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Research Article

Alpha-lipoic acid and its protective impact against gastric ischemia-reperfusion injury in rats

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Abstract
Purpose: *Gastric ischemia-reperfusion (IR) injury is a pathological event starting with tissue deoxygenation and then proceeding with the production of free oxygen radicals (FOR). For this reason, antioxidants are tested within the scope of gastric IR injury treatment. α-Lipoic acid (ALA) to be investigated in this study in terms of its protective effect against gastric IR injury is an endogenous antioxidant molecule synthesized from octanoic acid and cysteine in the cells. The aim of this study was to investigate the effect of ALA on IR induced gastric injury in male albino Wistar rats biochemically and histopathologically.*
Materials and Methods: *In the ALA+gastric ischemia-reperfusion group (AGIR) group, 100 mg/kg ALA was administered orally through a catheter. In the gastric ischemia-reperfusion group (GIR) and sham-operated group (SG), distilled water was administered as solvent. After 30 minutes of ALA and distilled water administration, all of the animals kept under anesthesia by intraperitoneal (ip) 25 mg/kg sodium thiopental sodium application and making the rats sniff xylazine at appropriate intervals. In order to induce IR lesions, the celiac artery of the animals in the AGIR and GIR groups was compressed with a clip to provide one hour of ischemia and three hours of reperfusion. The celiac artery of the SG group was closed with stitches without clipping. Then the rats were killed with a high dose (50 mg/kg) thiopental anesthesia. The biochemical and histopathological analyses were conducted on the gastric tissues removed from the killed rats.*
Results: *An increase for the amount of malondialdehyde (MDA), and a decrease in endogenous total antioxidant glutathione (tGSH) and superoxide dismutase (SOD) levels were found in the tissue of gastric treated with GIR. ALA prevented the increase in MDA and decrease in tGSH and SOD levels in gastric tissue caused by IR. In addition, ALA alleviated severe gastrointestinal hemorrhage, polymorpho nuclear leukocyte infiltration, edema, dilated congested blood vessels and destruction in the stomach caused by IR.*
Conclusion: *These findings indicate that ALA protects stomach against oxidative damage of IR. It has been suggested that ALA could beneficial in the treatment of gastric IR injury.*
Keywords: *Alpha-lipoic acid, gastric ischemia-reperfusion, protective impact.*

1. Introduction

Ischemia-reperfusion (IR) injury which is a pathological process begins with tissue deoxygenation and continues with the production of free oxygen radicals (FOR) [1]. Since most of the FORs causing lipid peroxidation (LPO) are produced during the reperfusion period. They are known as reperfusion mediators. Therefore, cell membrane lipids are mainly oxidized during the reperfusion period[2]. As is known, LPO analysis is evaluated by the measurement of malondialdehyde (MDA) amount because MDA is the final product of LPO event[3]. Polymorphonuclear leukocytes (PNL) are also crucial in exacerbating reperfusion injury; PNLs release NADPH oxidase, elastase and myeloperoxidase enzymes with oxidant activity found in the content of azurophilic granules[4]. PNLs are significant in the formation of lesions after gastric IR and accumulate in gastric mucosa after injury[5].

In clinical practice, gastric IR damage can be seen in a variety of pathological conditions including vascular rupture, gastrointestinal disease, and hemorrhagic shock in various surgical interventions[6]. Gastric IR injury is a critical and significant problem that develops in more than 80% of patients undergoing surgical procedure. Gastric IR may cause multiple organ failure and death as a result of ceased blood supply to an organ or tissue and subsequent blood flow to the affected region[7]. For this reason, studies on the treatment of gastric IR injury have been carried out. All of this information suggests that drugs with antioxidant activity could be effective in the treatment of gastric IR damage.

