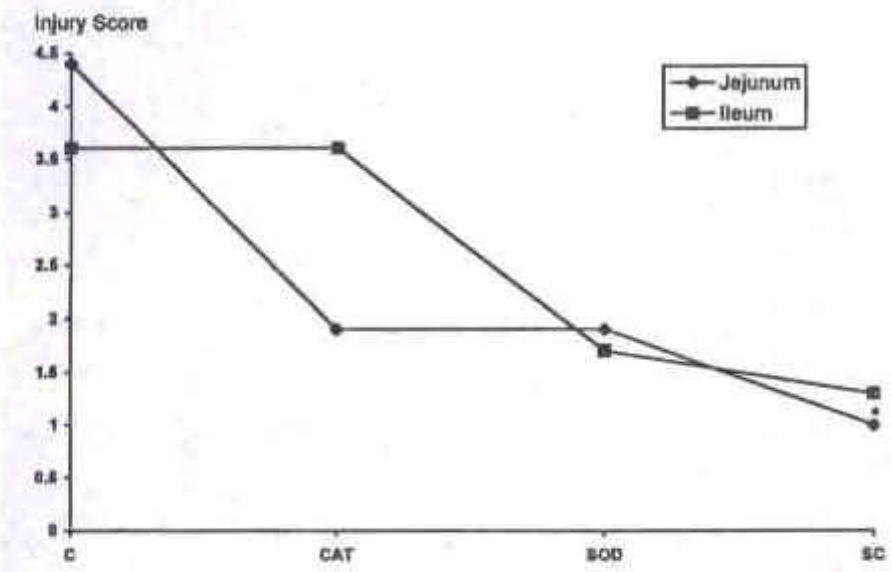

Figure 3. Histopathological injury scores. Jejunal and ileal injury scores are shown separately in control (C), catalase (CAT), superoxide dismutase (SOD) and superoxide dismutase and catalase (SC) groups. Marks indicate the means of the jejunal and ileal scores (4.4±0.66 vs 3.6±1.02 for group C, 1.9±1.37 vs 3.6±1.11 for group CAT, 1.9±1.58 vs 1.7±1.10 for group SOD and 1.0± 0.44 vs 1.3±0.90 for group SC). The jejunal score of the SC group is significantly lower (*p<0.05) when compared to the other treatment groups (1.0±0.44 vs 1.9±1.58, and 1.9±1.37).

Discussion

Reactive oxygen metabolites generated upon reintroduction of molecular oxygen in ischemic tissue have been agreed as the initiating event in reperfusion injury. During hypoxia energy rich phosphates are reduced from ATP to hypoxanthine (5). Xanthine oxidase (XO) is the rate limiting enzyme in nucleic acid degradation that has the ability to generate H_2O_2 and O_2- during the oxidation of hypoxanthine or xanthine. Xanthine dehydrogenase (XD) is converted to the oxidant-producing XO form during tissue ischemia (6). The univalent reduction of oxygen produces the O_2-. $2O_2$ may be formed as a result of the divalent reduction of O_2- or the dismutation of O_2- (7). The third radical species derived from moleculer oxygen is the hydroxyl radical (.OH) which is formed by the interaction of O_2- and H_2O_2 (Haber-Weiss reaction) and is a potent oxidizing agent (4, 7, 8, 9).

Another potential source of reactive oxygen metabolities in postischemic tissues are polymorphonuclear (PMN) leukocytes. It has been shown that the removal or inhibition of PMN attenuated the changes in microvascular permeability associated with reperfusion injury (4).

Since the gut possesses large quantities of the xanthine dehydrogenase - oxidase enzyme system necessary for the production of oxygen free radicals, intestine is one of the most sensitive tissues to reperfusion. Conversion of the XD to the XO is completed in one minute in the intestine, but it takes one hour in other tissues (10). The jejunum is more susceptible to I/R injury than the mid-small bowel and the latter is more sensitive than ileum. The lesions vary due to the duration and severity of the pathology (11).

Endogenous antioxidant enzymes such as CAT and SOD are normally present in the cells in high concentrations. The useful effects of these somewhat specific substances which detoxify free oxygen radicals have served as indirect evidence for admitted roles of free radicals in assorted pathophysiological conditions. SOD significantly attenuated reperfusion-induced increases in microvascular permeability. Furthermore, SOD administration before reperfusion significantly attenuates mucosal villi and crypt epithelium necrosis that occurs during prolonged ischemia (8, 12, 13). CAT has proved to be protective in many models of I/R (14). Experimental studies indicates that XO-generated reactive oxygen metabolites attract PMN to postischemic tissue. XO inhibitors and free radical scavengers have been shown to decrease both the number of PMN in reperfused tissue and associated microvascular injury (15, 16).

Biochemical serum markers have been studied in several studies about intestinal ischemia. To date no single serum marker specific to intestinal ischemia has been found. Serum CK, LDH, AST, ALP ve IP are some of these markers (17). Only if the BB fraction of CK is > 20 ng/ml, could it be demonstrated that this was 100% specific for intestinal ischemia, but the sensitivity of the test was reported as 63% (18). Jamieson at al. also reported that an increase in serum concentration of IP was an important indicator of mesenteric ischemia (19). Recently, Sisley and Gewertz identified the enterocyte enzyme alkaline phosphatase as a specific marker of reperfusion (20). The enzyme activity levels decreased in reperfusion while remaining unaffected by ischemia alone. Furthermore, the extent of the depression in ALP activity was parallel to the severity of reperfusion damage, providing a means of quantifying the reperfusion component of an I/R injury.

In our study, histopathologically, the jejunum and ileum were affected to the same extent and were protected to the same extent by the various treatments. The

combination of SOD and CAT proved to be the most effective in the prevention of I/R injury. SOD and CAT given alone conferred some protection but not to the same extent as the combination. The SC treatment was found to be most effective possibly due to potentialization of their oxygen radical scavenger effects. This results are in agreement with these of previous studies (3, 8, 11, 12, 16).

Improvements were seen in the histopathological injury scores, but no improvement was seen in the biochemical parameters measured at the end of the reperfusion period with the different treatments. Increases of the serum CK, LDH, AST and IP, and decrease of ALP in groups carried on their levels during the reperfusion period. This may reflect the fact that these enzymes are released from cells or depressed earlier in the injury cycle and persist in the circulation during the short-term reperfusion period. On the other hand, although these biochemical parameters may be relatively good indicators of ischemia alone they may not necessarily be indicators of reperfusion.

In conclusion, the combination of SOD and CAT was effective in decreasing or preventing intestinal reperfusion injury. This was manifest in the histopathological injury scores but not in the biochemical measurements in the short-term reperfusion period.

The results of this animal model study of intestinal I/R suggest that these agents may be effective in minimizing injury. Future developments may lie in producing agents such as these which will be safe for patients but only after an effective diagnostic tool is developed to guide the timing of their administration.

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