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Research Article

MESNA HAS ANTIOXIDANT POTENTIAL ON INTESTINE, LIVER, KIDNEY AND LUNG INJURY INDUCED BY INTESTINAL ISCHEMIA/REPERFUSION

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Abstract: An ischemia/reperfusion (IR) injury of small intestine is a serious and common condition that is a result of the blockage of the superior mesenteric artery (SMA) due to some significant clinical problems. In the present study, we evaluated the effect of Mesna on systemic injury induced by IR in small intestine and liver, kidney and lung of rats. Thirty-two Wistar albino female rats were randomly divided into four groups as control, ischemia, IR (Sham) and IR+Mesna. Ischemia period was executed by clamping SMA for 2 h and after when reperfusion was permitted by removing the clamp from SMA for 2 h. Superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) activities were measured spectrophotometrically in the tissues. Microscopic examination was performed with Hematoxylin-Eosin (H&E) staining for light microscopy and double staining (Uranyl acetate and Reynolds'lead citrate) for electron microscopy. Oxidative damage was determined in ischemia ($p < 0.05$) and IR groups ($p < 0.05$) by Chiu score in small intestine and also antioxidant enzyme activities in all groups. Significant recovery in SOD, GPx and CAT activities in IR+Mesna group was determined but the effect of oxidative damage was not reduced significantly histopatologically. Mesna treatment caused recovery in SOD, GPx and CAT activities but not achieved an improvement in the histopathologic findings in level of light microscopy in IR damages but contains the signs of improving at electron microscopy level in Mesna dose and application time of IR.

Özet: İnce bağırsağın iskemi/reperfüzyon (İR) hasarı, önemli klinik problemler nedeniyle superior mezenterik arterin (SMA) tıkanmasının bir sonucu olarak ortaya çıkan ciddi ve yaygın bir durumdur. Bu çalışmada, Mesna'nın ince bağırsak, karaciğer, böbrek ve akciğerde İR ile indüklenen sistemik hasar üzerindeki etkisi değerlendirildi. Otuziki Wistar albino dişi sıçan, rastgele dört gruba ayrıldı: Kontrol, iskemi, İR (Sham) ve İR+Mesna. Superior mezenterik arter (SMA) 2 saat klemplenerek iskemi ve ardından klemp SMA'dan çıkarılarak 2 saat reperfüzyon uygulandı. İnce bağırsak, karaciğer, böbrek ve akciğer dokularında, antioksidan enzim aktiviteleri spektrofotometrik olarak ölçüldü. Mikroskobik inceleme için hematoksilen-eozin (H&E), elektron mikroskobik inceleme için çift boyama (uranil asetat ve Reynolds kurşun sitrat) yapıldı. İskemi ($p < 0.05$) ve IR gruplarında ($p < 0.05$) ince bağırsakta Chiu skoru ve ayrıca tüm gruplarda antioksidan enzim aktiviteleri ile oksidatif hasar belirlendi. IR+Mesna grubunda SOD, GPx ve CAT aktivitelerinde anlamlı iyileşme saptandı ancak oksidatif hasarın etkisi histopatolojik olarak önemli ölçüde azalmadı. Mesna uygulaması, SOD, GPx ve CAT aktivitelerinde düzelme sağladı ancak histopatolojik bulgularda ışık mikroskobi düzeyinde İR hasarında anlamlı bir iyileşmeye neden olmadı, ancak uygulanan İR süresi ve Mesna dozunda, ultrastrüktürel olarak iyileşme belirtileri içerdi.

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Introduction

Ischemia/reperfusion (IR) is an oxidative stress process that is characterized by the overproduction of reactive oxygen species and activation of leukocytes (Ypsilantis *et al.* 2008). The intestine is one of the organs susceptible to IR injury (Granger & Korthuis 1995). Problems encountered in operations including intestinal transplantation and basic vascular surgery, especially in clinical conditions such as strangulated hernia, volvulus, invagination, shock, sepsis, and arterial embolism, are the most important indicators of this susceptibility (Ypsilantis *et al.* 2008). It is known that regional and systemic injuries occur when the circulatory disorder occurs in the intestine

and these injuries are surgically corrected (Savas *et al.* 2003, Giakoustidis *et al.* 2006). Following injuries, multiple organ failure may develop. Organs most affected by intestinal IR injury are the liver, lungs, and kidneys (Savas *et al.* 2000, Stallion *et al.* 2005, Giakoustidis *et al.* 2006).

