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Effect of tramadol hydrochloride on ischemic colon anastomosis healing

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ABSTRACT

Aims: This study aimed to investigate the possible effects of tramadol hydrochloride administration on the healing of colonic anastomosis after colonic ischemia-reperfusion injury in rats.

Methods: Twenty-four Wistar albino male rats were divided into the control group (rats underwent resection-anastomosis after ischemia-reperfusion injury in the colon and saline was given intraperitoneally, n=12); and the TRA group (rats underwent resection-anastomosis after ischemia-reperfusion injury in the colon and tramadol hydrochloride was given, n=12). After colon ischemia-reperfusion injury was produced, the distal part of the descending colon was cut to full thickness, and the lumen was completely closed one by one with 6/0 polypropylene sutures. Then, 0.5 ml saline was administered to the control group and 30 mg/kg tramadol hydrochloride was administered to the TRA group intraperitoneally every day for five days. After sacrificing all the rats, the burst pressures were recorded, and blood serum and colon tissue samples were analyzed biochemically.

Results: Biochemical analysis of blood serum samples revealed that IL-6 (p=0.026), MPO (p=0.001), CAT (p=0.024), and AST (serum p=0.002) levels were different between the two groups. Biochemical analysis of the tissue samples revealed that glutathione reductase (p=0.006), AST (p<0.001) and ALT (p=0.004) levels were different between the two groups.

Conclusion: At the end of this study, it was thought that tramadol usage as an analgesic drug may not have a negative or positive therapeutic effect on colon ischemia-reperfusion injury and colon anastomosis in rats. Therefore, it was thought that tramadol hydrochloride could be used as an analgesic drug option in pain management in such patients.

Keywords: Colon anastomosis, hypoxia-reperfusion injury, tramadol

INTRODUCTION

The colon is the part of the gastrointestinal tract most prone to ischemia. Colonic ischemia is more common in elderly patients due to atherosclerotic events and some other comorbid conditions. Therefore, colon resection and anastomosis have been performed more frequently in the same age group[1] Ischemic colitis may progress to transmural necrosis and develop into gangrenous colitis requiring surgical intervention by disruption of colonic blood flow due to occlusive or nonocclusive causes of mesenteric blood flow. Reactive oxygen radicals can cause reperfusion injury leading to lipid peroxidation and cell necrosis in case of reoxygenation after developing mucosal damage.[2] Although the safety of colorectal resection and anastomosis surgery has improved with advances in surgical technique, antibiotic prophylaxis, preoperative preparation, and postoperative period, major morbidity rates still range between 20-35% and 30-day mortality rates between 2-9%.[3] The most important complication in colorectal surgery is anastomotic leakage (5-15%) with a high mortality rate (10-32%).[4,5]

Agents that stimulate tissue healing or reduce inflammation are much more needed to prevent adverse anastomotic leakage, especially in tissues damaged by ischemia-reperfusion.[6] Studies are investigating the effect of drugs used for postoperative analgesia in patients undergoing segmentary colon resection and anastomosis under emergency or elective conditions. However, the effect of tramadol hydrochloride, which is considered a weak opioid analgesic causing serotonin and noradrenaline reuptake inhibition and used to treat moderate to severe pain in the postoperative period, is unknown on anastomotic healing.[6-8]

This study aimed to investigate the possible effects of tramadol hydrochloride administration on the healing of colonic anastomosis after colonic ischemia-reperfusion injury in rats.

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METHODS

Ethics

The study was conducted after the approval of the Kırıkkale University Animal Experiments Local Ethics Committee (Date: 24.12.2021, Decision No: 54). All procedures were carried out following the ethical rules and principles.

In this study, 24 Wistar albino male rats weighing 220-280 g were used. Rats were divided into groups by simple random sampling method as follows;

- Control group (resection-anastomosis was performed after ischemia-reperfusion injury was induced in the colon and saline was given intraperitoneally, n=12)
- TRA group (resection-anastomosis was performed after ischemia-reperfusion injury was induced in the colon and tramadol hydrochloride was given intraperitoneally, n=12)

Surgery

The same surgeon performed all surgical procedures. All rats were anesthetized intramuscularly with 50 mg/kg ketamine hydrochloride (Ketalar®, Pfizer). After a median abdominal incision of approximately 3 cm, the vascular structures, including the vessels and collaterals supplying all colon, were clamped with an atraumatic bulldog clamp. Blood flow was interrupted for 45 minutes and ischemia was induced in the entire colon. At the end of the time, the clamp was opened, blood flow was observed again in the colon, and reperfusion was waited for 45 minutes.[9,10] Following reperfusion, the distal part of the descending colon was cut to full thickness. The lumen was completely closed one by one with 6/0 polypropylene sutures (Figure 1). After the abdominal incision was sutured continuously, the rats were taken to their cages after the dressing procedure and the follow-up was started.

