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EXPERIMENTAL STUDY

The preventive effects of atorvastatin and N-acetyl cysteine in experimentally induced ischemia-reperfusion injury in rats

Yasar M[1], Erdi I[1], Kaya B[2]

Duzce University Medical Faculty-Department of General Surgery Duzce, Turkey. **myasar59@gmail.com**

ABSTRACT

AIM: We investigated the effects of atorvastatin and N-acetyl cysteine in decreasing ischemia–reperfusion damage after detorsion of a volvulus of the cecum and ascending colon.

METHODS: Wistar albino rats (250–300 g) were divided into four groups. A cecal-ascending colon volvulus was created by the intestinal clockwise 720° rotation. At the end of one hour, the bowel was detorsioned. Group I (n = 7) was the sham (laparotomy) group, Group II (n = 7) the control (no treatment, volvulus or detorsion), Group III (n = 7) (N-acetyl cysteine administered) , and Group IV (n = 7) (atorvastatin administered) group. Blood samples were collected from each group via peripheral veins and centrifuged one hour after detorsion. The parameters of ischemia including malondialdehyde, glutathione peroxidase, catalase, and superoxide dismutase were then observed in the serous fluid.

RESULTS: Malondialdehyde and superoxide dismutase increased in the control group, whereas they were reduced in the Group III and Group IV ($p = 0.005$; $p = 0.008$, respectively).

The glutathione peroxidase levels revealed no significant differences ($p > 0.05$), whereas the catalase levels of the group III was higher than in each of the other three groups ($p < 0.001$). Histopathological evaluation detected reduced lesioning of the organ in the groups which were given atorvastatin and N-acetyl cysteine.

CONCLUSION: Atorvastatin and of N-acetyl cysteine have a similar preventive effect in experimental ischemia–reperfusion injury *(Tab. 8, Fig. 6, Ref. 24)*. Text in PDF *www.elis.sk.*

KEY WORDS: atorvastatin, N-acetyl cysteine, ischemia–reperfusion injury, volvulus.

Introduction

Colon volvulus is one of the causes of acute abdomen which requires urgent and specific diagnosis. Sigmoid colon volvulus is a very common disorder which responds to colonoscopic detorsion. In unresponsive cases, surgical resection, Hartman procedures and other surgical techniques are applied (1, 2).

Intestinal vascular ischemia also occurs due to the mechanical bowel obstruction in volvulus. Ischemia results when the organ or tissue perfused by insufficient blood flow develops reversible or irreversible cell and tissue damage (3). Following ischemia, the restoration of the blood flow in the region (reperfusion) takes place rapidly, along with the delivery of molecular oxygen into the cells together with reactive oxygen species (ROS) derivatives. In order to prevent irreversible cell damage, blood flow must be restored to the organs and tissues. However, reperfusion can cause more damage to the tissues and organs already damaged by ischemia (4).

[1]Duzce University Medical Faculty-Department of General Surgery Duzce, Turkey, and [2]FSM Training and Research Hospital Department of General Surgery Istanbul, Turkey

Address for correspondence: Yasar M, Dr, Duzce University, Medical Faculty, Department of General Surgery, Ducze, Turkey.
Phone: +0532.7716576

N-acetyl cysteine (NAC) is an intracellular glutathione (GSH) precursor and markedly increases glutathione S-transferase activity in the liver. This activity is the foundation of the antioxidant, anticarcinogenic and antimutagenic effects of the agent. The antimutagenic effect of NAC on bacterial test systems as well as its mucolytic and antioxidant effects have long been known (5–7). Under hypoxic conditions, a decrease in blood and tissue GSH levels and an increase in lipid peroxidation products have been reported (8).

Atorvastatin is one of the HMG-CoA reductase inhibitor statins. Statins are known to be anti-inflammatory, to exhibit protective effects in atherosclerosis and to lower serum lipid levels. Additional effects, including the reduction of cytokines, the secretion of adhesion molecules and the proliferation of smooth muscle cells, have also been demonstrated (9–11). Moreover, statins have the pleiotropic effects of reducing vascular inflammation and improving endothelial function, the antithrombotic effects of regression and stabilization of atherosclerotic plaque, as well as oncoprotective effects. They have also been found to decrease arterial compliance and improve insulin resistance.

