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The effect of colchicine and low-dose methotrexate on intestinal ischemia/reperfusion injury in an experimental model

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ABSTRACT

Aim: Intestinal ischemia/reperfusion (I/R) injury is a serious clinical condition. Colchicine and low-dose methotrexate have anti-inflammatory features. An experimental model was conducted to investigate the effect of colchicine and methotrexate on intestinal I/R injury.

Methods: Twenty-four rats were included. Only laparotomy was done in control group (CG, n = 6). In experimental groups, superior mesenteric artery was occluded. After 1 h ischemia, reperfusion (1 h) was started by de-occlusion. 30 min before reperfusion, saline in sham group (SG, n:6), colchicine (1 mg/kg) in colchicine group (CNG, n:6), and methotrexate (0.1 mg/kg) in methotrexate group (MTXG, n:6) were infused intraperitoneally. Small intestines were harvested for evaluation of intestinal mucosal injury (Chiu score) and oxidative stress markers (nitric oxide: NO, malondialdehyde: MDA, superoxide dismutase: SOD).

Results: Biochemically, MDA levels were significantly low in CG compared to SG, CNG, and MTXG ($p < 0.05$). NO levels were significantly low and SOD levels were significantly high in CG compared to MTXG ($p < 0.05$). Histopathologically, Chiu score was significantly low in CG compared to SG, CNG, and MTXG ($p < 0.05$), and significantly high in MTXG compared to SG and CNG ($p < 0.05$).

Conclusion: The present experimental model caused I/R injury in rat intestines. Contrary to literature, it was found that methotrexate worsens and colchicine does not attenuate intestinal I/R injury.

Ischemic injury of intestines is a medical and surgical emergency with high mortality rate [1,2]. This serious clinical condition can be a consequence of a mesenteric emboli, midgut volvulus, invagination, necrotizing enterocolitis or small bowel transplantation [1,2].

It causes both structural and functional damage associating with severe intestinal cellular injury. During this process, inflammatory factors and cytotoxic substances are released in intestinal tissue [2–4]. The return of oxygenated blood to the ischemic tissue worsens the injury by release of free oxygen radicals and aggravated inflammatory reactions [2,3]. Therefore, resuscitation of this fatal inflammatory reaction is as important as restoration of the blood flow.

Colchicine (CN) is an anti-inflammatory agent which regulates cytokine production, decreases the tumor necrosis factor-α (TNF-α), and inhibits leukocyte functions [5]. It has been used for several diseases such as gout, familial Mediterranean fever, and Behcet's disease [5]. Recently, it was reported that it has beneficial effects in necrotizing enterocolitis [5]. However, its effect on intestinal ischemia reperfusion (I/R) injury has not been studied previously.

Methotrexate (MTX), a folic acid antagonist, inhibits DNA and RNA synthesis by inhibiting dihydrofolate reductase enzyme [6]. It has been used in treatment of several neoplastic diseases in high doses. However, in low doses, it has anti-inflammatory effect which allows it to be used in the treatment of some inflammatory diseases such as rheumatoid arthritis, psoriasis, and Crohn's disease [6]. But, its effects on intestinal I/R injury have not been reported so far.

Therefore, we conducted an experimental intestinal I/R model to investigate the effect of CN and MTX on intestinal I/R injury.

1. Materials and methods

The study was approved by the Local Ethical Committee and the experiments were performed under the recommendations of the laboratory animal care committee.

Twenty-four Wistar albino adult rats, weighing 250 ± 50 g were included to the study. The rats were kept in standard cages in 22 °C room temperature and 12 h day/night cycle with tap water and standard food ad libitum.

The rats were randomly divided into 4 groups including six animals in each. In CG, after anesthetization, 2 cm of small intestine 15 cm proximal to ileceacal valve was sampled without any intervention except laparotomy (CG, n: 6). Intestinal ischemia and reperfusion (I/R) were performed in other groups by occluding and de-occluding the superior mesenteric artery (SMA) similar to the way defined by Dundar et al. [7]. Before de-occluding SMA, saline in Sham group (SG, n: 6), colchicine (CN) in colchicine group (CNG, n: 6), and

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methotrexate (MTX) in MTX group (MTXG, n: 6) were injected intraperitoneally.

1.1. Experimental protocol

The rats were anesthetized with intraperitoneal ketamine hydrochloride (50 mg/kg, Ketalar, Eczacıbası, Istanbul, Turkey). The rats were kept warm and allowed spontaneous breathing during surgery. The abdominal wall skin was shaved and cleaned with 10% povidone iodine. Then, a midline laparotomy was performed and SMA was exposed (Fig. 1). In experimental groups, SMA was occluded with 0/0 catgut suture. The ischemia was accepted to be achieved when the small intestines became purple in color and the pulse of intestinal arteries stopped. The drugs were not like crystalloid liquid and so were given intraperitoneally to achieve a systemic response without causing venous emboli. Since we planned to give the drugs intraperitoneally before reperfusion, we closed the abdominal wall after occlusion of SMA.

