EXPERIMENTAL STUDY

Melatonin exhibits supportive effects on oxidants and anastomotic healing during intestinal ischemia/reperfusion injury

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ABSTRACT

BACKGROUND: The aim of this study was to investigate the effects of melatonin on intestinal anastomosis after intestinal ischemia/reperfusion injury (IRI).

METHODS: Thirty Wistar albino rats of both sexes were divided into 3 groups: sham, control, and treatment. IRI was performed by clamping the superior mesenteric artery (SMA) for 30 minutes, followed by reperfusion. The sham rats received only manipulation of the SMA. Melatonin (10 mg/kg) was administered to the treatment group, and the control group was given a vehicle injection. Both the treatment group and the control group further underwent ileal resection of a 1-cm segment and anastomosis. On the postoperative seventh day, the anastomotic burst pressure, hydroxyproline level, histological indices of wound healing, and oxidative parameters of catalase (CAT), superoxide dismutase (SOD), total glutathione (T-GSH), and glutathione peroxidase (GSH-Px) levels were measured. A one-way analysis of variance and chi-square test were used for the categorical data.

RESULTS: Melatonin treatment led to a significantly higher burst pressure ($p=0.027$ and $p<0.001$, respectively). The 2 antioxidant enzymes, CAT and SOD, were at the highest level in the sham and melatonin groups and the lowest level in the control group ($p=0.001$ and $p=0.002$, respectively). Melatonin treatment resulted in a significantly higher level of both enzymes compared with the control group ($p=0.026$ and 0.003, respectively). The GSHpx and total GSH levels were slightly elevated in the treated rats, but the difference was not statistically significant ($p=0.205$ and 0.216, respectively). Fibroblast infiltration, capillary formation, and epithelialization were significantly better in the melatonin-treated animals. The granulocyte and mononuclear infiltration scores were similar between all groups.

CONCLUSION: It was concluded that melatonin had marked effects on intestinal anastomotic healing during intestinal IRI.

Keywords: Anastomosis; ischemia/reperfusion injury; melatonin.

INTRODUCTION

Despite the evidence of marked progression in perioperative care and technical advancements, anastomotic leaks still constitute serious problems with marked levels of morbidity and mortality.[1] Anastomotic safety is affected by several factors, including the surgeon's experience, suture material and tightness, blood supply, and the nutritional and medical status of the patient.[2] However, sufficient tissue oxygenation may be the most important factor. In addition to mesenteric embolism, bowel obstruction and trauma are related to intestinal ischemia/reperfusion injury (IRI), which should be treated immediately.[2,3]

Several studies have reported that intestinal IRI also leads to delays in intestinal anastomotic healing due to local or systemic effects.[2–4] Intestinal ischemia/reperfusion (IR) causes an exaggerated systemic response via the release of gut-derived toxins or inflammatory mediators, such as reactive oxygen species (ROS), cytokines, arachidonic acid products, and the expression of adhesion molecules during reperfusion.[5] Thus, tissue injuries increase throughout reperfusion.[6]

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Recent studies have shown that antioxidants, such as N-acetylcysteine, superoxide dismutase (SOD), catalase (CAT), selenium, and vitamins E and C, can prevent IR-associated adverse effects of oxygen radicals and ROS and improve wound healing.[7,8] Melatonin, the pineal hormone, is a powerful antioxidant and free radical scavenger of ROS, including the hydroxyl and peroxyl radicals, as well as singlet oxygen and nitric oxide.[9] Melatonin exerts a tissue protective effect by increasing the antioxidant enzyme levels via its specific receptors and protects the cell from death.[10] Other beneficial effects of melatonin are mitogen activated protein kinase (MAPK) and nuclear factor-kappa (NF-K) activation, iNOS expression, and nitrite production.[11] NF-K plays a role in the modulation of DNA transcription, which is a critical point in the regulation of cellular processes, such as DNA repair, and situations requiring cellular growth, such as sepsis. Melatonin also inhibits lipopolysaccharide induced increase in MAPK activation, which promotes tissue inflammation and injury.[12] Melatonin has also been reported to play a preventive role in IRI in the heart, kidney, liver, lung, and intestines.[13] For these reasons, in the present study, we evaluated the effects of melatonin on intestinal anastomoses during intestinal IRI.