In this study, it was thought that mortality could be reduced by preventing the tissue damage caused by the oxygen radicals generated in the process of reperfusion following detorsion of a volvulus. Atorvastatin and N-acetyl cysteine for reducing ischemia–reperfusion injury were used in experimental model.

Tab. 1. Experimental animal groups and procedures.

Group No	No. of Animals	Group Type	Experimental Procedures
I	7	Sham	Laparotomy
II	7	Control	Volvulus + Detorsion
III	7	Positive Control	Volvulus+Detorsion+N-acetylcysteine, 300 mg/kg, i.p., half an hour before detorsion
IV	7	Treatment	Volvulus+Detorsion+atorvastatin injectable 30 mg/kg, i.p., half an hour before detorsion

Materials and methods

Experimental animals

For this experimental study, the necessary permits and approvals were obtained in accordance with the Animal Research Ethics Committee decision no. 2013/44, dated 12/02/2014. Our experimental animal trials were carried out in the Experimental Animal Center, while the other investigations were conducted in the biochemistry, pathology and pharmacology laboratories of our faculty.

For the study, 28 healthy, mixed male and female albino Wistar rats, 250–300 gm in weight, were selected and kept under appropriate temperature and feeding conditions. The rats were randomly divided into four groups of seven each. For ten days prior to the start of the experiment, the rats were given water and standard feed and housed in individual cages, allowing them to adapt to ambient conditions (Tab. 1).

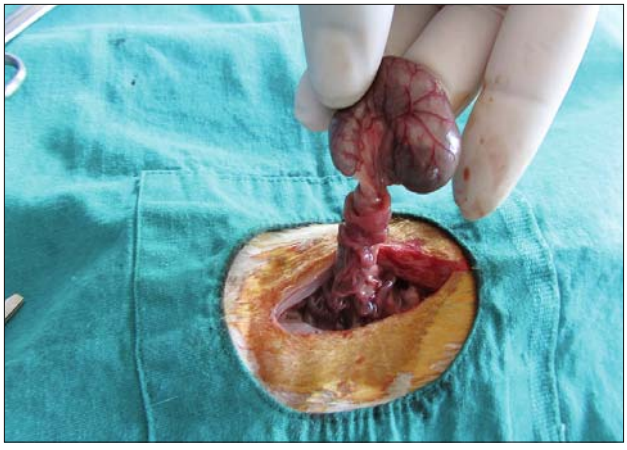

Fig. 1. Creation of cecum-ascending colon volvulus in the experimental rats.

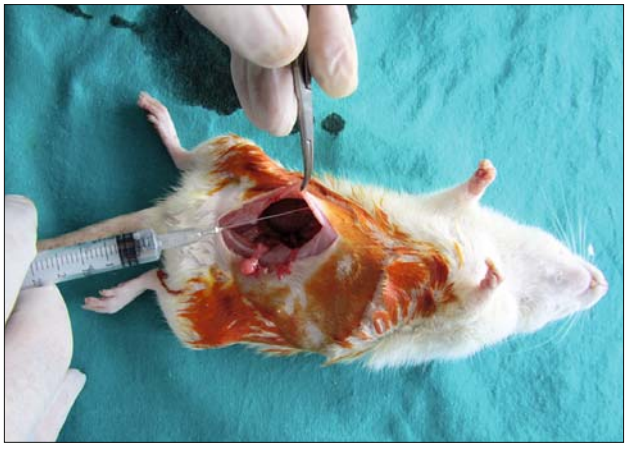

Fig. 2. Intraperitoneal administration of atorvastatin in median laparotomy rats.

Surgical procedures

Group I (Sham group): After proper cleaning and sterilization of the area with 10 % povidon iodine solution (Baticon®, Adeka, Turkey), the rats were anesthetized using Ketamine hydrochloride (Ketalar Eczacıbaşı, Istanbul, Turkey), 50 mg/kg i.p. and Xylazine (vial) (Rompun Bayer Ilac, Turkey), 10 mg/kg. A midline laparotomy incision of about 3 cm was made and then closed with 3–0 silk. Peripheral venous blood samples (about 2 cc) were taken.

Group II (Control Group): After sterilization and anesthesia, laparotomy + volvulus + detorsion were performed (Fig. 1). One hour after reperfusion, a blood sample was taken and the laparotomy closed.