IR injury of the small intestine is a serious and common condition and is the result of the blockage of the superior mesenteric artery (SMA) due to many factors. Moreover, causes that do not lead to arterial embolism such as systemic hypertension, vasoconstriction, blood viscosity disorders, arteriosclerosis, and hypotension also lead to small bowel ischemia (Ayvaz *et al.* 2009).

Mesna, a thiol compound, is used to prevent hemorrhagic cystitis caused by chemotherapeutics such as ifosfamide and cyclofosfamide in the chemotherapy protocols of cancer patients (Haselberger & Schwinghammer 1995, Siu & Moore 1998, El-Baset *et al.* 2021). The uroprotective effect of this compound arises from its binding ability to toxic metabolites secreted and concentrated by the kidney. Due to the sulfhydryl group it contains, Mesna is also an effective oxygen radical scavenger (Ypsilantis *et al.* 2006, 2008, Berkesoglu *et al.* 2020). Studies also revealed that Mesna has effective therapeutic properties in experimental colitis models (Shusterman *et al.* 2003) and reduces IR injury, due to its antioxidant properties, in the kidney, liver and lung (Kabasakal *et al.* 2004, Sener *et al.* 2005, El-Baset *et al.* 2021).

The effects of numerous antioxidant substances on intestinal IR injury were investigated. There are studies on the use of antioxidants, of which therapeutic or protective effects are observed, to reverse IR injury (Itagaki *et al.* 2009, Tunc *et al.* 2009, Turut *et al.* 2009, Hosgorler *et al.* 2010, Gedik *et al.* 2018). These antioxidant agents include vitamin C, vitamin E, mannitol, melatonin, bilirubin, allopurinol, caffeic acid, zinc, carnitine and erdosteine (Mallick *et al.* 2004, Tunc *et al.* 2009, Turut *et al.* 2009, Hosgorler *et al.* 2010, Shafik 2013).

Here, we hypothesized that Mesna may prevent the hazardous effects of intestinal IR on small intestine, liver, lung and kidney. This is the first report on investigation on the ultrastructural effects of Mesna on liver, lung and kidney after intestinal IR injury.

Materials and Methods

Animals and experimental design

Thirty-two female Wistar albino rats weighing 200-250 g were obtained from the Experimental Animal Centre of Trakya University, Edirne, Türkiye. All animals were fed daily with tap water and a standard pellet diet for rats under optimum laboratory conditions (temperature: 22±2°C; humidity: 50-55%; light/dark period: 12 h/12 h). The animals were starved for 12 h before the experiments. The study was approved by the local animal ethics committee (TÜDYEK 2010/010).

The experimental animals were randomly divided into four groups (n=8 in each group) as follows: Control, ischemia, IR (Sham) and IR+Mesna (150 mg/kg), intraperitoneally (i.p.). Mesna was dissolved in 2 ml saline (w/v). All surgical procedures were performed under xylazine/ketamine (10/90 mg/kg, i.p.; Eczacıbaşı, İstanbul, Türkiye) anesthesia and sterile conditions. A midline laparotomy incision was performed after preparation of the abdominal wall with 10% povidone-iodine solution. In the IR (Sham) group, a midline laparotomy incision was performed after preparation of the abdominal wall with 10% povidone-iodine solution. The superior mesenteric artery (SMA) was dissected, with no further treatment, and then the abdomen was closed. 2 ml physiologic serum was administered instead of Mesna just before closing the abdomen. In IR+Mesna group, an atraumatic microvascular clamp was placed across the SMA at the origin from the aorta, as described by Megison *et al.* (Megison *et al.* 1990). Mesenteric ischemia was confirmed when the mesenteric pulsations of the jejunum and ileum were lost and the intestinal segment became pale. In the ischemia group, the rats were subjected to 2 h of intestinal ischemia. In the IR group (Sham), after 2 h of ischemia, a relaparotomy was performed, and the microvascular clamp on the artery was removed for 2 h for reperfusion. Mesenteric reperfusion was confirmed by the return of pulsation and color of the jejunum and ileum. In the Mesna group, surgery was performed as in the IR group (Sham) and IR+Mesna (Mesna Eczacibasi Company, Istanbul, Türkiye) Mesna was administered i.p. just after the clamp was removed. The abdominal incisions in all groups were closed with interrupted 4-0 silk suture after the bowel was returned to the cavity.

During the surgical procedures, the rats were warmed with a heating lamb and their body temperature was kept at approximately 37°C. At the end of the procedure, stitches were opened. Small intestine (jejenum), lung, kidney and liver tissue samples were obtained for histological analysis.

For biochemical analysis, the intestinal segment (jejenum), liver, kidney and lung tissues were dried with a drying paper after washing with 0.9% NaCl and then rolled with an aluminum foil. Tissue samples were stored at -80°C until further analysis.