MATERIALS AND METHODS

Procedure and Evaluation

Ethical committee approval for the experimental protocol was obtained from Gaziosmanpasa University Faculty of Medicine before initiating the study. All rats received professional human care at Gaziosmanpasa University Experimental research center. Thirty Wistar albino rats of both sexes, weighing 250–320 g, were fasted for 12 h before experiments but had free access to water only. All rats were housed at 2–3 per wire cage with a 12:12-h light:dark cycle, kept at a constant temperature of 22°C–23°C, and fed with standard rat chow. The rats were divided into three groups (sham, control, and treatment groups; n=10 in each group).

Surgery and Experimental Protocol

Before the operative process, anesthesia was induced by an intraperitoneal injection of ketamine hydrochloride (75 mg/kg; Ketalar, 500-mg flacon; Pfizer, Istanbul) and xylazine hydrochloride (10 mg/kg; Rompun 2% flacon; Bayer, Istanbul). After skin preparation, a midline laparotomy of 2–3 cm was made. The superior mesenteric artery (SMA) was denuded from its attachments and occluded immediately distal to the aorta with collateral interruption for 30 min using an atraumatic microvascular clamp as described elsewhere.[14–17]

Group 1 (Sham): The SMA and ileal branches were dissected free, but not occluded.

Group 2 (Control): A vehicle (1% alcohol in saline; 1 ml/kg) injection was intraperitoneally administered to the Control group 30 min prior to the operation.[15] The SMA and ileal branches were dissected free and occluded.

Group 3 (Melatonin treatment): Melatonin (Sigma, St. Louis, MO, USA) was dissolved in absolute ethanol, and further dilutions were made using saline. The final concentration of ethanol was 1%. Melatonin (10 mg/kg) was intraperitoneally administered to the treatment group 30 min prior to the operation.[15] The SMA and ileal branches were dissected free and occluded.

During the ischemic episode, the abdominal incision was temporarily closed to prevent hypothermia. Both the control and treatment groups further underwent a reperfusion by releasing the clamps, followed by ileal resection. A 1-cm segment of ileum was resected 5 cm proximal to the cecum. The resected segment was reconstructed by a single layered, end-to-end ileo-ileal anastomosis using 5–0 polypropylene (Prolene, Ethicon) interrupted inverting sutures.[18] The fascia and skin were closed by 4–0 monofilament polypropylene (Prolene, Ethicon) running sutures. All animals were resuscitated using a subcutaneous injection of saline (8–10 ml/kg) to the dorsal area.

Blood and Tissue Sample Collection

The rats in the sham, control, and treatment groups were sacrificed on the seventh day as per the study design. Blood and tissue samples were obtained for biochemical analysis and histopathological evaluation of anastomotic tissue. The rats were sacrificed in accordance with laboratory conditions by intraperitoneally administering a high-dose of the anesthetic pentothal (200-mg/kg thiopental sodium, 0.5 G IE Ulugay, Istanbul).

Burst Pressure (BP)

A laparotomy through the previous incision was made, and a 5–6 cm portion of a ileal segment with anastomosis was resected. The luminal content was cleaned by gentle saline flushing. One end of the ileal segment was tightly sutured with 3–0 silk, and the other end was attached to a mercury manometer using a tubing piece with an infusion pump. The intestinal segment was place in a saline-filled container, and air was pumped at a constant pressure of 10 mmHg/s. The pressure at which the reading suddenly declined or air bubbles were observed was recorded as BP, as previously described.[19] The possible upper limit of the apparatus was 300 mmHg. Measurements higher than 300 mmHg were accepted as 300 mmHg.

Determination of Hydroxyproline Levels

Hydroxyproline levels were determined using Reddy and Enwemeka's technique with some modification. The samples (50–100 µg) in 4 N NaOH were hydrolyzed by autoclaving at 120°C for 20 min. The hydrolysate was then cooled, neutralized with 4 N HCl, and centrifuged at 12,000 ×g for 10 min. Further, 1 mL of chloramine-T was added to 1 mL of supernatants and mixed gently, and the oxidation was allowed to proceed for 20 min at room temperature. Next, 1 mL of

Ehrlich's reagent was added to each sample and mixed gently, and the chromophore was allowed to develop by incubating the samples at 65°C for 15 min. The absorbance of each sample was read at 560 nm using a spectrophotometer. Hydroxyproline levels were calculated from L-hydroxyproline standard curve, and results were expressed in micrograms of hydroxyproline per milligram of wet tissues.[20]