Group III (N-acetyl cysteine): After sterilization and anesthesia, laparotomy + volvulus + detorsion were performed + N-acetyl cysteine injectable (Asist ampoule, Hüsnü Arsan, Turkey), 300 mg/kg, i.p., was administered half an hour prior to detorsion, One hour after reperfusion, a blood sample was taken and the laparotomy closed with 4–0 silk.

Group IV (Atorvastatin): For this group, after sterilization and anesthesia, laparotomy + volvulus + detorsion were performed. Atorvastatin injectable (prepared in the pharmacology laboratory), 40 mg/kg, i.p., was administered half an hour prior to detorsion (Fig. 2). One hour after reperfusion, a blood sample was taken and the laparotomy was closed with 4–0 silk.

During the post-operative period, paracetamol injectable (Parol vial, Turkey) 100–300 mg/kg, s.c., was administered once every 4 h as an analgesic. Oral feeding was carried out.

One day later, after sterilization and under anesthesia, relaparotomy was performed on all groups, pathological specimens and control blood samples of arterial blood were taken and the animals were sacrificed.

In our experimental volvulus model, transmural occlusion was presented along with complete venous obstruction and partial arterial obstruction. Ischemia and perfusion times were equal and evaluated as one hour.

Biochemical analysis

Blood samples were centrifuged and transported while observing the blood cold chain. Ischemia parameters of the serum malondialdehyde (MDA), glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD) were investigated and a statistical analysis was performed.

Tab. 2. Snyder's semi quantitative histopathological evidence scale used to classify the morphological changes in the experimentally induced ascending colon volvulus in the rats.

1) Hemorrhage and edema in each separate layer of the intestine (mucosa, submucosa, muscularis) (Scored from 0–4)
0: None
1: Hemorrhage / edema upon very careful inspection
2: Easily detected but infrequent hemorrhage / edema
3: Extensive hemorrhage / edema, but normal structure undamaged
4: Normal structure disrupted with extensive hemorrhage / edema
2) Degenerative changes and desquamation in the surface epithelium (Scored from 0–3)
0: No degenerative changes or desquamation
1: No desquamation, but degenerative changes present
2: Desquamation in a small portion of the surface epithelium
3: Infrequent desquamation in the surface epithelium
(Degenerative changes: deteriorated nucleus/cytoplasm ratio; cytoplasm coagulation deposits; reduction in mucus secretion; prominent nucleoli)
3) Necrosis and gangrene in the intestinal wall (Scored from 0–3)
0: Absence of necrosis or gangrene
1: Necrosis extending into the surface epithelium muscularis mucosa
2: Necrosis advanced beyond the muscularis mucosa and into the submucosa
3: Deep necrosis extending into the muscularis propria.

Measurement of the total (Cu-Zn and Mn) SOD activity was performed according to the method of Sun et al (12) as modified by Durak et al (13). In this method, the SOD activity is based on the reduction of the xanthine/xanthine oxidase system with the produced superoxitin nitroblue tetrazolium (NBT). Kits obtained from Cell Bioplast and Usnc Life Science, Inc., and a Shimadzu spectrophotometer were used in the analyses.

Histopathological examination

For the histopathological examination, the cecum, ascending colon and distal ileum of all the sacrificed rats were removed, washed with physiological serum, placed in containers of formaldehyde and sent to the pathology laboratory. Antimesenteric cecal tissue samples were taken and fixed on paraffin blocks. The 4 µm-thick sections were stained with hemotoxilin–eosin and prepared for the light microscope by a single pathologist. A blind assessment was carried out using the Snyder scale for the classification of morphological changes.[14] The semi quantitative histopathological changes, including hemorrhage, edema, degenerative changes and desquamation and presence or absence of necrosis, in every layer of the intestine were scored (Tabs 2 and 3, Figs 3–6).

Tab. 3. Histopathological results.