Biochemical assay

Determination of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) activities in tissue samples

Common extraction procedure was applied for determination of SOD, GPx and CAT activities. Tissue samples of small intestine, liver, kidney and lung (0.5 g of each) were homogenized with 5 volumes per weight of 0.05 M phosphate buffer of pH 7.0 containing 1% (w/v) Triton X-100 by glass-glass homogenizer for 5 min. Extract was centrifuged for 20 min at 10,000 g (+4°C). The supernatant (S1) was used in the assay for GPx. CAT was measured in the same fraction (S1) after addition of

0.2 volume ethanol (1% v/v) and incubation in cold for 15 min. An aliquot of the S1 supernatant was precipitated on ice with 0.3 volume chloroform/ethanol (3:5 v/v), stirred on ice for 15 minutes and centrifuged at 10,000 g for 15 minutes. The final supernatant (S2) was used in the assay of SOD activity (Aksnes & Njaa 1981).

SOD and GPx activities were measured with a spectrophotometer (Shimadzu-1240, Japan) by using the Ransod kit (Randox Cat. No; SD 125, United Kingdom BT29 4QY) and the Ransel kit (Randox Cat. No; RS 505, United Kingdom BT29 4QY), respectively.

One unit SOD activity was defined as the amount of enzyme that causes 50% inhibition of the rate of reduction of I.N.T. (2-(4-iodophenyl)-3-(nitrophenol)-5-phenyltetrazolium chloride) under the assay conditions (37°C, pH 7.0). One unit GPx activity was defined as the amount of enzyme that oxidized 1 µmol NADPH to NADP in one minute at 37°C. One unit CAT was defined as the amount of enzyme that decomposed 1 µmol H_2O_2 per minute at 30°C and pH 7.0 (Aebi 1974).

Histological examinations

Light Microscopy

The dissected tissue samples from small intestine, liver, kidney and lung were fixed in 10% formalin and embedded in paraffin. The tissues were cut into 5 µm sections and stained with Hematoxylin-Eosin (H&E) for histological examination with a light microscope (Nikon E-100, Japan). Systematic random samplings for morphological analysis were done from each group using ten cross-sectional areas and five slides. Damage of different parts of intestinal mucosa was evaluated in a blind test by two independent researchers. Mucosal lesions of the intestine were graded on a scale from 0 to 5 as described by Chiu *et al.* (Chiu *et al.* 1970) according to the following criteria: grade 0: normal mucosa; grade 1: development of subepithelial space at the apex of the villus; grade 2: extension of subepithelial space with moderate separation of mucosa; grade 3: extensive epithelial separation with a few denuded villi; grade 4: denuded villi with exposed dilated capillaries, and grade 5: disintegration of lamina propria with hemorrhagic ulceration.

Electron Microscopy

Samples of 1-2 mm in size from small intestine, liver, kidney and lung were fixed 4% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2-7.3) for 1 h in room temperature and after primary fixations, tissues were washed in 0.1 M phosphate buffer overnight. The tissues were postfixed with 1% osmium tetroxide in phosphate buffer for 1 h at +4°C. The postfixed tissues were then washed in 0.1 M phosphate buffer and dehydrated using graded ethyl alcohol and, finally, 1% osmic acid. Then they were processed by acetone and propylene oxide series. Finally, samples were embedded in Epon 812/Araldite resin. Thin sections were obtained by an ultramicrotome (Leica EM-UC 6, Germany) and collected on copper grids for double staining (Uranyl acetate and Reynolds'lead citrate). Stained sections were observed by a transmission electron microscope (FEI TecnaiTM G2 Spirit/Biotwin, serial no:12TN47B/1043) (Cerkezkayabekir *et al.* 2010).

Statistical analysis

Statistical analyses were performed using the SPSS 20 software. Data were expressed as the mean ± standard deviations (SD). The Kruskal Wallis test and Mann Whitney *U* were selected for multiple and paired comparisons respectively. Statistical significance was defined as $p < 0.05$.

Results

Biochemical Results

Significant changes occurred in antioxidant enzyme activities following ischemia and IR in the small intestine. In the IR group (Sham), while the SOD activity increased ($p < 0.05$), the GPx activity ($p < 0.05$) and CAT activity ($p < 0.05$) decreased. In the IR+Mesna group, the SOD activity decreased, and GPx and CAT activities increased and reached the control group levels. There was no significant difference between the IR+Mesna group and control groups in the small intestine in terms of SOD, GPx, and CAT enzyme activities (Table 1).

