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Research Article

Effect of Gallic Acid on Distant Organ Stomach in Intestinal Ischemia Reperfusion Injury

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Abstract

Aim: To examine the effects of gallic acid (GA) in the stomach in rats undergoing intestinal ischemia/reperfusion (IR) with Beclin -1 immunostaining.

Material-Metod: 24 male Wistar albino rats were divided into control, IR and IR+GA groups. For the IR protocol, 60 minutes of ischemia and reperfusion were applied. The small intestines of the rats in the control group were removed by laparotomy and the abdomen was closed without applying anything else. Ischemia and reperfusion was applied to the superior mesenteric artery (SMA) for 60 minutes with a clamp in rats in the IR group. The blood flow was stopped with a clamp on the SMA of the rats in the IR+GA group and ischemia was applied for 60 minutes. 50 mg/kg gallic acid was given intraperitoneally to the animals, 60 minutes of reperfusion was performed, and the abdomen was closed. The stomach tissues were placed in paraffin blocks after routine histological follow-ups. Five micrometer-thick sections were taken from the paraffin blocks and stained with Hematoxylin-eosin and Beclin-1 immunostaining.

Results: In the IR group, degeneration of gastric folds, apoptosis of epithelial cells and degeneration of lamina muscularis were observed. In the IR + GA group, gastric folds improved, but cell infiltration in the lamina propria and degeneration in the muscular layer were observed. Beclin-1 expression was positively observed in the surface epithelial cells and gastric glands in the control group. In the IR group, it was observed that the gastric folds were positive on the surface cells and negative in the submucosa, while in the IR + GA group, it was intensely expressed in the gastric epithelium and gastric glands.

Conclusion: By increasing the expression of beclin-1, GA treatment induced autophagy in gastric mucosal cells, triggered the destruction of damaged cells and provided restoration of the mucosal layer.

Keywords: Beclin-1, gallic acid, apoptosis

INTRODUCTION:

Ischemia is a reversible or irreversible cell and tissue injury due to insufficient blood flow. This leads to the accumulation of some toxic metabolites and cell death with the decrease of cellular energy. Reperfusion is the re-establishment of blood flow for cell regeneration and clearance of toxic metabolites. When blood flow is restored in the same tissue, excessive amounts of free oxygen radicals are released and much more tissue damage occurs [1].

Intestinal ischemia-reperfusion injury (IRI) is a common and serious condition. This event can occur frequently in mesenteric embolism/thrombus, midgut volvulus, necrotizing enterocolitis, cardiopulmonary disease, intestinal transplantation, sepsis, trauma and shock [2]. Intestinal ischemia/reperfusion injury is not only caused by interruption of blood flow, but also by reactive oxygen species (ROS).), is a complex and multifactorial pathophysiological process involving the effects of reactive nitrogen species (RNS), inflammatory cytokines, nitric oxide (NO), and polymorphonuclear lymphocytes (PMNL). The resulting ROS (H2O2,O2-,OH-,NO-) changes intracellular molecules, damages cellular lipids, proteins and DNA, disrupts intestinal epithelial homeostasis and ultimately induces apoptosis [3, 4].

Intestinal ischemia/reperfusion injury is a serious condition with high mortality, including mucosal barrier damage and bacterial translocation, which initially occurs in the gut and also triggers systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS)[5]. Distant organ failure is a common complication after intestinal IR injury. Harmful substances such as ROS and RNS released after intestinal ischemia induce a systemic inflammatory response and may affect the function and integrity of distant organs such as the respiratory system, liver, heart and kidney[6].

Autophagy is a cellular mechanism that enables the cell to adapt and survive in physiological stress situations such as starvation, hypoxia, and energy depletion. While misfolded proteins and damaged organelles are eliminated by autophagy, cellular integrity is maintained by recycling of cellular building blocks and energy sources [7]. Autophagy is significantly increased under ischemia conditions and is associated with both beneficial and harmful effects. While autophagy functions as a cell death mechanism, it plays an important role in cell/tissue homeostasis by regulating

inflammation, oxidative stress and apoptosis in ischemic injuries and various diseases [8, 9]. Recent studies show that defective autophagy in some ischemic diseases potentiates proinflammatory cytokine production and causes accumulation of damaged cytoplasmic components such as mitochondria, resulting in excessive inflammatory response, oxidative stress and apoptosis. Therefore, autophagy activation was found to ameliorate ischemic injuries by directing the degradation of damaged intracellular components and recycling of amino acids to reduce cell death [10].