Group & Animal No.	Morphological Changes & Scores			
Group I (Sham)	Edema	Hemorrhage	Degenerative changes and desquamation	Necrosis and gangrene
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
Group II (Control)	Edema	Hemorrhage	Degenerative changes and desquamation	Necrosis and gangrene
1	4	3	3	3
2	2	2	1	0
3	4	3	3	3
4	4	3	3	3
5	1	1	1	0
6	1	0	1	0
7	3	2	3	3
Group III (Positive Control)	Edema	Hemorrhage	Degenerative changes and desquamation	Necrosis and gangrene
1	1	1	2	2
2	1	0	0	0
3	2	2	3	2
4	3	1	3	2
5	1	0	0	0
6	3	2	3	2
7	2	3	2	2
Group IV (Treatment)	Edema	Hemorrhage	Degenerative changes and desquamation	Necrosis and gangrene
1	1	1	0	0
2	1	2	0	0
3	1	2	0	0
4	1	3	2	2
5	1	2	2	2
6	1	0	0	0
7	1	1	0	0

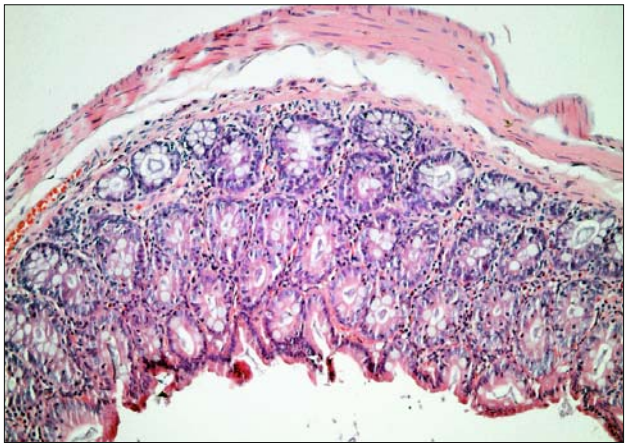

Fig. 3. Normal bowel tissue (H & E × 200).

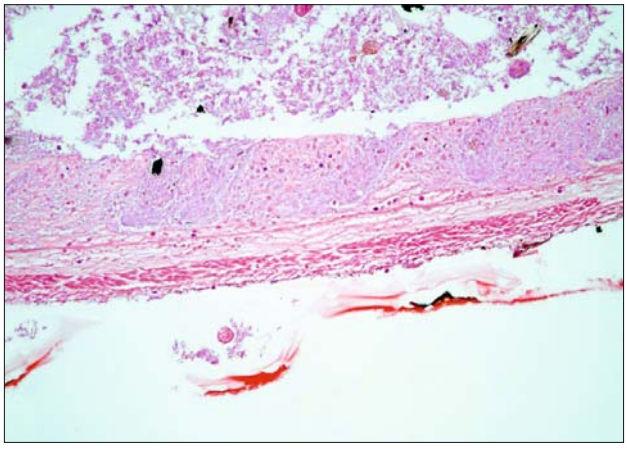

Fig. 4. Extensive necrosis and desquamation of the muscular layer (H & E × 200).

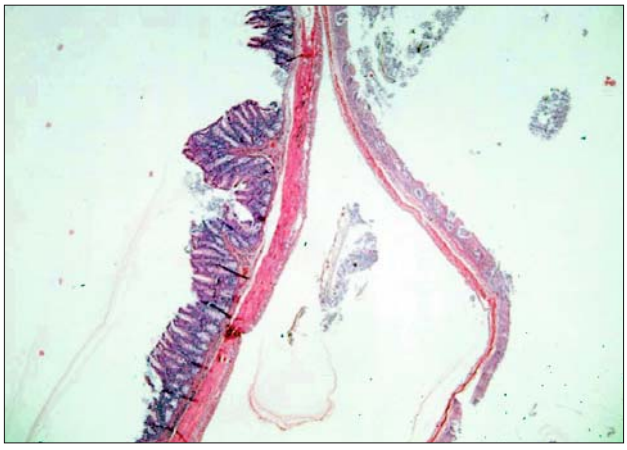

Fig. 5. Bowel tissue: necrotic on the right side and healthy on the left side (H & E × 40).