Table 1. Activities of SOD, GPx and CAT in small intestine, liver, lung and kidney

	Groups	SOD (U/ml)	GPx (U/L)	CAT (U/ml)
Small intestine	Control	158.61±1.07	15.93±1.40	237.50±23.37
	Ischemia	164.77±2.22[a]	14.30±4.56	261.56±28.62
	IR (Sham)	170.17±3.49[a,b]	8.27±1.19[a,b]	182.81±27.50[a,b]
	IR+Mesna	155.21±6.98[c]	11.50±3.16[c]	142.81±4.31[c]
Lung	Control	171.30±4.34	7.29±1.46	230.63±22.27
	Ischemia	171.81±5.32	11.77±3.33[a]	322.81±57.98[a]
	IR (Sham)	173.49±3.96	10.51±2.23[a]	328.75±55.66[a]
	IR+Mesna	168.61±2.35[c]	14.72±3.51[c]	332.81±24.03[a]
Liver	Control	191.60±1.94	50.18±6.62	874.37± 42.04
	Ischemia	188.07±3.89	57.18±12.04	475.94± 23.53[a]
	IR (Sham)	182.63±2.76[a]	64.12± 6.56[a]	446.56± 46.40[a]
	IR+Mesna	194.63±3.36[c]	47.17± 10.40[c]	483.43± 89.29[c]
Kidney	Control	183.86±7,30	37.01±4,72	300.31±27.20
	Ischemia	180.00±3.22	29.02±4.70[a]	340.93±32.80
	IR (Sham)	182.63±2.76	22.43±1.66[b]	357.81±35.94[a]
	IR+Mesna	181.96±5.99	23.97±2.87[a]	334.06±27.68[c]

Data are means±SD, n=8, $p < 0.05$, a represents comparison of control with other groups, b represents comparison of ischemia with IR group, c represents comparison of IR with IR+Mesna group.

The SOD and CAT activities decreased significantly, and the GPx activity increased in the liver in the IR group (Sham) ($p < 0.05$). In the IR+Mesna group, while the SOD and CAT activities increased significantly, the GPx activity decreased ($p < 0.05$). There was no significant difference in SOD and GPx activities between the IR+Mesna and control groups in the liver. However, the CAT activity was significantly lower in the IR+Mesna group compared to the control ($p < 0.05$) (Table 1).

The GPx activity decreased, and the CAT activity increased in the kidney in the IR group compared to the control, but the SOD activity remained the same. The GPx and CAT activities decreased in the IR+Mesna group ($p < 0.05$), but the SOD activity did not change (Table 1).

The GPx and CAT activities increased, and the SOD activity did not change significantly in the lung in the IR group compared to the control. The GPx and CAT activities increased, and the SOD activity did not change in the Mesna group compared to the IR group (Table 1).

Histological Results

Light Microscopy Findings

In all tissues examined, control groups were observed with normal histological structures (Table 2). The degeneration in the small intestine was assessed according to the Chiu score. According to the criteria of Chiu, the ischemia and IR group (Sham) had significant degeneration in the small intestine ($p < 0.05$). Histopathologic degeneration level was not significantly changed in the IR+Mesna compared to IR groups (Fig. 1).

In addition to the criteria of Chiu, it was determined that mononuclear cell inflammation and mucosa appeared to be flat, far from their characteristic structure (Fig. 2; 1st line, A, B, and C). In the IR+Mesna group, degenerative changes due to ischemia and reperfusion were observed to continue (Fig. 2, 1st line, D).

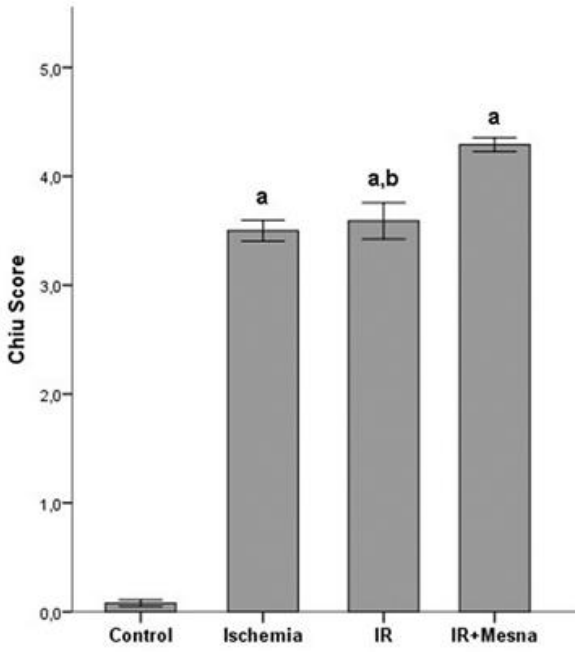

Fig. 1. The bars represent Chiu score of small intestine. **a** represents comparison of control between other groups ($p < 0.05$), **b** represents comparison of ischemia between IR groups ($p < 0.05$).