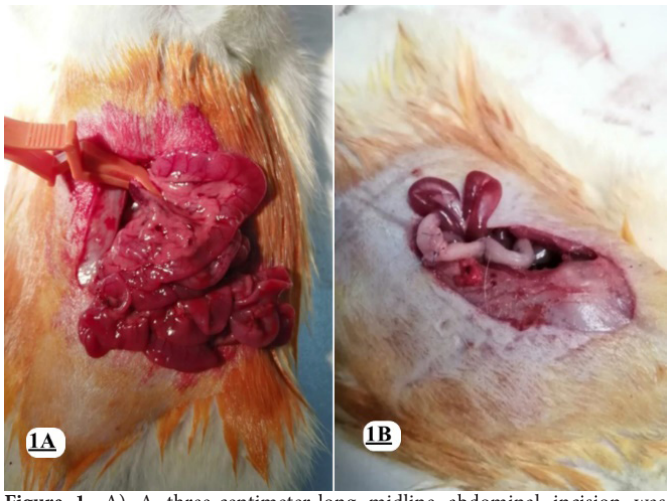

Figure 1. A) A three-centimeter-long midline abdominal incision was performed, and the vascular trunk of the colon was clamped with bulldog clamps to provide ischemia in the colon for 45 minutes. B) After the clamps were removed and reperfusion was allowed for 45 minutes, the descending colon was transected using a scalpel blade, and a single-layer colonic anastomosis was performed with 8 sutures using 6-0 polypropylene

In the postoperative period of the rats, 0.5 ml saline was administered intraperitoneally to the control group and 30 mg/kg tramadol hydrochloride was administered intraperitoneally to the TRA group every day for five days. On the sixth postoperative day, all rats were anesthetized with 50 mg/kg ketamine hydrochloride intramuscularly. The colonic anastomosis line was resected 3 cm each proximally and distally by entering the abdomen through the previously created median incision. After 8-10 ml blood sample was obtained from each rat vena cava, all rats were sacrificed by intracardiac blood puncture. The blood samples were centrifuged at 3000 rpm for 10 min and the serum samples were stored at -80°C.

Anastomotic Burst Pressure Measurement

The lumen of the resected intestinal segments of all rats was cleaned from fecal content with warm saline. Saline was infused into the lumen with an infusion pump (Braun® Infusomat Space) at a constant rate. A manometer connected to the infusion pump with a three-way cannula was used to measure the pressure as the pressure increased in the lumen. The pressure increase was monitored on the manometer monitor and the burst pressure value at the burst time was noted for each intestinal segment (Figure 2). After the burst pressures were recorded, the anastomotic line was stored at -80°C for biochemical analyses.

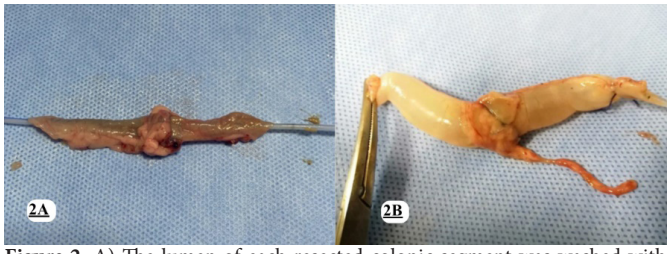

Figure 2. A) The lumen of each resected colonic segment was washed with saline for fecal removal. B) The system pressure was increased via saline infusion in a controlled manner, and the bursting pressure was measured when air bubbles were seen in the water-filled container

Biochemical Evaluation

Biochemical tests performed on tissue samples were aimed to evaluate wound healing, cross-linking of collagen fibrils, and endothelial damage. Tissues stored at -80° C were allowed to reach room temperature and homogenized with phosphate-buffered saline. Then, the homogenized tissues were centrifuged, and the supernatants were obtained. Then these supernatants were subjected to the ELISA kits' methods and tissue levels of hydroxyproline (Catalog No: E0511Ra, BT-Lab), myeloperoxidase (MPO) (Catalog No: Otto3048, Ottoscientific), malondialdehyde (MDA) (Catalog No: Otto1001, Ottoscientific) and caspase-3 (Catalog No: E1648Ra, BT-Lab) were measured using a spectrophotometer (Thermo Scientific Multiskan FC, 2011-06, USA).