Statistical analysis

The quantitative variable identifier mean, standard deviation and median values are given in Table 4. The normality of the distribution of these variables was assessed using the Shapiro–Wilk test. The Kruskall–Wallis test was used to compare the groups in terms of edema, hemorrhage, desquamation, necrosis and gangrene variables. The investigation of variations in the group test results was aided by applying the Dunn test. A one-way analysis of variance (ANOVA) was used to examine the group variations in terms of the parameters measured on Day 1 and Day 2. The differences shown by the ANOVA results were evaluated by the Tukey test. The paired t-test was used to compare the groups separately in terms of the average parameters measured on days 1 and 2. The ANOVA was used to compare the averages of the groups in terms of the different values measured on days 1 and 2. The results of the statistical evaluations were found to be statistically significant ($p \leq 0.05$). The PASW (ver. 18) program was used for the calculations.

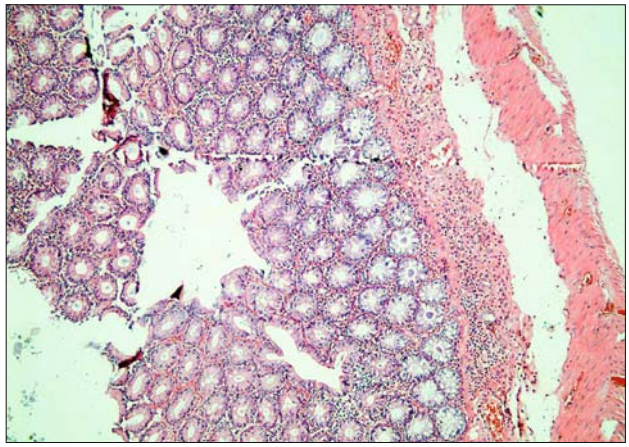

Fig. 6. Mild edema in the mucosa (H & E × 200).

Results

Table 4 shows a comparison of the resulting descriptive statistics and p values of the groups (sham, control, group III, and group IV) in terms of the variables of edema, hemorrhage, desquamation, necrosis and gangrene. Significant differences were determined among the study groups in terms of median values for edema, hemorrhage, desquamation, necrosis and gangrene, at $p < 0.05$ each. A detailed examination of the differences shows that the median edema value for the sham group was significantly lower than the values of the positive control group and the control group ($p = 0.003$; $p < 0.001$). The hemorrhage median for the sham group was significantly lower than the treatment group and control group ($p = 0.048$; $p = 0.007$). When desquamation differences are examined, the sham group median was determined as significantly lower than the positive control and control groups ($p = 0.043$; $p = 0.005$). When the medians groups are compared in terms of necrosis medians, only the sham group median was found to be significantly different from the control group ($p = 0.05$). No significant difference was observed among the other groups in terms of necrosis medians ($p > 0.05$ per comparison). After 24 h, one test subject each from the control and positive control groups died.

The descriptive statistics and p values obtained from the comparison of the groups in terms of MDA, GSHPX, CAT and SOD

Tab. 4. Statistical comparison of histopathological results*.

	Group	Average	Median	Standard Deviation	Minimum	Maximum	p
Edema	Sham	0.0000	0	0.00000	0.00	0.00	< 0.001
	Control	2.7143	3	1.38013	1.00	4.00	
	Positive control	1.8571	2	0.89974	1.00	3.00	
	Treatment	1.0000	1	0.00000	1.00	1.00	
Hemorrhage	Sham	0.0000	0	0.00000	0.00	0,00	0.007
	Control	2.0000	2	1.15470	0.00	3.00	
	Positive control	1.2857	1	1.11270	0.00	3.00	
	Treatment	1.5714	2	0.97590	0.00	3.00	
Desquamation	Sham	0.0000	0	0.00000	0.00	0.00	0.002
	Control	2.1429	3	1.06904	1.00	3.00	
	Positive control	1.8571	2	1.34519	0.00	3.00	
	Treatment	0.5714	0	0.97590	0.00	2.00	
Necrosis and Gangrene	Sham	0.0000	0	0.00000	0.00	0.00	0.033
	Control	1.7143	3	1.60357	0.00	3.00	
	Positive control	1.4286	2	0.97590	0.00	2.00	
	Treatment	0.5714	0	0.97590	0.00	2.00	

* Kruskal–Wallis test

Tab. 5. Comparison of group biochemistry results for the first day (Day 1)*.