Electron Microscopy Findings

In all tissues examined, control groups were observed with the normal ultrastructural structures (Table 3). It was determined that irregularity in chromatin distribution, impairment in the integrity of cytoplasm, vacuolization, and deletion in the crista of mitochondria occurred in the small intestine both in the ischemia and IR groups (Sham) (Fig. 3; 1st Line, B). In the IR group, it was observed that the integrity of the nucleus and cytoplasm was also impaired (Fig. 3; 1st line, C). It was determined that in the IR+Mesna group, in addition to the IR group, the adhesion loss occurred between cells and the tissue integrity was impaired.

Table 2. Histopathologic findings from lung, liver and kidney by light microscopy.

	Ischemia (B) and IR (Sham) (C)	IR (Sham)+Mesna (D)
Lung (Fig. 2; 2nd line)	Mononuclear cell inflammation Regression of alveolar structure Increasing of connective tissue	Morphological changes in ischemia and IR groups are remained
Liver (Fig. 2; 3th line)	Mononuclear cell inflammation Degeneration of endothel Degeneration of hepatocytes Vacuolization	Stasis and leukocytic margination Mild inflammation
Kidney (Fig. 2; 4th line)	Expansion in Bowman's capsule Hypertrophy of epithelia of tubules Loss of tubular integrity	Hypertrophy of epithelia of tubules Loss of tubular integrity