After 1 h of ischemia, the catgut nodes were opened carefully to de-occlude SMA and reperfusion period began. The reperfusion period lasted for 1 h in all experimental group animals. Thirty minutes before reperfusion period, intraperitoneal injections of 1 ml serum physiologic in SG, 1 mg/kg colchicine (Colchicum-Dispert, Dr.F.Frik, Istanbul, Turkey) in CNG, and 0.1 mg/kg MTX (Methorexate, Kocak Farma, Istanbul, Turkey) in MTXG were infused into the peritoneal cavity.

At the end of each procedure, the rats were sacrificed by exsanguination. Small intestinal samples (2 cm of small intestine from 15 cm proximal to ileoceacal valve) were harvested in all groups for histopathological and biochemical examinations.

1.2. Biochemical examination

All samples were kept at −80 °C. Tissue was homogenized (Labor Technique, Müllheim, Germany) with 0.9% NaCl solution 1 mL in ice, and then it was centrifuged at 1500 *g* for 10 min at 4 °C. Supernatants were used for malonyl dialdehyde (MDA), total nitrite/nitrate (NO), superoxide dismutase (SOD) and protein determinations. Protein level was measured using the method of Lowry et al. [8].

1.3. Determination of NO

NO levels were measured by a spectrophotometric method as described by Miranda et al. [9]. Nitrate was reduced to nitrite with vanadium (III). The nitrite level was measured by using Griess reagents which reflect the total amount of nitrate and nitrite in the sample. Standards were accepted as serial dilutions of Na nitrate (Merck, Germany). The results were expressed in μM/mg protein.

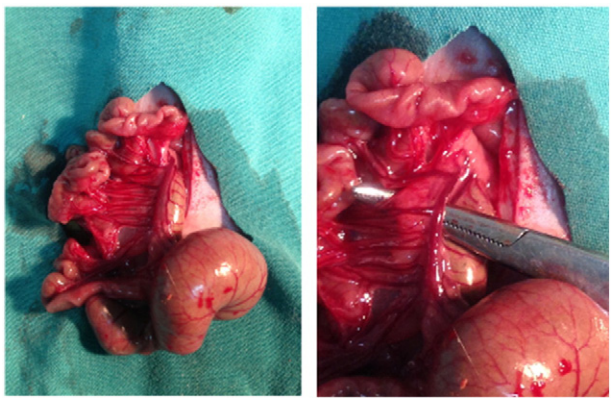

Fig. 1. The experimental model. Left: Small intestines and Right: Superior mesenteric artery (SMA) being exposed.

Table 1
Histopathologic grades of intestinal tissue (Chiu scoring system [11]).

Grade	Histopathologic finding
0	Normal mucosal villi
I	Development of a subepithelial space, usually at the tip of the villus, with capillary congestion
II	Extension of the subepithelial space with moderate lifting of the epithelial layer
III	Massive epithelial lifting down the sides of villi
IV	Denuded villi with lamina propria, dilated capillaries exposed, increased cellularity of the lamina propria
V	Digestion and disintegration of the lamina propria, hemorrhage and ulceration

1.4. Determination of MDA

MDA levels indicate lipid peroxidation and were measured by the method described by Armstrong and Al-Awadi [10]. The calibration curve was prepared with 1, 1, 3, 3-tetraethoxypropane (Sigma, St Louis, MO) standards of 1- to 25-nmol/L dilutions. The results were expressed in nM/mg protein.

1.5. Determination of SOD

SOD levels were determined by using quantitative enzyme linked immunoabsorbent assay kit which is commercially available (Cayman Chemical Company). The results were expressed in U/mg protein.

1.6. Histopathological examination

Intestinal samples were fixed with 10% formalin and embedded in paraffin. Tissues were sectioned in 4–5 μm pieces. Then they were stained with routine hematoxylin and eosin stain. The specimens were examined under a light microscope (Lecia, Germany) by the same pathologist who was blind to the study. Histopathologic findings were graded according to Chiu scoring system (intestinal mucosal injury score) [11] (Table 1).

1.7. Statistical analyses

Results were analyzed with Statistical Package for the Social Science version 15.0 (SPSS 15.0). All data were expressed as median with inter-quartile ranges. The difference between two groups was evaluated with Kruskal Wallis test. The p values lower than 0.05 were considered as significant.

2. Results

The results of biochemical and histopathological examinations are given in Table 2. The median levels of NO were significantly low in control group animals (CG) compared to MTX treated ones (MTXG) ($p < 0.05$). The median levels of MDA were significantly low in CG compared to SG, CNG, and MTXG ($p < 0.05$). The median levels of SOD, indicator of the anti-oxidant activity, were significantly high in CG compared to MTXG ($p < 0.05$). These results suggest that the present intestinal I/R model caused oxidative damage in intestinal tissue, but not more than the one caused by MTX (Fig. 2).

The harvested intestines were fragile, edematous and discolored in experimental groups macroscopically. The samples were examined histopathologically for intestinal injury and graded with Chiu score (Table 2). The Chiu scores were consistent with the macroscopic examination. The scores were significantly low in CG compared to SG, CNG, and MTXG ($p < 0.05$). The Chiu scores of MTXG were significantly higher than those of SG and CNG ($p < 0.05$). The microscopic images of intestines from each group are given in Fig. 3.