Group		Average	Standard Deviation	Minimum	Maximum	p
MDA	Sham	1.09286	0.235319	0.672	1.310	0.002
	Control	2.67386	0.997492	0.731	3.522	
	Positive control	1.18214	0.198211	0.945	1.511	
	Treatment	2.08343	1.174203	0.502	4.180	
GSHPX	Sham	0.25243	0.068593	0.129	0.322	0.751
	Control	0.26514	0.063154	0.155	0.349	
	Positive control	0.28671	0.080135	0.190	0.407	
	Treatment	0.28729	0.071542	0.200	0.386	
CAT	Sham	0.13329	0.032113	0.080	0.173	<0.001
	Control	0.12671	0.018581	0.093	0.149	
	Positive control	0.37429	0.054503	0.291	0.449	
	Treatment	0.18114	0.070172	0.129	0.285	
SOD	Sham	13.32629	0.978441	11.981	14.437	<0.001
	Control	17.69029	1.179833	16.006	19.134	
	Positive control	13.81571	0.679664	13.006	15.112	
	Treatment	13.64586	1.606397	11.036	15.700	

* One-way ANOVA

Tab. 6. Comparison of group biochemistry results for the second day (Day 2)*.

Group		Average	Standard Deviation	Minimum	Maximum	p
MDA_2	Sham	1.00714	0.257173	0.624	1.225	0.008
	Control	2.56986	1.145517	0.512	3.722	
	Positive control	1.25686	0.248714	0.948	1.593	
	Treatment	2.02000	1.230621	0.420	3.752	
GSHPX_2	Sham	0.32800	0.045607	0.248	0.395	0.423
	Control	0.43257	0.200967	0.207	0.854	
	Positive control	0.37386	0.078334	0.276	0.534	
	Treatment	0.35917	0.070559	0.274	0.456	
CAT_2	Sham	0.18971	0.054540	0.121	0.264	<0.001
	Control	0.20200	0.053460	0.129	0.278	
	Positive control	0.41814	0.096670	0.258	0.505	
	Treatment	0.23633	0.052914	0.177	0.304	
SOD_2	Sham	14.13371	0.777516	12.852	14.845	<0.001
	Control	18.14557	1.771975	15.128	19.968	
	Positive control	14.44014	0.639128	13.854	15.784	
	Treatment	14.20100	0.621447	13.433	15.152	

* One–way ANOVA

Tab. 7. Comparison of first and second day results for the each group separately*.

Group		The average of the differences (1st day – 2nd day)	The standard deviation of the differences	p
Sham	MDA – MAD2	0.085714	0.124531	0.118
	GSHPX – GSHPX2	–0.075571	0.063235	0.020
	Cat – Cat2	–0.056429	0.038061	0.008
	SOD – SOD2	–0.807429	0.554628	0.008
Control	MDA – MAD2	0.104000	0.266491	0.342
	GSHPX – GSHPX2	–0.167429	0.196473	0.065
	Cat – Cat2	–0.075286	0.041624	0.003
	SOD – SOD2	–0.455286	1.336460	0.402
Positive Control	MDA – MAD2	–0.074714	0.093952	0.080
	GSHPX – GSHPX2	–0.087143	0.046280	0.002
	Cat – Cat2	–0.043857	0.068282	0.140
	SOD – SOD2	–0.624429	0.322799	0.002
Treatment	MDA – MAD2	0.133500	0.230043	0.214
	GSHPX – GSHPX2	–0.070500	0.033321	0.004
	Cat – Cat2	–0.046500	0.073001	0.179
	SOD – SOD2	–0.897500	1.230511	0.134

* Paired samples t-test

Tab. 8. Comparison of groups in terms of changes (difference) occurred between the first and second day*.

Group		n	Averag e	Standard Deviation	Minimum	Maximum	p
MDA differences	Sham	7	–0.08571	0.124531	–0.304	0.059	0.210
	Control	7	–0.10400	0.266491	–0.362	0.403	
	Positive control	7	0.07471	0.093952	0.003	0.270	
	Treatment	6	–0.13350	0.230043	–0.428	0.180	
GSHPX differences	Sham	7	0.07557	0.063235	0.017	0.210	0.337
	Control	7	0.16743	0.196473	0.052	0.609	
	Positive control	7	0.08714	0.046280	0.009	0.149	
	Treatment	6	0.07050	0.033321	0.026	0.110	
CAT differences	Sham	7	0.05643	0.038061	0.024	0.110	0.730
	Control	7	0.07529	0.041624	0.027	0.136	
	Positive control	7	0.04386	0.068282	–0.091	0.130	
	Treatment	6	0.04650	0.073001	–0.047	0.173	
SOD differences	Sham	7	0.80743	0.554628	0.305	1.801	0.836
	Control	7	0.45529	1.336460	–2.404	1.698	
	Positive control	7	0.62443	0.322799	0.111	1.023	
	Treatment	6	0.89750	1.230511	–0.795	2.815	