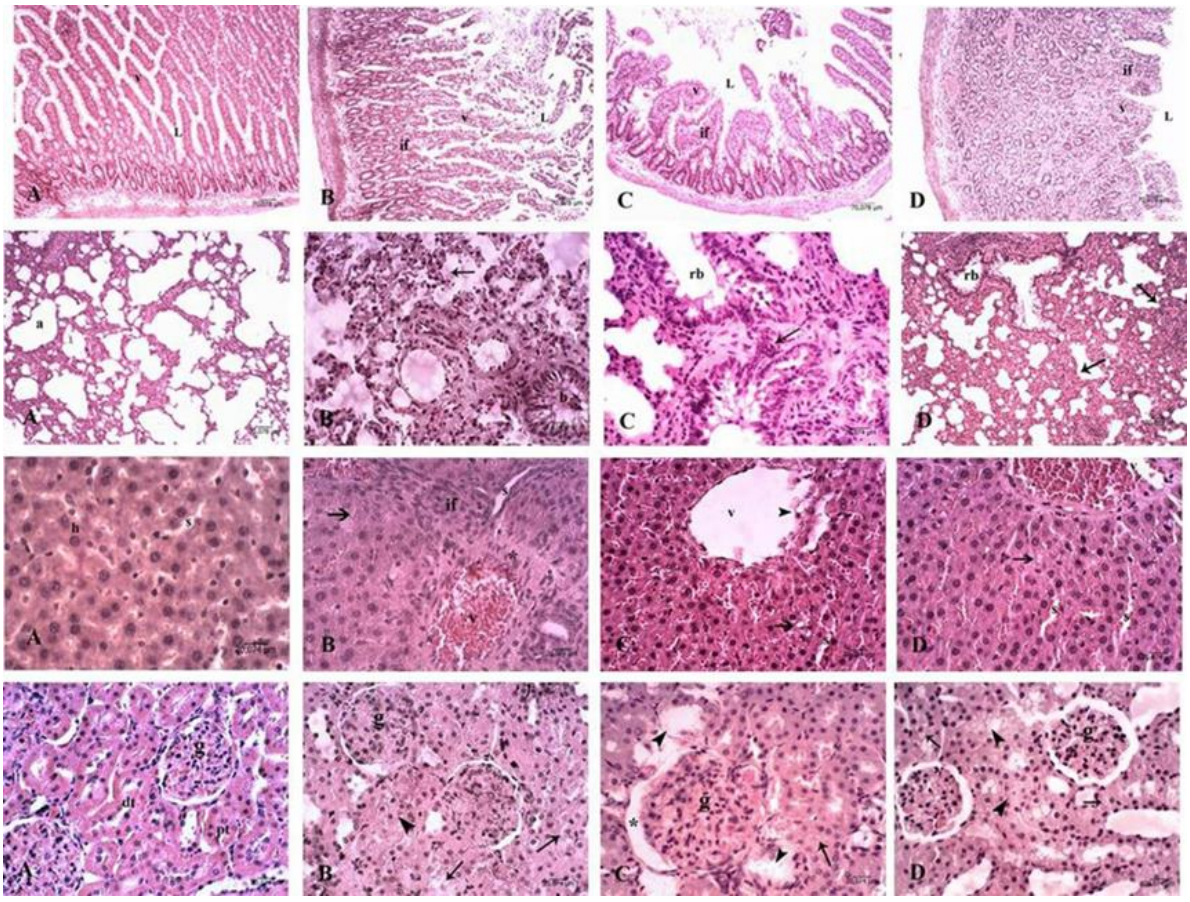

Fig. 2. Micrographs represent control (A), ischemia (B), IR (C) and IR+Mesna (D) groups from small intestine (1st line), lung (2nd line), liver (3th line) and kidney (4th line) by light microscope. In small intestine (1st line); V: villus, L: lumen, if: inflamation, in lung (2nd line); a: alveol, b: bronchiole, rb: respirator bronchiole, in liver (3th line); h: hepatocyte, s: sinuzoid, v: vein, if: inflammation, *: degeneration of hepatocyte, arrow: vacuolisation, arrow head: degeneration of endothel, in kidney (4th line); g: glomerulus, dt: distal tubule, pt: proximal tubule, arrow: hypertrophy of epithelia of tubules, arrow head: loss of tubular integrity, *: expansion in Bowman's capsule.

Table 3. Ultrastructural findings from lung, liver and kidney by electron microscopy.

	Ischemia (B) and IR (C)	**IR+Mesna (D)**
Lung (Fig. 3; 2nd line)	Irregularity in chromatin distribution Disruption of integrity between the nucleus and cytoplasm, Loss of cytoplasm around the nucleus and vacuolization Myelin figures	Disruption of integrity between the nucleus and cytoplasm, Loss of cytoplasm around the nucleus, Myelin figures
Liver (Fig. 3; 3th line)	Irregularity in chromatin distribution Loss of cytoplasm Vacuolization Deletion in mitochondrial crista Losses and irregularity in the Disse space of microvilli Loss of endoplasmic reticulum integrity Expanding between the inner and outer membranes of the nucleus	Ultrastructural changes in ischemia and IR groups are remained
Kidney (Fig. 3; 4th line)	Loss of cytoplasm, Deletion in mitochondrial crista, Irregularity in chromatin distribution, Loss of integrity in mitochondria, Cell membrane damage,	Disruption of integrity between the nucleus and cytoplasm, Loss of cytoplasm around the nucleus, Vacuoles from degenerated organelles