Gallic acid (3,4,5-trihydroxy benzoic acid) is one of the phenolic acids found in many plants such as green tea, grapes, raspberry, pineapple, banana, lemon, strawberry, and red wine. Gallic acid is known to have various properties such as antioxidant, antiviral, anti-inflammatory, anti-cancer. Numerous studies have shown that polyphenolic compounds exhibit a number of pharmacological properties in the gastrointestinal tract, act as anti-secretory, cytoprotective and antioxidant agents, and as a treatment for gastric ulcers. proved to be an alternative [11, 12]

Our aim in our work; The aim of this study is to detect the damage in the stomach in intestinal ischemia reperfusion and to examine the effect of gallic acid in preventing this damage.

MATERIAL AND METHOD:

Before starting the experimental procedure, 90 mg/kg intramuscular ketamine hydrochloride (Ketalar; Pfizer, Istanbul, Turkey) and 8 mg/kg xylazine (Rompun; Bayer, Istanbul, Turkey) were given general anesthesia. Three groups, eight rats in each group, were formed and the following procedures were applied to the groups.

1. Sham (control) group: All rats will be fixed on the operating table and kept in the supine position after anesthesia administration. After shaving the abdominal skin of the rats, it would be cleaned with an antiseptic solution and then laparotomy was performed with a midline incision. The superior mesenteric artery (SMA) was reached after the small intestines were reached by entering the abdominal cavity and the small intestines were temporarily removed. The abdomen was closed again without any other procedure.

2. IR group: All rats were operated after anesthesia administration.

He was fixed on the table and kept in the supine position. After shaving, the abdominal skin of the rats was cleaned with an antiseptic solution, and then laparotomy was performed with a midline incision. Afterwards, an atraumatic microvascular clamp was placed on the SMA, and blood flow was stopped, and then 60 minutes of ischemia and then 60 minutes of reperfusion was applied.

3.IR+GA group: All rats were fixed on the operating table after anesthesia and kept in the supine position. After shaving the abdominal skin of the rats, it was cleaned with an antiseptic solution and then laparotomy was performed with a midline incision. After entering the abdominal cavity, reaching the small intestines and temporarily removing the small intestines, the SMA was reached and the artery was carefully dissected and isolated from the surrounding tissues. Then, an atraumatic microvascular clamp was placed on the SMA, and blood flow was stopped, then ischemia was applied for 60 minutes. 50 mg/kg gallic acid was given to the animals intraperitoneally and reperfusion was performed for 60 minutes.

After all experimental stages were finished (at the end of the ischemia-reprfusion procedure), the animals were sacrificed by intracardiac blood collection under general anesthesia. The abdomen of the rats was opened and the stomach was resected. Tissues were stored in zinc-formalin solution for routine histological follow-up.

After the excised stomach tissues were fixed in zinc-formal for 72 hours, routine paraffin tissue follow-up was performed. 5 micron sections were taken from the obtained paraffin blocks with a rotary microtome, and Hematoxylin-Eosin counterstaining was performed to examine the tissue histopathology[13]. Vacuolization, interstitial edema, inflammation, vascular stasis, hemorrhage, leukocyte infiltration, epithelial degeneration parameters were evaluated. Statistical significance was analyzed by performing pathological scoring for these parameters

Semi-quantitative immunohistochemistry is a powerful method used to detect protein expression and localization in tissues. In this study, the method of Crowe et al. was exemplified, and free ImageJ Fiji (version 1.5; WS Rasband et al., National Institute of Health, USA, http://imagej.nih.gov/ij) software was used for semi-quantitative immunohistochemistry analysis [14]. In the program based on the contrast of chromogen DAB and hematoxylin counterstaining, the intensity of brown (expression areas) is determined and its ratio to the color intensity in the whole area is determined. In this way, Beclin1 expression in the groups was measured semi-quantitativel

All statistical analyzes were performed with IBM SPSS Statistics 25 (IBM®, version 25.0.0.0, US) software. The normal and homogeneous distribution of the data was analyzed with the analysis Shapiro-Wilk test. Kruskal Walllis and Mann Whitney U tests were used for comparison between groups. Those with $p<0.05$ were considered statistically significant.

RESULTS:

Statistical Findings:

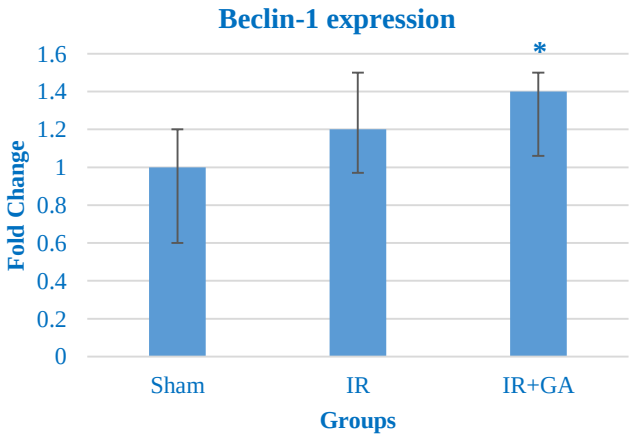

Figure 1: Beclin1 signal intensity in groups was analyzed with the ImageJ program. The expression variation between groups is shown in Figure X.