Histopathology

For histopathological analyses, the anastomosis-site specimens were fixed in 10% neutral buffered formalin and embedded in paraffin. Paraffin-embedded tissues were sectioned at 5 µm and stained with hematoxylin and eosin. The specimens were examined by a blinded pathologist. During histopathological analysis, we semi-quantitatively assessed healing parameters (fibroblast infiltration, capillary formation, and epithelization) and inflammatory changes (granulocyte and mononuclear cell infiltration) in each specimen by giving a score of 0–3 for each parameter, as mentioned by Nursal et al.[21]

Determination of Protein Levels and Homogenization

Protein levels in the tissues were measured by the Bradford method.[22] The absorbance was measured at 595 nm using a UV-VIS spectrophotometer. Bovine serum albumin was used as the protein standard. Tissues for enzyme activity studies were homogenized (PCV Kinematica Status Homogenizator) in ice-cold phosphate-buffered saline (pH 7.4). The homogenate was sonified in three cycles (20-s sonications and 40-s pause on ice) using an ultrasonifier (Bronson sonifier 450). The homogenate was centrifuged (15,000 ×g, 10 min, 4°C), and cell-free supernatant was immediately subjected to enzyme assay.

CAT Assay

CAT activity was measured at 37°C using the rate of disappearance of hydrogen peroxide (H_2O_2) at 240 nm (ε240=40 M^{-1} cm^{-1}). One unit of CAT activity is defined as the amount of enzyme catalyzing the degradation of 1 µmol of H_2O_2 per min at 37°C and specific activity corresponding to transformation of substrate (in µmol) (H_2O_2, min/mg protein).[23]

SOD Assay

SOD (Cu, Zn-SOD) activity in the supernatant fraction was measured using xanthine oxidase/cytochrome c method,[24] where 1 unit of activity is the amount of enzyme needed to cause half-maximal inhibition of cytochrome c reduction. The amount of SOD in the extract was determined as unit of enzyme/mg protein, utilizing a commercial SOD as the standard.

Glutathione Peroxidase (GSH-Px) Assay

GSH-Px activity was determined using a coupled assay with glutathione reductase by measuring the rate of NADPH oxidation at 340 nm using H_2O_2 as the substrate.[25] Specific activity is given as the amount of NADPH (µmol) disappeared per min per mg protein.

Total Glutathione (GSH) Assay

The formation of 5-thio-2-nitrobenzoate is followed spectrophotometrically at 412 nm.[23] The amount of GSH in the extract was determined as nmol/mg protein using a commercial GSH as the standard.

Statistical Analysis

Statistical evaluation of numeric variables was performed by one-way ANOVA, followed by post hoc Tukey. Non-numeric variables were evaluated using χ^2 test. A p value of <0.05 was considered to be statistically significant.

RESULTS

No intraoperative or postoperative death occurred in the rats, and we did not observe any infective complication in the sham, control, and melatonin groups during 7-day follow-up. All results are shown in Table 1.

A graphical demonstration of BPs and hydroxyproline (OH-

Table 1. Quantitative values of burst pressures, OH-proline levels, and antioxidant levels in all groups

	Sham group	Treatment group (Melatonin)	Control group	p
	Mean±SD	Mean±SD	Mean±SD	
Burst pressures (mmHg)	221±24.70	216.00±21.18	181.25±33.56	<0.05
OH-proline levels (µg/mg)	7.27±0.69	6.74±0.46	5.39±0.51	<0.001
Catalase	475.83±88.19	429.61±55.94	343.11±61.19	<0.05
SOD	5.31±0.66	5.48±1.30	3.87±0.88	<0.05
GSH-px	11.90±3.12	12.55±3.68	14.54±3.22	>0.05
Total GSH	15.09±2.49	15.73±2.88	17.05±1.96	>0.05

OH-proline: Hydroxyproline; SOD: Superoxide dismutase; GSH: Glutathione; SD: Standard deviation.

Melatonin application during intestinal IR resulted in better anastomotic healing, as proven by higher burst pressures, OH-proline levels, and catalase and SOD levels. Of particular interest, GSH-px and total GSH levels were slightly high in melatonin-treated rats. However, these differences were not significant.

proline) levels are shown in Figure 1. Both the sham- and melatonin-treated rats exhibited significantly higher BPs than the control rats (221±24.7, 216±21.2, and 181.25±33.56 mmHg, respectively; p=0.009). The post hoc evaluation revealed that the sham and melatonin groups were significantly higher than the control group (p=0.011 and 0.027, respectively), but there was no difference between the sham and treatment groups (p=0.906). The mean OH-proline levels were markedly higher in the sham and melatonin groups than in the control group (7.27±0.7, 6.74±0.46, and 5.39±0.51 μg/mg, respectively; p<0.001).

