Outcomes of oxytocin treatment on intestinal ischemia-reperfusion injury in rats

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ABSTRACT

Ischemia-reperfusion injury is a clinical condition that poses life-threatening risks and can be caused by diseases or operations such as trauma, shock, and gastric dilatation volvulus. The objective of this study was to examine the effect of oxytocin on intestinal damage in rats induced by experimental ischemia-reperfusion injury. Three groups of Wistar albino rats were established: a control group (CTR, n=6), an intestinal ischemia-reperfusion group (I-IR, n=6), and an intestinal ischemia-reperfusion with oxytocin group (I-IR+Oxt, n=6). The I-IR+Oxt group received an intraperitoneal injection of 1 mg/kg oxytocin 30 minutes before anesthesia. In the I-IR and I-IR+Oxt groups, the superior mesenteric artery was ligated for 1 hour to induce ischemia-reperfusion injury, followed by one hour of reperfusion by opening the ligatures. At the end of the reperfusion period, the rats were euthanized, and blood and intestinal tissue samples were collected. From the blood samples, ALT, ALP, AST, LDH, BUN, creatinine, IL-1β, and TNF-α concentrations were evaluated. Tissue samples were analyzed for IL-1β, TNF-α, and MDA activity. Serum and tissue IL-1β and TNF-α concentrations were higher in both the I-IR and I-IR+Oxt groups compared to the CTR group. However, these levels were found to be lower in the I-IR+Oxt group compared to the I-IR group. The histopathological analysis showed that the I-IR+Oxt group had better epithelial regeneration and less inflammatory cell infiltration compared to the I-I/R group. In conclusion, oxytocin inhibited the release of IL-1β and TNF-α and the harmful effect of I/R on intestinal cells.

Introduction

Numerous diseases and operations can result in intestinal ischemia-reperfusion (I-I/R) injury, including neonatal necrotizing enterocolitis, mesenteric ischemia, embolism or volvulus, trauma, shock, aortic aneurysm repair, and rejection of intestinal transplantation in human medicine (13, 18). In veterinary medicine, ischemic reperfusion injury is observed in cases such as gastric dilatation-volvulus (GDV), mesenteric torsion, intestinal incarceration, and colic in the gastrointestinal system or abdominal compartment syndrome (11, 15, 17). Intestinal epithelial cells are susceptible to the effects of ischemia, and correcting the impact of ischemia is essential for restoring blood flow and preserving the structure of single-layer epithelial cells (8).

The nonapeptide neurohypophysial hormone oxytocin (Oxt), produced by the hypothalamic supraoptic and paraventricular nuclei, binds to the Oxt receptor. The effects of Oxt on various vascular beds can act as either vasoconstrictors or vasodilators, which stimulate uterine contractions during sexual activity and labor as well as myoepithelial contractions in the mammary gland during lactation (5). In experimentally induced duodenal and gastric ulcers, Oxt exerts an antiulcer effect primarily due to its moderate anti-secretory activity (2). Additionally, Oxt has been reported to protect the colon from acetic acid-induced and stress-induced colitis (7). And has also been shown that the release of Oxt decreases macrophage activity, tumor necrosis factor-alpha (TNF-α), and interleukin-1beta (IL-1β) levels (20). The rats, due to their

structural similarities, are often used in experimental-based modeling of diseases and metabolic or inflammatory reactions that may also occur in humans and other mammals. For this reason, we selected rats as our animal of choice for our study on I-I/R injury. Additionally, recently there have been numerous studies in both veterinary and human medicine to test different therapeutic strategies for preventing or treating ischemia-reperfusion injury. Therefore, in this study, it was investigated whether Oxt administration has an anti-inflammatory and protective effect against experimentally induced I-I/RI.