*: IR vs IR+GA

Histopathological Findings:

Histochemical analysis of the stomach is followed by hematoxylin-eosin staining in Figure 2. In the control group, it was determined that the gastric folds were smooth, the lamina propria and the lamina muscularis were regular, and the gastric glands were in normal distribution (Figure 2a). In the IR group, degeneration of gastric folds, apoptotic degeneration of epithelial and surface cells, loss of integrity in the epithelial layer, and degeneration of smooth muscle fibers in the lamina muscularis were observed (Figure 2b). In the IR+GA group, gastric folds improved, but cell infiltration in the lamina propria and degeneration in the muscular layer continued. Dilatation and congestion were observed in the vessels (Figure 2c). It can be said that gallic acid treatment is effective in the mucous layer of the stomach, but has a weak effect in correcting the pathologies in the other layers.

Beclin-1 immune reactivity of stomach tissues in the groups is shown in figure 3. In the control group, Beclin1 expression was positively observed in the surface epithelial cells and cells of the gastric glands. Negative Beclin-1 expression was observed in cells in the lamina propria (figure 3a). In the IR group, Beclin-1 expression was especially observed in the tunica mucosal layer. In the cytoplasm of the cells on the surface of the gastric folds (Beclin-1 expression was positive. Beclin-1 expression was negative in the tunica submucosa (Figure 3b). In the IR+GA group, Beclin-1 expression was intensely observed in the surface cells of the tunica mucosal layer and gastric glands. Beclin-1 expression was more intense than the other groups (Figure 3c).

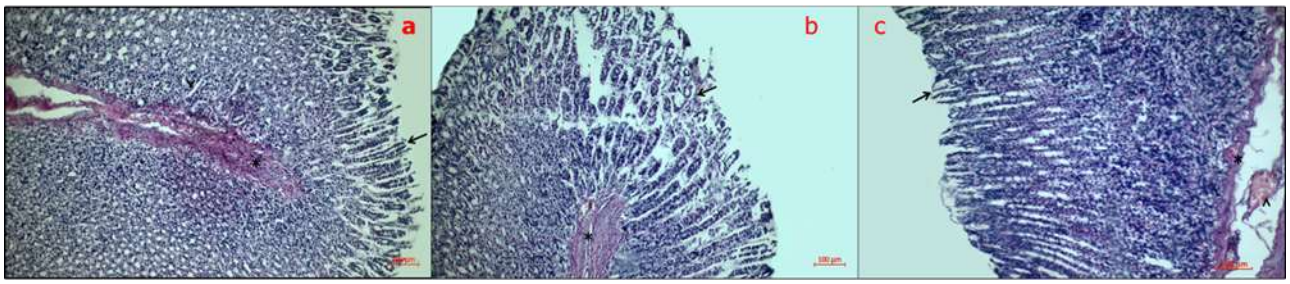

Figure 2. **hematoxylin-Eosin stainig**: a) Control group: gastric folds are smooth (arrow), lamina propria and lamina muscularis are regular (asterisk), and gastric glands are normal (arrowhead). b) IR group: loss of integrity in the epithelial layer (arrow), degeneration of smooth muscle fibers in the lamina muscularis (asterisk). c) IR+GA group: Gastric folds improved (arrow), dilatation and congestion were observed in vessels (arrowhead).

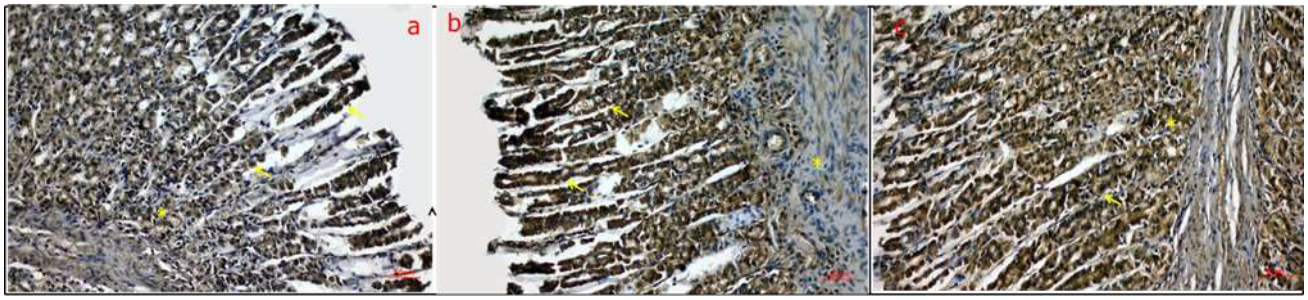

Figure 3. Beclin-1 immunostaining: a) Control group: positive in surface epithelial cells (arrow) and cells of gastric glands (asterisk). b) IR group: positive expression (asterisk) in the tunica submucosa, positive in the cytoplasm of cells on the surface of the gastric folds (arrow). c) IR+GA group: Positive expression in surface cells (arrow) and gastric glands (asterisk) in the tunica mucosal layer.