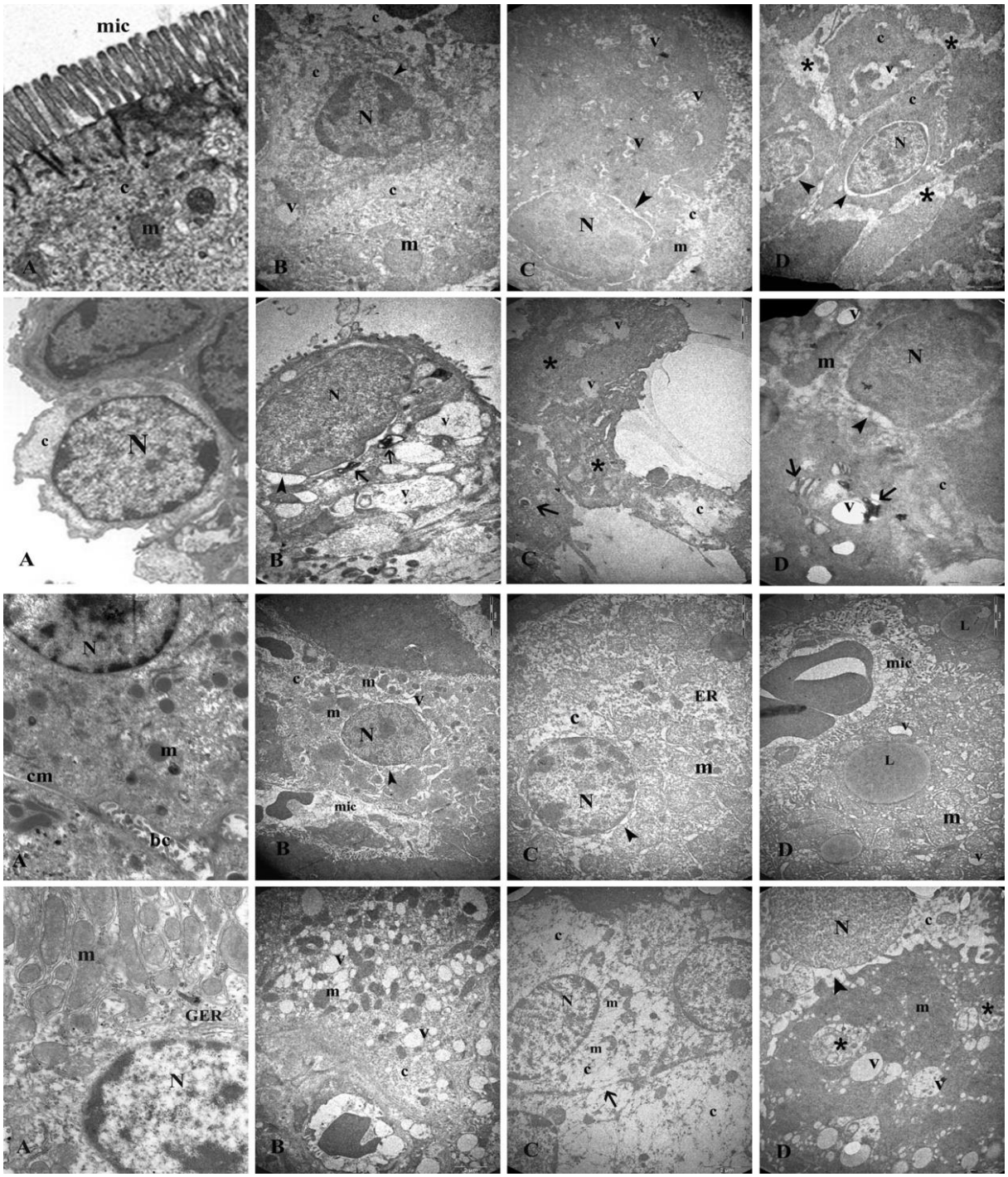

Fig. 3. Micrographs represent control (A), ischemia (B), IR (C) and IR+Mesna (D) groups from small intestine (1st line), lung (2nd line), liver (3rd line) and kidney (4th line) by electron microscope. In small intestine (1st line); mic: microvillus, m: mitochondria, c: cytoplasm, N: nucleus, v: vacuolisation, arrow head: integrity of the nucleus and cytoplasm, *: impairment in the integrity of cytoplasm, in lung (2nd line); arrow: myelin figures, in liver (3th line); cm: cell membrane, bc: bile canaliculi, mic: microvillus, ER: endoplasmic reticulum, L: lipid, in kidney (4th line); GER: granular endoplasmic reticulum, c: loosing of cytoplasm, *: degeneration of organelles. The scale bar corresponds to 1 nm in all micrographs.

Discussion

Intestinal ischemia and the following inevitable reperfusion are considered as an emergency condition in clinical practices. Cases treated in the early period and with an appropriate approach can be protected from local effects such as intestinal perforation and systemic effects such as shock. Unfortunately, despite the advanced technology of today, the rings of this complex chain of events have not been clearly revealed (Ypsilantis *et al.* 2006, Ypsilantis *et al.* 2008, Ayvaz *et al.* 2009, Wang *et al.* 2021).

Significant changes in the markers of oxidative stress in the ischemia and IR group (Sham) in all tissues

examined in this study that has been conducted by applying 2-hour intestinal ischemia and 2-hour reperfusion are the indicator that the IR model has been applied appropriately (Table 1). The light microscopy examination in the small intestine was assessed according to the criteria of Chiu and confirmed the results of the model. The electron microscopic examination as a gold standard method was performed to evaluate the effect of Mesna on both the small intestine and other tissues.

The biochemical and light and electron microscopy examinations in the small intestine and other distant organs were considered as degenerative results of ischemia. Thus we confirmed that the first step of our IR model has been accomplished. We observed the activity of antioxidant enzymes and histopathological changes to show the regenerative effect of Mesna as an antioxidant. Mesna treatment may provide a protection of the intestinal tissue, by virtue of its sulfhydryl group, which may act as a scavenger of reactive oxygen species. Many researchers have previously reported significant changes in the activities of SOD, CAT and GPX enzymes in intestinal ischemia triggered by various agents (Chen *et al.* 2008, Tunc *et al.* 2009, Yao *et al.* 2009, Ioannis *et al.* 2015, Borges *et al.* 2018, Gedik *et al.* 2018).