Materials and Methods

Experimental Protocol and Animals: The local animal ethics committee approved our study protocol (2021-130). Eighteen outbred male Wistar albino rats, weighing between 200 and 250 g, were used in this investigation. All rats were housed in a room with a regulated temperature (22±2°C), humidity (60%), and a 12-hour light/dark cycle. Rats had unrestricted access to water and food pellets. Rats were allocated into three groups: control group (CTR, n=6), intestinal ischemia-reperfusion group (I-I/R, n=6), and intestinal ischemia-reperfusion+ Oxytocin group (I-I/R+Oxt, n=6). In I-I/R+Oxt groups, 1 mg/kg of Oxt was given intraperitoneally 30 minutes before the anesthesia (9). All groups were fixed in the supine position after receiving intraperitoneal injections of a combination of Xylazine (2% Vetaxyl 20 mg, Vetagro ®) and Ketamine (2% Ketamine, Dutchfarm®, 100 mg/kg) to induce anesthesia. A ventral midline incision was used to access the abdominal cavity. The abdominal cavities of the rats in the CTR group were opened, but no interventions or applications were applied. By ligating the superior mesenteric artery for 1 hour, ischemia was established in the I-I/R and I-I/R+Oxt groups, and the small intestine color alterations were noticed. The intestines were reperfused for one hour by opening the ligature (21). The rats were euthanized using an overdose of Xylazine+Ketamine anesthesia at the end of the reperfusion periods. The small intestines were excised, and blood samples were collected from all groups.

Sampling: Serum was isolated following complete coagulation of blood samples taken from rats and placed into serum separator tubes. Fresh intestinal tissue samples were initially homogenized, and the homogenates were centrifuged at +4ºC 10000 g x 10 minutes, after which the supernatant was collected.

Blood analysis: Alanine aminotransferase (ALT, U/L), alkaline phosphatase (ALP, U/L), aspartate aminotransferase (AST, U/L), lactate dehydrogenase (LDH, U/L) enzyme activities, and blood urea nitrogen (BUN, mg/dL) and creatinine (Crea, mg/dL) levels were detected in sera using commercial clinical chemistry assay kits.

Rat-specific enzyme immunoassay kits were assayed to measure concentrations of IL-1β (pg/mL) and TNF-α (pg/mL) in sera and tissue homogenizates (Rat TNF-α ELISA Kit Catalog No: E-EL-R0019; Rat IL-1β Catalog No: E-EL-R001, Elabscience Biotechnology Inc., TX, USA). To assess lipid peroxidation levels, malondialdehyde (MDA) levels were evaluated in intestinal tissue homogenizates using commercially available test kits (TBARS Assay Kit, Item No. 10009055, Batch No. 0510196 and 0502129, Cayman Chemicals, Michigan, USA) (19). Tissue TNF- α, IL-1β, and MDA concentrations were detected performing Coomassie brilliant blue method (3).

Histopathological analysis: Intestine samples were fixed in a formaldehyde solution. Tissues were routinely treated with alcohol and toluene, fixed to paraffin blocks, and stained with hematoxylin-eosin. The light microscope's bright field mode (Zeiss-Axio Scope A1, Carl Zeiss, Gottingen, Germany) was used to examine histopathological sections. Each criterion was graded using a semi-quantitative system as 0: no, 1: mild, 2: moderate, and 3: severe.

Statistical analysis: GraphPad Prism 9 was used for the statistical analysis (GraphPad Software, San Diego, CA, USA). Means (±1 SD) were used to describe all results. The Shapiro-Wilk test was used to evaluate the statistically normal distribution of data. IL-1β and TNF-α levels in intestines and serum were compared using a one-way analysis of variance (ANOVA). Further analysis for binary comparisons was conducted using Tukey's test. P values less than 0.05 were considered significant.

Results

Serum ALT, AST, ALP, LDH, BUN, and Crea levels were examined in rats' experimentally developed I-I/R model to ascertain the effects of ischemia-reperfusion injury (Table 1). While there were significant increases in ALT, AST, ALP, and LDH activities, Crea, and BUN levels in the I-I/R group compared to the CTR group ($P<0.001$ and $P<0.01$). The increased values were less in the I-I/R+Oxt group compared to the I-I/R group.

In the blood samples, IL-1β and TNF-α levels as cytokines were evaluated in measuring the response to ischemia-reperfusion injury (Table 1). IL-1β ($P<0.01$) and TNF-α ($P<0.001$) levels were significantly higher in the I-I/R group compared to the CTR group. However, it was observed that IL-1β and TNF-α levels were significantly reduced in the I-I/R+Oxt group in comparison to the I-I/R group, with $P<0.05$ and $P<0.01$, respectively. Similarly, it

was determined that IL-1β and TNF-α levels obtained from tissue samples were significantly higher in the I-IR group compared to the CTR group, respectively P<0.0001 and P<0.5. Alternatively, IL-1β and TNF-α levels were found to be lower in the I-IR+Oxt group compared to the I-IR group, respectively P< 0.05 and P<0.0001 (Figure 1 a, 1b).