The highest levels of the two antioxidant enzymes CAT and SOD were observed in the sham and melatonin groups and the lowest levels were observed in the control group (p=0.001 and 0.002, respectively). The levels of these enzymes showed similar results in the sham and melatonin groups (p=0.317 and 0.919, respectively). However, melatonin application led to significantly elevated levels of CAT and SOD compared with the levels in the control group (p=0.026 and 0.003, respectively) (Fig. 2). The other two enzymes, GSH-Px and GSH showed alterations similar to those of CAT and SOD. Their levels were the highest in the control group and lowest in the sham group. However, statistical analyses revealed no significant difference in GSH-Px and GSH levels between the control and sham groups (p=0.205 and 0.216, respectively) (Fig. 3).

Fibroblast infiltration was significantly different among the groups (p=0.007). An evaluation within the groups showed similar values in the sham and treatment groups. However, both the sham and treatment groups showed a significant difference in fibroblast infiltration compared with the controls (p=0.024 and 0.012, respectively). Similarly, capillary forma-

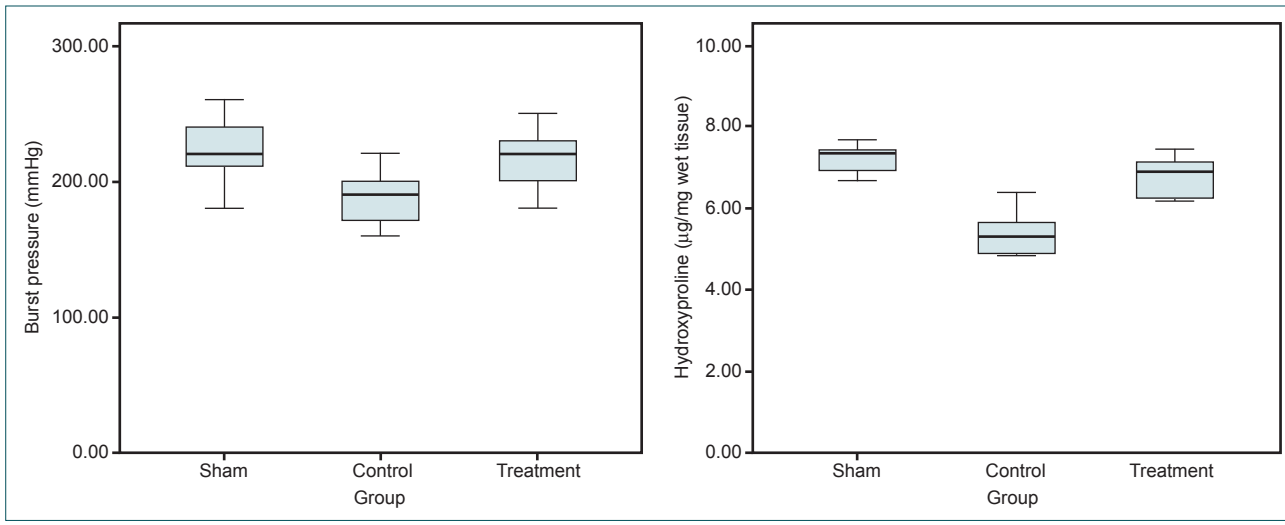

Figure 1. Burst pressures and anastomotic OH-proline levels. Footnote of Figure 1: Burst pressures and OH-proline levels in sham- and melatonin-treated rats were similar (p=0.109) but higher than those the untreated controls. Melatonin application markedly increased both burst pressures and OH-proline levels over those in the controls.