* One-way ANOVA

averages measured on the first day (Day 1) are given in Table 5. Upon examination of the table, no significant difference among the groups can be seen in the GSHPX averages ($p > 0.05$). However, significant differences can be observed among the group averages for MDA, CAT and SOD. Close examination of the differences shows that the MDA of the control group was significantly higher than the MDA averages of the sham and positive control groups ($p = 0.005$; $p = 0.008$). Similarly, the CAT average of the positive control group was significantly higher ($p < 0.001$ per comparison) than in the other three groups (sham, control and treatment). Although the SOD average for the control group was found to be significantly higher than the averages of the sham, positive control and treatment groups ($p < 0.001$ each), no significant difference was observed among the other groups in terms of SOD averages ($p > 0.05$).

The descriptive statistics and p values obtained from the comparison of the groups in terms of MDA, GSHPX, CAT and SOD averages measured on the second day (Day 2) are shown in Table 6. Upon examination of the table in terms of GSHPX averages, no significant difference was determined among the groups ($p > 0.05$). However, significant differences were seen among the group MDA, CAT and SOD averages. When the differences were examined, the control group MDA average was found to be significantly higher than the MDA averages of the sham and positive control groups ($p = 0.010$; $p = 0.036$). Similarly, the positive control group CAT average was significantly higher than the CAT averages ($p < 0.001$) per comparison of the other three groups (sham, control, treatment). Although the SOD average of the control group was found to be significantly higher than the SOD averages of the sham, positive control and treatment groups ($p < 0.001$ each), no significant difference was found among the other groups in terms of SOD averages ($p > 0.05$).

The p values are included in the examination of possible differences between the measurements for each group taken separately

on days 1 and 2. As seen in Table 7, the sham group averages for CAT, GSHPX and SOD taken on Day 2 were significantly higher than those taken on Day 1 ($p = 0.020$; 0.008 and 0.008, respectively). However, no significant difference was seen between the MDA averages measured on days 1 and 2 ($p > 0.05$). No significant difference was found between the Day 1 and the Day 2 MDA, GSHPS and SOD averages for the control group ($p > 0.05$ each). In contrast, the Day 2 CAT average for the control group was significantly higher than that of Day 1 ($p = 0.003$). The Day 1 and Day 2 MDA and CAT averages for the positive control group were similar ($p > 0.05$); however, the GSHPX and SOD averages measured on Day 2 were significantly higher ($p = 0.002$ for both). The MDA, CAT and SOD averages for Days 1 and 2 were similar in the treatment group. However, the treatment group GSPX average measured on Day 2 was significantly higher ($p = 0.004$).

The descriptive statistics and p values obtained from the comparison of the groups in terms of differences in the MDA, GSHPX, CAT and SOD averages for days 1 and 2 are given in Table 8. The table shows that the groups did not differ significantly in their variations of the averages between Day 1 and Day 2 ($p > 0.05$ for each).

Discussion

Colon volvulus was defined for the first time in 1836 by Rokitansky as "the anormal axial rotation of a segment of the large bowel around its mesentery causing an acute closed loop obstruction" (1). As a result of this obstruction, the endoluminal pressure increases, leading to the development of ischemia, gangrene and finally, perforation of the colon. Colon volvulus is life-threatening, and must be quickly and accurately diagnosed and treated in the most appropriate manner (1, 2). It is recognized that the most important predisposing pathological factors for volvulus are a narrow mesenteric base and a long and mobile colon segment structure. Other predisposing factors include chronic constipation, extended bed rest, colonic motility disorders, megacolon, advanced age, neuropsychiatric disorders, predisposing anatomical factors, previous abdominal surgery, pregnancy, living at high altitudes, Chagas' disease, Hirschsprung disease and scleroderma (15, 16).