Lipid peroxidation and oxidative tissue damage were assessed in the tissue samples using MDA analysis (Figure 1c). Comparing the I-I/R group to the CTR group, MDA levels were much higher in the I-I/R group (P<0.01). However, it was observed that MDA levels decreased in the I-I/R+Oxt group compared to the I-I/R group (P<0.001).

According to histopathological examination and semi-quantitative scoring system, we observe that the CTR group had a regular epithelial and glandular structure, and almost no inflammatory cell infiltration (Figure 2a, Table 2). However, epithelial and glandular structures in the I-I/R group had a large number of inflammatory cells, and destructive morphological degeneration (Figure 2b, Table 2). Alternatively, in the I-I/R+Oxt group, it was observed that epithelial regeneration of both epithelium and glands structure and decreased inflammatory cell infiltration compared to the I-I/R group (Figure 2c, Table 2).

Table 1. Plasma ALP, ALT, AST, LDH, BUN, Creatinine, IL-1β and TNF-α activities in the control (CTR), intestinal ischemia/reperfusion (I-I/R) and intestinal ischemia/reperfusion +Oxytocin (I-I/R+Oxt) groups.

Parameters (n=6)	CTR	I-I/R	I-I/R+Oxt
ALP (U/L)	66.6 ± 12.4	143.3 ± 4.2 ****	100.4 ± 9.9 $^{+}$
ALT (U/L)	40.1 ± 5.79	1375 ± 281 ****	105.1 ± 20.9 $^{+++}$
AST (U/L)	99.1 ± 14.1	2692 ± 542 ****	192.7 ± 24.9 $^{++++}$
LDH (U/L)	1532 ± 223	7131 ± 1021 ****	1832 ± 157 $^{++++}$
BUN (mg/dL)	34.1 ± 3.5	81.5 ± 5.1 ****	58.4 ± 5.5 $^{**, ++}$
Creatinine (mg/dL)	0.47 ± 0.05	0.95 ± 0.06 ***	0.54 ± 0.09 $^{++}$
IL-1β (pg/mL)	391.3 ± 26.8	830.9 ± 30.2****	679.8 ± 21$^{****,++}$
TNF-α (pg/mL)	260.6 ± 30.9	553.4 ± 40.5****	371 ± 37.6^{++}

** P<0.01; *** P<0.001, **** P<0.0001 compared with the control (CTR) group. + P < 0.05, ++ P<0.01, +++ P<0.001, ++++ P<0.0001 compared with the I-I/R group.

Table 2. Histopathological scoring results of control (CTR), intestinal ischemia/reperfusion (I-I/R), and intestinal ischemia/reperfusion +Oxytocin (I-I/R+Oxt) groups.

Parameters (n=6)	CTR	I-I/R	I-I/R+ Oxt
Desquamation of villus tip	0.43 ± 0.07	2.76 ± 0.07****	1.63 ± 0.03$^{****,++++}$
Hyperplasia of intestinal glands	0.36 ± 0.06	2.48 ± 0.09****	1.55 ± 0.07^{++++}
Inflammatory cell infiltration	0.48 ± 0.04	2.53 ± 0.07****	1.67 ± 0.07^{++++}

Each of the criteria was scored semi-quantitatively as 0: none, 1: mild, 2: moderate, 3: severe. **** P<0.0001 compared with the control (CTR) group. ++++ P<0.0001 compared with the I-I/R group.