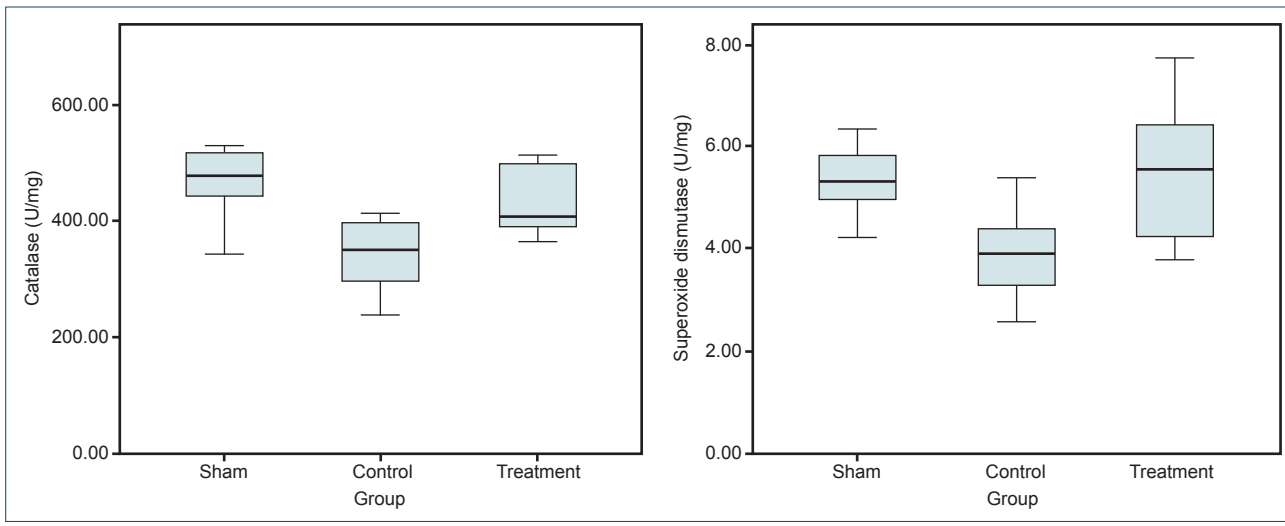

Figure 2. CAT and SOD levels in all groups. Footnote of Figure 2: Melatonin application significantly increased the levels of the antioxidant enzymes CAT (p=0.001) and SOD (p=0.002).

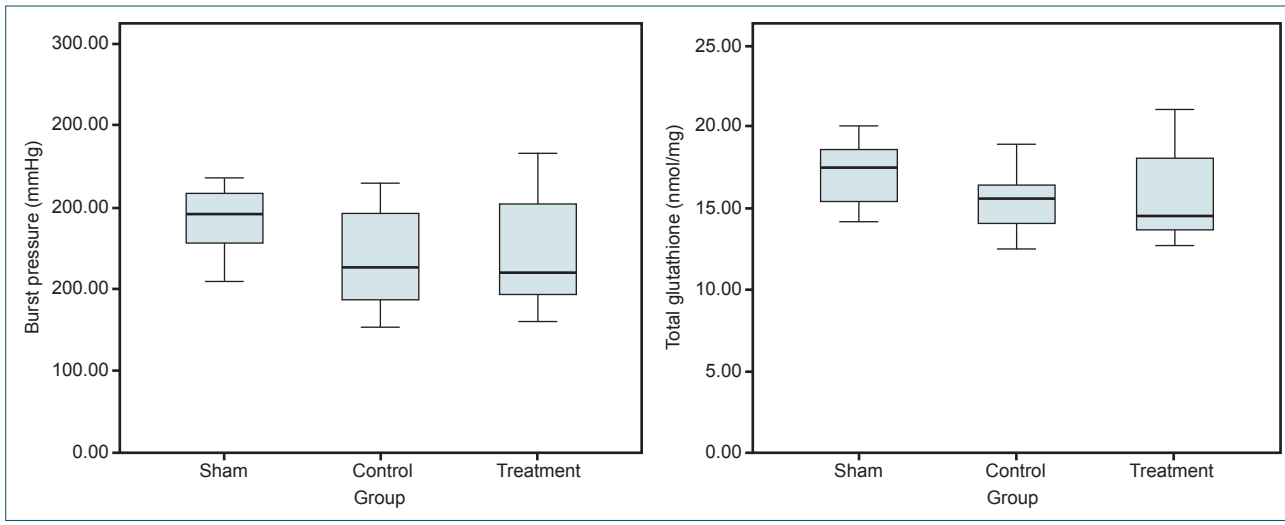

Figure 3. Glutathione peroxidase and total glutathione levels.