In the present study, SOD enzyme activity in ischemia and reperfusion groups was significantly increased as we expected depending on the results of previous similar studies (Tunc *et al.* 2009, Yilmaz *et al.* 2013). A significant reduction in SOD activity in tissues following the Mesna administration indicates that superoxide radicals are scavenged by SOD and that Mesna has a protective effect at the biochemical level.

GPx and CAT catalyze the reduction reaction of organic hydroperoxides or H_2O_2 to H_2O. It was observed that the GPx and CAT activity decreased significantly in the small intestine following IR. However, in the IR+Mesna group, the GPx activity increased and reached the control level, but the CAT activity remained unchanged compared to IR group. This result shows that H_2O_2 in the tissue is removed by the GPx activity, another H_2O_2 reducer, as reported in previous studies (Ayvaz *et al.* 2009, Tunc *et al.* 2009, Yildiz *et al.* 2010, Molina-Jijon *et al.* 2012, Ozturk *et al.* 2012). In contrary, it was reported previously in a study conducted with revastrol that the decrease in CAT level in IR was due to the fact that free radicals that occurred secondarily during IR were antioxidant and antilipoperoxidative (Yildiz *et al.* 2009). We think that there may be a similar effect by the GPx activity, scavenger of H_2O_2, following the Mesna treatment.

Microscopic evaluations performed in the intestine show that significant injury occurred in the ischemia and IR group (Sham) (Fig. 2). However, it was observed that this damage was not improved as expected in the IR+Mesna group and significant recovery in tissue morphology was not observed.

The SOD and GPx activities reached the control level in the liver, the CAT activity was not change significantly in the IR+Mesna. However, we observed by light and electron microscopy evaluations that Mesna did not have any regenerative effect on tissue morphology.

In the kidney, SOD and GPx activity did not change, but the CAT activity increased in the IR group. It can be considered that the kidney was less affected by the intestinal IR process than the liver. It was also determined that the CAT activity decreased to the control level by the effect of Mesna. It was observed in the microscopic evaluation that despite decreasing, the morphological damage continued in IR+Mesna group.

The SOD activity did not change, but the GPx and CAT activity increased in the lung in the IR group. It was determined that only the CAT activity decreased to the control level in the IR+Mesna group but the GPx activity reached a level higher than IR group. This finding indicates that, as in the small intestine tissue, the detoxification of H_2O_2 is performed by GPx instead of the decreased CAT activity. It was observed in the microscopic examinations that the morphological damage slightly decreased in IR+Mesna group but not fully recovered.

In previous studies evaluating the effects of Mesna on IR damage, the mechanism of the therapeutic property identified in the small intestine (Ypsilantis *et al.* 2008, Sanal *et al.* 2017, El-Baset *et al.* 2021) and different tissues (Yilmaz *et al.* 2013, Ioannis *et al.* 2015, El-Baset *et al.* 2021) was attributed to the antioxidant property of Mesna.

We also found that Mesna has a protective role in the axis of arginase activity, NO amount, iNOS and eNOS immunoreactivity in its intraperitoneal administration of 150 mg/kg single dose following I/R in the small intestine (Sanal *et al.* 2017).

It was reported that Mesna increases the levels of antioxidant enzymes such as GPx and SOD in brain damage in rat models, reduces nitric oxide, nitric oxide synthase, and xanthine oxidase levels and that its administration immediately after brain damage histopathologically protects the brain injury (Yilmaz *et al.* 2013). It was observed that when Mesna is administered alone orally or intraperitoneally in experimentally induced ulcerative colitis in rats, it significantly reduces the colorectal tissue damage (Ioannis *et al.* 2015).

Ypsilantis *et al.* (2008) suggested that its protective effect is associated with the inhibition of NF-kB activation of the intestinal mucosa (Ypsilantis *et al.* 2008).

The limitations of this study are the number of animals recommended by the local animal ethics committee and the lack of simultaneous testing of different doses of Mesna. There are several studies in which lower doses of Mesna were applied. Therefore, Mesna doses less than 150 mg/kg were not administered. According to the results of our study, we think that the therapeutic effect can be observed in more detail histologically in trials with higher doses of Mesna.

Conclusion

We, in the present study, examined, for the first time, the effects of Mesna both biochemically and ultrastructurally in IR process in terms of both small intestine and liver, kidney and lung as distant organs. It is clear, considering biochemical parameters, that the Mesna administration leads to therapeutic changes against intestinal IR injury. Biochemical parameters that improve in the early period may be associated especially with the oxygen radicals scavenging effect of Mesna. We believe that this study will make a significant contribution to the literature and will become a source for novel related studies of intestinal IR with ultrastructural assessments not found in other studies.

Ethics Committee Approval: This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Trakya University (TÜHDYEK-2010/10).

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