DISCUSSION:

The absence or low blood flow to tissues and organs is called ischemia (I), and their re-blooming is called reperfusion (R). Both ischemia and reperfusion can cause damage to tissues separately. The harmful effects of free oxygen radicals (ROS) formed as a result of ischemia are increased by reperfusion into the tissues [15]. Intestinal ischemia, whose mortality varies between 60-80%, is one of the most important abdominal emergencies [16].

Gallic acid (GA), a class of phenolic compounds also known as 3,4,5-trihydroxybenzoic acid, is found in a variety of plants, vegetables, nuts, and fruits [17]. GA, which is among the polyphenol groups, is a low molecular weight tri-phenolic compound with anti-inflammatory and anti-oxidative activities [18]. In addition, GA is known to have many distinct pharmacological effects, including anti-tumor, anti-bacterial, anti-diabetes, anti-obesity, anti-microbial and anti-myocardial ischemia [19]. GA anti-inflammatory effect; It has been revealed that it inhibits proinflammatory cytokines IL1-6, TGF beta and TNF alpha, and inhibits the NF-Kb pathway and increases IL4 release [20]. Again, the antioxidant and antiapoptotic effect of GA; preventing cellular damage caused by ROS, glutathione peroxidase (GPX) expression, attenuate the presence of free radicals, and its ability to regulate Bax, Bcl-2 and Caspase-3 [19, 21].

Nouri, A. et al. applied a toxic substance to the kidney and then examined the effects of GA. As a result of the experiment, it was observed that GA had anti-inflammatory and antioxidant effects on the kidney and protected the kidney [17]. In an experiment by Lei Zuhu et al. on 60 male mice, it was shown that when GA was added to the treatment of mice with ulcerative colitis, the viability of intestinal epithelial cells increased and it helped to treat colitis by showing an anti-inflammatory effect [19]. Again, in a study in rats, the anti-inflammatory and anti-apoptotic effects of gallic acid against

mucosal inflammation and erosions caused by gastric ischemia-reperfusion were investigated. It was concluded that gallic acid had a protective effect against ischemia-reperfusion injury of the gastric mucosa by decreasing the protein expression of iNOS and caspase-3 [22]. As a result, cellular damage occurred in the gastric mucosa. GA administration had important roles in reducing inflammation and preventing partial cell degeneration by interfering with the apoptotic process. In the treatment group, it was observed that gastric folds improved, but cell infiltration in the lamina propria and degeneration in the muscular layer continued. Dilatation and congestion were observed in the vessels. It can be said that drug treatment is effective in the mucous layer of the stomach, but has a weak effect in correcting the pathologies in the other layers.

Beclin-1 is one of the main proteins involved in the autophagy process. Beclin-1 plays important roles in many cellular processes such as the initiation and development of autophagy, endocytosis, adaptation to stress, aging and cell death. Beclin-1 is localized in the intracytoplasmic endoplasmic reticulum, mitochondria and nuclear membrane. Beclin-1 mainly regulates autophagy by forming a complex with proteins such as class III phosphatidylinositol-3 kinase (PI3K III) and Bcl-2 [23, 24]. In the immunohistochemical examination of Zeng et al. after cardiac IR, it was observed that Beclin-1 expression increased intensively in the ischemia area [25]. In recent studies, intestinal IR damage was created and Beclin-1 expression was shown to increase significantly in the ischemia group [26].In our study, mild Beclin-1 expression was observed in the gastric glands and gastric epithelial cells in the control group, while intense Beclin-1 expression was observed in the intestinal ischemia group. It was observed intensely especially in the surface cells of the tunica mucosa layer and gastric glands.

CONCLUSION:

Beclin-1 is an important regulator of autophagy. In conclusion, GA treatment increased the expression of Beclin-1 and induced autophagy in gastric mucosal cells, triggering the destruction of damaged cells and providing restoration of the mucosal layer. More studies are needed on distant organ damage of GA.

Conflict of Interest

The author declared that there was no conflict of interest during the cause of this study and producing and submitting this manuscript for publication.

Author contribution

S.O.B, O.K. and F.A contributed equally to manuscript drafting, writing, data collection, conceptualization and observation. All authors read and approved the final version of the manuscript.

Data availability

All generated data were presented in this study.

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