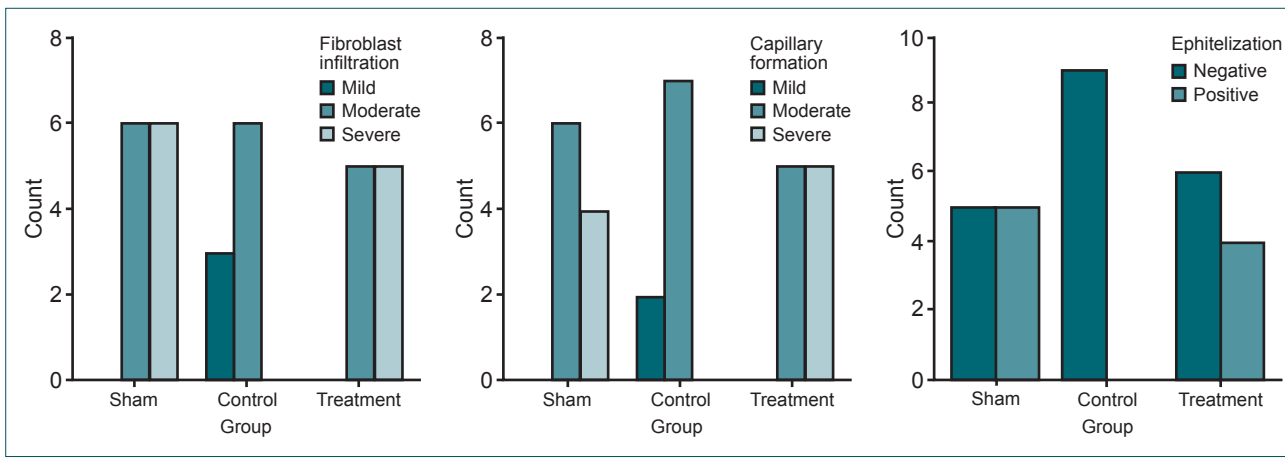

Figure 4. Fibroblast proliferation, capillary formation, and epithelization. Footnote of Figure 4: Fibroblastic proliferation was significantly improved in the treatment group, p=0.012. Capillary formation, an indicator of wound healing, increased over time in the treatment group, with significant difference, p=0.021. Epithelization showed significantly better results in the treatment group, p=0.015.

tion and epithelization were significantly higher in the sham and treatment groups than in the control group. Graphical analyses of predictive histological parameters are shown in Figure 4. Granulocyte and mononuclear infiltration scores were similar between all groups (p=0.278 and 0.485, respectively).

DISCUSSION

IRI is an important clinical problem that is associated with high rates of morbidity and mortality in surgery. In many vascular operations (e.g., embolectomy for SMA occlusion, repair of traumatic vascular lacerations or abdominal aortic aneurysm, treatment of hypovolemia due to bleeding, and organ transplantations), a concomitant gastrointestinal anastomosis may be necessary. The construction of a gastrointestinal anastomosis during these situations may be hazardous, in part because of the reperfusion of ischemic tissues. IR has a negative effect on the healing of intestinal anastomoses. Preoperative or intraoperative IRIs may also affect wound healing and decrease anastomotic durability.[3] Most of the general factors suggest that the vasoconstrictive effects are the cause of local ischemia and dehiscence of an anastomosis. Perfusion and the state of local oxygenation are some of the most important factors for healing gastrointestinal anastomoses. The wound healing process is fast in optimally perfused tissue, and sufficient oxygen should reach the wound for proper healing. Related to this issue, Kologlu et al.[19] investigated and compared the effects of local IRI and remote IRI on the healing of colonic anastomoses. They concluded that segmental small intestinal, unilateral lower extremity, and unilateral renal IR significantly delay anastomotic healing in the right colon. Similarly, Kuzu et al.[3] showed that reperfusion stress after SMA ischemia cause a delay in the anastomotic healing process in the left colon.

Many materials and drugs, such as resveratrol, thymoquinone, and melatonin, have been evaluated with regard to their protective effects on intestinal IRI.[26,27] Ozban et al.[17] concluded

that melatonin prevents the harmful effects of IRI on intestinal tissues in a rat model of SMA occlusion. Similarly, our study showed that melatonin, when administered prior to ischemia, has protective effects on intestinal anastomotic healing in a rat model of SMA occlusion.