On the other hand, frozen blood serum samples were also thawed at room temperature. Then, those serum samples were centrifuged, and the supernatants were obtained. Then, these supernatants were subjected to the ELISA kits' methods and serum tumor necrosis factor-α (TNF-α) (Catalog No: E0764Ra, BT-Lab), interleukin-6 (IL-6) (Catalog No: E0135Ra, BT-Lab), catalase (CAT) (Catalog No: E0869Ra, BT-Lab), glutathione reductase (Catalog No: E1085Ra, BT-Lab), superoxide dismutase (SOD) (Catalog No: RLD0123, REL ASSAY), and nitric oxide (NO) (Catalog No: E-BC-K035-S, Elabscience) were measured using a spectrophotometer (Thermo Scientific Multiskan FC, 2011-06, USA). Furthermore, serum aspartate aminotransferase (AST) (Catalog No: OttoBC127, Ottoscientific), and alanine aminotransferase (ALT) (Catalog No: OttoBC128, Ottoscientific) levels were measured using a fully automatic biochemistry device (Mindray brand BS300).

Statistical Analysis

Shapiro-Wilk test was performed to test the normal distribution of the study data. The parametric data was evaluated using the independent samples T test ($p<0.05$). The nonparametric data was analyzed using the Mann-Whitney U test ($p<0.05$).

RESULTS

The mean anastomotic burst pressure was measured at 130.4 (96-170) mmHg in the control group, and 106.6 (80-130) mmHg in the TRA group. However, anastomotic burst pressure values were not different between the groups ($p=0.183$) (Table).

Tissue hydroxyproline, MPO, MDA, and caspase-3 level values were not different between the groups ($p>0.05$). On the other hand, serum IL-6 ($p=0.026$), CAT ($p=0.024$), and AST ($p=0.002$) levels were different between the groups (Table).

DISCUSSION

Oxygenation is the most important factor in collagen synthesis produced by fibroblasts.[11,12] Studies showed that hypoxia occurring in the tissue after intestinal anastomosis adversely affects collagen synthesis and that the wound site heals faster in hyperoxia-treated tissue.[13] Increased reactive oxygen radicals in the damaged tissue after ischemia-reperfusion disrupt cellular functions, delay wound healing, and cause tissue necrosis, leading to anastomotic leaks. Prevention of anastomotic leaks can be aimed at preventing the generation of free oxygen radicals and accelerating wound healing.[14] In addition to hydroxyproline level, an important indicator of wound healing at the tissue level, collagen quality, and crosslinking properties have also been reported to be important in anastomotic wound healing.[14] In this study, burst pressure, a more reliable method comparing the rupture and tension method, was used to determine the wound healing of colon anastomosis. However, at the end of the study, it was found that tramadol hydrochloride administration did not affect burst pressure. In addition, hydroxyproline level values in tissue samples were similar between the two groups in our study. With these findings, it was thought that tramadol hydrochloride administration did not impair wound healing in terms of mechanical support in the tissue.

Activation of the cytokine network (such as IL-6 and TNF-α) has also been shown in intestinal ischemia-reperfusion injury models. In animal experiments, activated cytokines have also been reported to inhibit wound healing. Especially TNF-α stimulates the macrophages to synthesize IL-1, which inhibits wound healing. In addition, MPO can indicate the severity of inflammation and neutrophil migration.[15,16] In our study, MPO values measured at the colonic tissue were not different between the two groups. Furthermore, serum TNF-α level values were similar between the groups. However, serum IL-6 level values measured in the TRA group were lower than in the control group. With these findings, it was thought that tramadol hydrochloride could have a slightly anti-inflammatory effect on colon hypoxia-reperfusion injury in rats.

Catalase is the enzyme that converts hydrogen peroxide synthesized by peroxisomes into water and oxygen. Glutathione peroxidase and glutathione reductase also contribute to the degradation of lower concentrations of hydrogen peroxide to water and oxygen by establishing a GSH/GSSG balance. In addition, glutathione reductase has known functions such as neutralizing free radicals and reactive oxygen products, protecting membrane proteins, and detoxifying some metabolic end products by conjugation. SOD enzyme provides the reduction of superoxide radical to hydrogen peroxide. On the other hand, malondialdehyde level is considered a marker of oxidative stress and lipid peroxidation.[17-19]

In our study, tissue malondialdehyde level values were similar between the two groups. In addition, serum glutathione reductase, SOD, and NO level values were not different between the two groups. However, serum CAT level values were lower in the TRA group compared to the control group. With these findings, it was argued that the antioxidant activity of tramadol hydrochloride in colon tissue and blood serum was almost negligible.