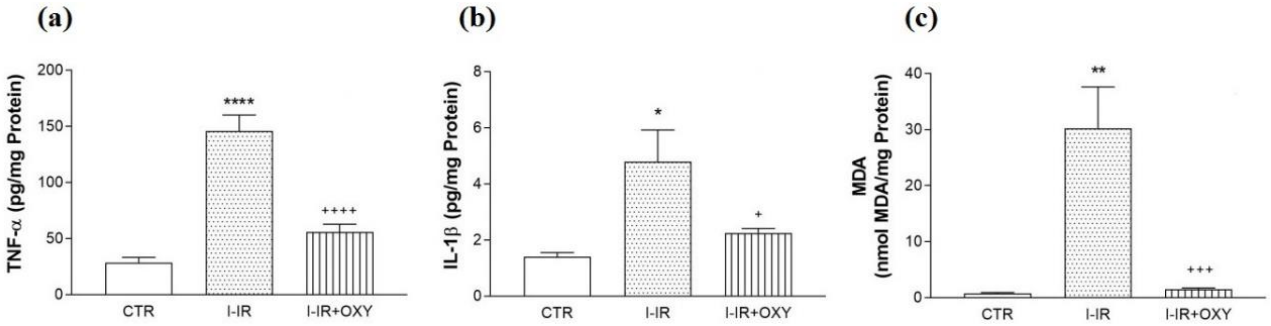

Figure 1. Intestinal tissue TNF-α (A), IL-1β (B), and MDA (C) activities in the control (CTR), intestinal ischemia/reperfusion (I-I/R), and intestinal ischemia/reperfusion +Oxytocin (I-I/R+Oxt) groups.*P<0.05, ** P<0.01; *** P<0.001, **** P<0.0001 compared with the control (CTR) group.+ P< 0.05, ++ P<0.01, +++ P<0.001, ++++ P<0.0001 compared with the I-I/R group.

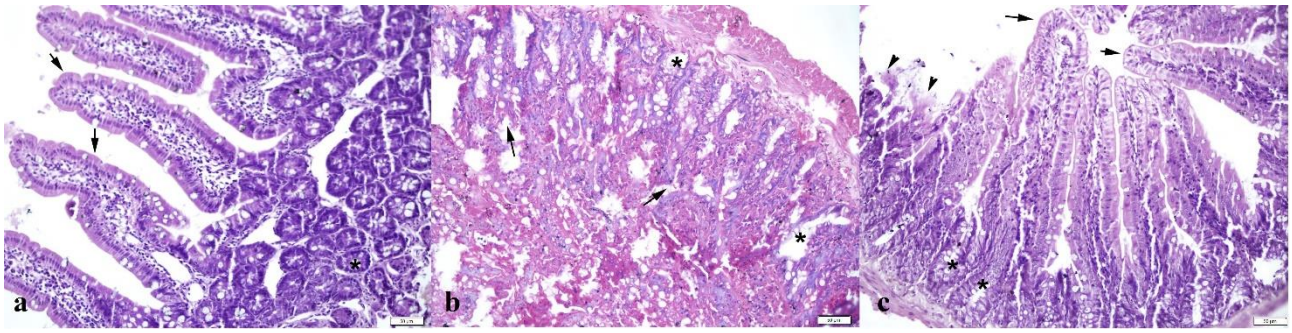

Figure 2. (a) Regular layout of epithelium (arrows) and gland (*) in the control group, (b) Severe desquamation of epithelium (arrows) and degeneration of glands (*) note the congestion of interstitium in the intestinal ischemia/reperfusion group, (c) Regeneration of epithelium (arrows) and renewal of intestinal glands (*) in the intestinal ischemia/reperfusion +Oxytocin group, inset (H&Ex200, 50μm).

Discussion and Conclusion

The primary function of the intestines is the digestion and absorption of nutrients, the removal of waste products, elimination of bacterial formations and toxins. One of the most important structures that enable it to perform these functions is the mucosal barrier of the intestine. After I-I/RI, the integrity of the mucosal barrier is impaired, and bacterial toxins pass into the systemic circulation, causing the release of cytokines with the inflammatory response that develops in the intestinal mucosa. At the same time, tissue damage occurs due to toxins, bacteria, and released cytokines in the systemic circulation (18). To defend against the adverse effects of ischemia-reperfusion injury or to reduce or prevent the production of components that contribute to the damage, numerous strategies are being developed (6, 27, 30). We investigated the effects of Oxt, an effective agent in ischemia-reperfusion injury, on important intestinal pro-inflammatory cytokines IL-1β and TNF-α, and thus its preservation impact on intestinal tissues in the experimentally induced I-I/R injury model in rats.

I-I/R injury is a complex pathophysiological cascade that includes the destruction of intestinal epithelial cells and enterocytes with high oxygen activity due to oxygen deficiency, causing the release of IL-1 and TNF-α, stimulation of inflammatory cells and the disruption of the mucosal barrier, leading to bacterial endotoxins entering systemic circulation (12). IL-1β and TNF-α, produced in the intestinal mucosal inflammatory cells and mediate the aforementioned metabolic changes, which cause local cell damage and trigger systemic inflammation in the early inflammatory response after I-I/R (1, 14). In this study, IL-1β and TNF-α values were found to be higher in I-I/R ($P<0.001$ and $P<0.01$) and I-I/R+Oxt ($P<0.01$ and $P<0.05$) groups, who underwent 1-hour ischemia and 1-hour reperfusion, compared to the CTR group. Histopathologically, villous desquamation, glandular and epithelial damage, and infiltration of the inflammatory cell scores were higher in the I-I/R and I-I/R+Oxt groups in comparison to the CTR group. Conversely, both histopathological findings and biochemical values were lower in the I-I/R+Oxt group compared to the I-I/R group. According to these results, a one-hour ischemia-reperfusion injury caused an increase in IL-1β and TNF-α levels, destructive morphological changes, and inflammatory cell infiltration of the intestine. In contrast, the low values in the Oxt administered group indicated that Oxt protected the intestinal mucosa through the reduction of inflammatory cell infiltration and suppressing pro-inflammatory cytokines IL-1β and TNF-α.