In the present study, we used a rat model of small bowel anastomosis in the setting of IRI. We hypothesized that IRI would be responsible for most of the anastomotic leaks and that melatonin could prevent the adverse effects of IRI and improve anastomotic healing. Melatonin, which has a short half-life, was administered 30 min prior to the procedure. Furthermore, to see the effect of melatonin on intestinal anastomosis, the rats were sacrificed at the end of 7 days.[3,15]

Although many methods to assess anastomotic healing have been defined, the most frequently used techniques are BP and tensile strength measurements.[4,28] An optimal tissue perfusion and oxygen delivery is essential for fibroblasts, macrophages, lysine, and proline hydroxylation for collagen synthesis. In the present study, for assessing anastomotic healing, we preferred intestinal BPs, a technique in which force is applied in all directions. Mechanical properties of anastomoses have also been assessed by measuring the hydroxyproline (OH-proline) levels. In vertebrates, almost the entire OH-proline content is present in collagen.[28] Collagen is the essential structural protein of the connective tissue and is responsible for the stability of the anastomosis and the elasticity of the tissue.[29] Therefore, we used the OH-proline level as an indicator of collagen metabolism. Our results demonstrated that melatonin has supportive effects on mechanical properties of anastomotic healing during IRI. Higher BPs in conjunction with higher OH-proline levels are confirmative of higher anastomotic strength in melatonin-treated rats. The improvements in mechanical properties of anastomotic healing in the melatonin group also paralleled the histological indices of wound healing, including fibroblast infiltration, capillary formation, and epithelization. These results suggest the ability of melatonin to prevent IRI in a rat model of intestinal anastomotic wound healing.

The beneficial effects of melatonin in IRI are further supported by the higher antioxidant enzyme activity in the treatment group. Reperfusion can be simplified as an oxygen burst in tissues undergoing reoxygenation. This is associated with a high rate of free radical generation and, if not faced with antioxidant systems, can lead to severe tissue damage. Experimental evidence suggests that ROS generation is significantly responsible for tissue damage during IRI.[6] Therefore, documentation of the protective activity of melatonin treatment prior to ischemia in conjunction with higher activities of the main antioxidative enzymes, CAT, SOD, and GSH-Px, allowed us to suggest that systemic melatonin treatment leads to increased anastomotic antioxidant enzyme activities. It has been previously reported that melatonin is an effective free radical scavenger[9,13] and acts as a positive regulator for main enzymes of the antioxidant system, including SOD, CAT, GSH-Px, and GSH reductase.[13] Our data paralleled these reports. We detected significant improvements in SOD and CAT levels in melatonin-treated rats. Melatonin-treated rats subjected to IR had more than 40% higher SOD activities than the control rats. A less significant difference of 25% was noted for CAT levels. These results are in accordance with previous reports showing that melatonin treatment significantly stimulates several antioxidant enzymes.[13] The remaining enzyme GSH-Px and total GSH levels were also improved, but these differences were not significant compared with the levels in the control rats. The data in the literature also fails to demonstrate an effect of melatonin on GSH-Px activity following IRI.

Recent studies have documented the protective role of melatonin on gastrointestinal mucosal healing. Celinski et al.[30] have shown beneficial effects of melatonin on the healing of gastric and duodenal ulcers and burn patients. They concluded that melatonin added to omeprazole treatment significantly accelerates the healing rate of Helicobacter pylori-infected chronic gastroduodenal ulcers over that obtained with omeprazole alone, and this likely depends on the significant increase in plasma melatonin and, possibly, leptin levels. Cabeza et al.[31] have reported that the free radical scavenger properties of melatonin mainly include superoxide anions, which are probably derived from the xanthine oxidase pathway, and that the increase in antioxidative enzymes significantly contributes to mediating the protection by the hormone against IR gastric injury.

Intestinal ischemia is a life-threatening abdominal emergency with an overall mortality rate of 60%–80%.[8] Restoration of the oxygen supply to ischemic tissues is essential for tissue repair because oxygen is critical to energy production and toxic metabolite removal. Some of the function lost after ischemia may be regained by reperfusion of the tissue, but reperfusion also accelerates the formation of oxygen-derived ROS. Therefore, in the clinical scenario of a patient suffering from acute mesenteric ischemia, a potentially therapeutic agent that is expected to block the deleterious effects of reperfusion, such as release of free oxygen radicals, should be given before restoring arterial flow. Therefore, in our experimental model, melatonin was administered 30 min before the start of reperfusion in the treatment group; the aim of this technique was to ensure that melatonin was in the animal's circulation. Adverse effects of melatonin are few, and it is generally regarded as safe in recommended dosages. In humans, for most non-sleep-related disorders, dosages of 10–50 mg daily have been safe and effective.[32] We used a melatonin dose of 10 mg/kg 30 min prior to the start of reperfusion, which was similar to that in other research in which experimental IR rat model investigations were performed with melatonin.[33] This dosage and timing seem to be reasonable when considering the relatively short serum half-life (30–60 min) and the total amount of melatonin in the gastrointestinal tract (up 400 times more than that in the pineal gland).[32]