Intestinal ischemia-reperfusion causes severe liver damage in both humans and animals. Studies have reported that tramadol decreases apoptosis in the liver and alleviates hepatic damage in liver ischemia-reperfusion injury.[20] In our study, serum AST level values, a hepatic damage indicator

Table. Comparison table of biochemical analysis of tissue and blood serum samples

		Control	TRA	
	Variable	Mean±SD/median (min-max)	Mean±SD/median (min-max)	p
	Burst pressure (mmHg)	130.4 (96-170)	106.6 (80-130)	0.183†
Tissue	Hydroxyproline	319.27 (153.34-417.27)	245.33 (89.25-1019.31)	0.242†
	Myeloperoxidase	44.25 (17.20-242.20)	30.55 (15.20-70.30)	0.178†
	Malondialdehyde	10.35 (3.28-46.19)	11.26 (2.75-32.13)	0.671†
	Caspase-3	0.32±0.14	0.29±0.06	0.539*
Serum	Tumor necrosis factor-α	18.56±2.84	18.06±2.83	0.669*
	Interleukin-6	5.40±1.28	4.33±0.89	0.026*
	Catalase	30.75 (8.90-50.17)	14.10 (9.68-37.16)	0.024†
	Glutathione reductase	5.22±1.18	4.43±1.37	0.148*
	Superoxide dismutase	387.17±108.04	404.83±72.23	0.642*
	Nitric oxide	11.95±2.60	11.98±1.80	0.975*
	Aspartate transaminase	104.25 (76.60-189.70)	139.30 (109.3-273)	0.002†
	Alanine transaminase	41.35 (28.70-58.10)	38.75 (25.90-108.40)	0.887†

*Independent samples T test, †Mann-Whitney U test, $p<0.05$, SD: Standard deviation, Min: Minimum, Max: Maximum

parameter in blood serum samples, were higher in the TRA group compared to the control group. However, serum ALT level values were similar between the two groups. With this finding, it was thought that AST values increased more in the TRA group than in the control group after colon ischemia-reperfusion injury. This increase was thought to be due to systemically administered tramadol hydrochloride.

On the other hand, caspase-3 is one of the effector caspases and is known to play a role in the apoptosis mechanism.[17] In our study, tissue caspase-3 level values were similar between the two groups. Thus, it was thought this experimental agent could not have an antiapoptotic effect on colon hypoxia-reperfusion injury in rats.

In conclusion, it was thought that this agent had no effect on collagen synthesis and had no anti-inflammatory, antioxidant, or antiapoptotic effect in colon ischemia-reperfusion injury in rats. In addition, the fact that the obtained burst pressure values were not found to be different between the two groups supported these thoughts. Thus, these findings thought that the use of tramadol as an analgesic agent did not create a negative or positive therapeutic effect in colon ischemia-reperfusion injury in rats. Therefore, it could be said that tramadol hydrochloride could be used as an analgesic drug option in pain management in such patients clinically.

Limitations

The study had some limitations. First, this study included the findings obtained at the end of 5 days of colon anastomosis following colon ischemia-reperfusion injury. Therefore, data regarding the chronic process were not included in this study. Second, the effects of tramadol hydrochloride were not compared to the other pharmacological agents in this study. Finally, histopathological (such as immunohistochemistry, electron microscopy) and biochemical (such as western blot) analysis methods that could reveal the anti-inflammatory, antioxidant, and possible anti-apoptotic mechanisms of action of tramadol hydrochloride in detail were not included in this study due to technical and/or financial limitations.

CONCLUSION

This study's results showed that tramadol hydrochloride usage as an analgesic drug may not have a negative or positive therapeutic effect on colon ischemia-reperfusion injury and colon anastomosis in rats. Therefore, it was thought that tramadol hydrochloride could be used as an analgesic drug option in pain management in such patients clinically.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of the the Kırıkkale University Animal Experiments Local Ethics Committee (Date: 24.12.2021, Decision No: 54).

Informed Consent

Since experimental animals were used in this study, a written consent form was not required.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

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Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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