I-I/R injury not only causes anatomical deformity and local inflammatory response in the intestine (1). Alterations in ALP, ALT, AST, LDH, BUN, and Crea levels are used to determine the degree of tissue damage (4, 28). However, the effects of Oxt on both kidney damage in hepatic ischemia-reperfusion injury and hepatic damage in kidney ischemia-reperfusion injury have previously been investigated. In these studies, a significant decrease in ALT, AST, LDH, BUN, and Crea levels were found using Oxt. Similarly, in the same studies, pro-inflammatory cytokine values were established to be low Oxt treated groups (10, 23, 29). In this study, ALP, ALT, AST, LDH, BUN, and Crea levels were also measured to determine tissue damage. While ALP, ALT, AST, LDH, BUN, and Crea enzyme activities were significantly increased in both I-I/R and I-I/R+Oxt groups compared to the CTR group, it was determined that Oxt administration caused a remarkable reduction in these values. Studies indicate that these parameters, which are frequently used in the determination of clinical ischemia-reperfusion damage, are more specific in the more advanced stages of the disease, and it has been stated that the increase in serum values is due to the inflammatory response (16, 22). An I-I/R injury study conducted by Alexandropoulos et al. (1) concluded that the levels of pro-inflammatory cytokines increase in the ischemia-reperfusion and cause damage to the tissue (1). In this study, IL-1β and TNF-α levels addressed that the damage in the intestine is due to

the formation of cytokines and concur with the findings of the mentioned studies. Therefore, we have shown that Oxt administration reduces the effects of ischemia-reperfusion injury by decreasing enzyme activity and reducing cytokine levels, which are both organ damage markers.

Any tissue damage, such as ischemia-reperfusion, is detected by macrophages and monocytes, causing the release of cytokines such as IL-1β and TNF-α. Cytokines activate inflammatory cells that trigger the peroxidation of membrane lipids. Lipid peroxidation affects the permeability of cell membranes, which ultimately results in cell lysis (26). MDA, a marker of lipid peroxidation, is widely used because of its sensitivity and reliability. Previous studies have established that MDA is a good marker of oxidative damage to lipids (24, 25). MDA, IL-1β, and TNF-α results obtained from intestinal tissue in the study show that the values of the I-IR+Oxt group are lower than the I-IR group. Both cytokine and MDA levels obtained in blood or tissue samples and histopathological examinations show that oxytocin is a protective agent against cytokines and reactive oxygen radicals that cause destructive effects in ischemia-reperfusion injury of the intestinal tissue.

In conclusion, local and systemic inflammatory responses that develop with I-I/R injury depend on the early diagnosis of the developing problem together with the success of the medical and/or operative treatment used to alleviate or eliminate the problem. A method that can be used as a medical treatment together with early diagnosis is Oxt. The efficacy of Oxt has previously been demonstrated in models of liver and kidney ischemia-reperfusion injury or stress colitis and colonic burn, but its efficacy has yet to be directly investigated with the experimentally established intestinal ischemia-reperfusion model. In this sense, our study was an initial design to establish the effect of Oxt on pro-inflammatory cytokines in I-I/R injury and its protective or preventive effect on damage after ischemia-perfusion. However, all aspects of biochemical pathways need to be investigated to clarify the full impact of Oxt in I-I/R injury, and future studies should be focused on this direction.

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Ethical Statement

The local animal ethics committee approved the study protocol (2021-130).

Conflict of Interest

The authors declare that there is no conflict of interest.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Author Contributions

ÇG, AÖŞ, and SS provided study conception and design. ÇG and AÖŞ performed experiments. SS, ŞÇ, and AÖŞ analyzed data. ÇG, AÖŞ, and SS interpreted the results and wrote the manuscript.

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