This study had some limitations. Despite the previously mentioned immune regulatory effects of melatonin, we could not demonstrate a difference between granulocyte and mononuclear cell infiltration scores. We could have tried to confirm our results with cytokine levels. However, this point remains to be proven in further studies. The lack of molecular biology studies is another limitation of our study. Despite these limitations, our results clearly showed that melatonin had systemic preventive effects on IRI during the wound healing process in ileal anastomoses in rats. These findings are promising in terms of the potential use of this drug in the treatment of patients undergoing intestinal anastomoses, under conditions in which remote ischemia may be a concern.

Conclusion

Our study showed that melatonin, when given prior to ischemia, has protective effects on intestinal anastomotic healing in a rat model of SMA occlusion. We believe that the effects are mainly based on its lipophilic nature and broad spectrum antioxidant characteristics. Melatonin is both a direct free radical scavenger and an indirect antioxidant because of its ability to promote the activities of a variety of antioxidative enzymes. Melatonin can be a suitable agent for use as a free radical scavenger during surgical operations associated with significant IRI. We believe that further clinical studies are needed to reveal the effectiveness of melatonin as a therapeutic agent in the clinical setting in mesenteric IRI.

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Conflict of interest: None declared.

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DENEYSEL ÇALIŞMA - *ÖZET*

Melatonin iskemi/reperfüzyon hasarı sırasında oksidanlar ve anastomoz iyileşmesi üzerine olumlu etkiler gösterir

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AMAÇ: Melatoninin iskemi/reperfüzyon hasarında (İRH) intestinal anastomoz iyileşmesi üzerine etkisini araştırmayı amaçladık.
GEREÇ VE YÖNTEM: Otuz Wistar-Albino sıçan üç gruba (Sham, kontrol ve tedavi) ayrıldı. Süperior mezenterik arter (SMA) klemplenip 30 dakika sonrasında açılarak İRH oluşturuldu. Sham grubunda sadece SMA manüplasyonu yapıldı. Tedavi grubuna melatonin (10 mg/kg) ve kontrol grubunda salin verildi. Tedavi ve kontrol gruplarında ek olarak 1 cm ileal segment rezeke edilerek anostomoz yapıldı. Ameliyat sonrası yedinci günde anastomotik patlama basıncı, hidroksiprolin seviyeleri, yara iyileşmesinin histolojik göstergeleri ve oksidatif parametrelerin düzeyleri araştırıldı. İstatistik için tek yönlü varyans analizi ve ki kare testi kullanıldı.
BULGULAR: Melatonin tedavisi patlama basıncında anlamlı yüksekliğe yol açtı (sırasıyla, p=0.027 ve p<0.00). Katalaz ve süperoksit dismütaz enzim seviyeleri sham ve melatonin gruplarında en yüksek, kontrol grubunda ise en düşüktü (sırasıyla, p=0.001 ve p=0.002). Melatonin tedavisi her iki enzim düzeylerinde anlamlı yükseklige yol açtı (sırasıyla, p=0.026 ve p=0.003). Glutatyon peroksidaz ve total glutatyon düzeylerinde tedavi grubunda hafifçe artış görülse de bu fark anlamlı değildi (sırasıyla, p=0.205 ve 0.216). Fibroblast infiltrasyonu, kapiller formasyon ve epitelizasyon melatoninle tedavi edilen sıçanlarda anlamlı olarak daha iyiydi. Granülosit ve mononükleer infiltrasyon skorları tüm gruplarda benzerdi.
TARTIŞMA: Deneysel modelde İRH sırasında melatonin uygulamasının intestinal anastomoz iyileşmesi üzerine belirgin olumlu etkileri olduğunu düşünüyoruz.
Anahtar sözcükler: Anastomoz; iskemi/reperfüzyon hasarı; melatonin.

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