After the volvulus-induced ischemia, restoration of the blood flow to that region (reperfusion) and the reintroduction of molecular oxygen along with reactive oxygen species (ROS) derivatives into the cells take place rapidly (3, 4).

It is known that the production of ROS derivatives resulting from intestinal ischemia–reperfusion (I–R) plays an important role in ischemic injury (17).

In this experimental study, the effect of atorvastatin was investigated in the rats exposed to I–R injury as a result of the volvulus. The structural features of NAC, used in the positive control group, were found to be similar to those of atorvastatin.

In addition to the mechanical intestinal obstruction of the volvulus, because of the vascular occlusions in question, many agents are available to prevent damage during reperfusion after torsion via colonoscopy or other procedures. One of these is atorvastatin. The effectiveness of atorvastatin in I–R injury has been observed clinically.

Time is important in dealing with I–R injury (18). Oxidants are formed in I–Rs lasting for as short a time as 2–5 min (18). In ischemia having a duration of up to 60 min, there is an increase in oxygen radicals, while in cases of ischemia that last more than 120 min, they are found to decrease (19). Because it is difficult to detect damage after the occurrence of reperfusion injury in ischemia cases of long duration, for this study, the ischemia duration was set as 60 min.

According to the histopathological results using the scores for edema, hemorrhage, degenerative changes, desquamation, necrosis and gangrene, the lesions in the control group increased, while a significant reduction was detected in the lesions in the positive control and treatment groups. Statistically, however, in the sham group, excepting the necrosis averages, no significant difference was found ($p > 0.05$ per comparison).

Following reperfusion, the oxygen radicals formed in the tissues lead to the peroxidation of phospholipid fat chains in the cell membrane, causing MDA to be produced (19).

Otamiri and Tagesson reported a 3–4-fold increase in the levels of mucosa and plasma MDA in rats after reperfusion of 5 min duration (20).

Naito et al. stated that the increased amount of MDA in the terminal ileum following I–R was significantly reduced with atorvastatin (21). Likewise, in this study, the MDA average in the control group was found to be significantly higher than the averages of the sham and positive control groups.

The effect of NAC on I–R injury was confirmed by several experiments carried out by Sun et al. Their study showed the effects of the NAC and indomethacin intestinal reperfusion model, and demonstrated that NAC ensured the integrity of the endothelial and epithelial barrier. Again, they determined that NAC was effective in preventing reperfusion injury (22). Another experimental study showed that NAC prevents reperfusion injury by impeding the adhesion molecules that inhibit peroxynitrite and that it provıdes for the reduction of neutrophils (23).

Demir et al. suggested that in I–R injury, there is an increase in the lipid peroxide levels in the liver, and that NAC application leads to a reduction in the lipid peroxide levels in the tissue (24).

In this study, the group given NAC (positive control group) exhibited less necrosis clinically, compared to the control group.

In the biochemical evaluation, the CAT average of the positive control group was significantly higher than the averages of the other three groups (sham, control and treatment).

MDA is the final product of lipid peroxidation, which is a marker of oxidative damage. Experimentally, in our study, a single dose of atorvastatin treatment given in the acute phase of the I–R injury created by the volvulus reduced the increased level of MDA.

The MDA average of the control group was found to be significantly higher than the MDA averages of the sham and the positive control group ($p = 0.005$, $p = 0.008$, respectively). However, no significant difference was seen between the MDA averages measured on days 1 and 2 ($p > 0.05$).

Although the SOD average of the control group was found to be significantly higher than the SOD averages of the sham, positive control and treatment groups ($p < 0.001$ each), in terms of the

SOD averages, no significant difference was seen among the other groups ($p > 0.05$). The SOD data, like those of MDA, presented high levels of I–R injury which dropped with the treatment of a single medical dose of NAC and atorvastatin.

Although no significant difference was detected in the GSH-Px and catalase values, the values were slightly high. Statistically, the CAT average of the positive control group was significantly higher than the averages of the other three groups (sham, control and treatment) ($p < 0.001$ per comparison). As for the GSHPX averages, among the groups, no significant difference was found ($p > 0.05$).

Conclusion

The effects of using atorvastatin were observed to be similar to those of N-acetyl cysteine in preventing experimentally induced ischemia–reperfusion injury after detorsion of the volvulus.Further comprehensive research needs to be carried out using other statins and materials.

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