ALA to be examined in this study for its protective effect against gastric IR injury has been synthesized from octanoic acid and cysteine in the mitochondria of plants and animal cells. It plays the role of the cofactor of pyruvate dehydrogenase in addition to α-ketoglutarate dehydrogenase enzymes in the body. Both reduced and oxidized forms of ALA have been reported to have antioxidant properties[8]. Antioxidant activities related to ALA cover FOR's direct inactivation and the renewal of endogenous antioxidants (endogenous glutathione)[9]. This information indicates that ALA can protect gastric tissue against possible IR damage. None of the studies have investigated the impact of ALA on gastric IR injury in the relevant literature. Therefore, the purpose of this research was to examine the effect of ALA on IR-induced gastric injury in rats biochemically and histopathologically.

2. Materials and Methods

2.1 *Experimental Animals*

18 Albino Wistar rats weighing between 280-295 grams were used in the experiment. The experimental animals were obtained from Ataturk University Medical Experimental Application and Research Center. The rats have been housed and fed in groups under appropriate room temperature (22 °C) and at proper conditions prior to the experiment. The study was carried out according to the National Guidelines for the Use and Care of Laboratory Animals. Moreover the approval for the study was obtained from the local animal ethics committee in Ataturk University, Erzurum, Turkey (Ethics Committee Number: 1-4. Dated: January 31, 2019)

2.2 *Chemicals*

Alpha lipoic acid which was preferred in the study was supplied from Solgar (United States), and thiopental sodium was supplied from İ.E ULAGAY (Turkey).

2.3 *Experimental Groups*

Animals have been categorized in three different groups: Gastric IR group in which ischemia-reperfusion was induced in the stomach (GIR), 100 mg/kg ALA + gastric ischemia reperfusion group (AGIR), and sham-operated group (SG).

2.4 *Experimental Procedure*

In the AGIR group, 100 mg/kg ALA was administered into the stomach orally through a catheter. In the GIR and SG groups, same volume of distilled water was administered as solvent with the same method. After 30 minutes of ALA and distilled water administration, all the experimental

animals were anesthetized by intraperitoneal (ip) 25 mg/kg sodium thiopental sodium application and animals were allowed to breath in xylazine at proper intervals. Then the animals were taken to the surgery. The period when the rats remained stable in the supine position was considered as the proper period for surgical intervention (10). For the entire period, all of the animals underwent laparotomy with a 2.5 cm long midline incision under sterile conditions. In order to induce IR lesions, the celiac artery of the animals in the AGIR and GIR groups was compressed with a clip and ischemia was induced for 1 hour. The celiac artery of the SG group was not clipped and the incision was sutured. Afterwards, the clips were removed and reperfusion was induced for 3 hours. Afterwards, all the rats were killed by high dose (50 mg/kg) thiopental anesthesia. Biochemical and histopathological analyses have been carried out on the gastric tissue removed from the animals which were killed at the end of the experiment.

2.5 Biochemical procedures

2.5.1 ***Malondialdehyde (MDA) analysis***

The method of Ohkawa H et al. used for MDA measurement[11] was based on the spectrophotometric measurement (at a wavelength of 532 nm) of the absorbance of the pink colored complex created by thiobarbituric acid (TBA) and MDA at a high temperature (95 °C). Centrifugation was performed on the homogenates at 5000 g for 20 minutes and these supernatants were used to determine the amount of MDA. 250 µl of homogenate, 100 µl of 8% sodium dodecyl into the test tubes and then they were vortexed. Incubation was performed on the mixture at 100 °C for 60 minutes, then 2.5 ml of n-butanol was added into the mixture and spectrophotometrically measured. The amount of red color formed was read at 532 nm using 3 ml cuvettes and the amount of MDA of the samples was sulfate (SDS), 750 µl of 20% acetic acid, 750 µl of 0.08% TBA and 150 µlof distilled water were pipetted identified while taking into account the dilution coefficients by using the standard graph prepared from the previously prepared MDA stock solution.

2.5.2 ***Total Glutathione (tGSH) analysis***

The measurement of GSH in total homogenate was carried out by using the method prepared by Sedlak and Lindsay with minor changes[12].Then the weight of the sample was measured and, it was homogenized in 2 mL of 50 mmol/L Tris–HCl buffer with 20 mmol/L Ethylenediaminetetra acetic acid (EDTA) and 0.2 mmol/L sucrose at pH 7.5. The mixture was precipitated with 0.1 mL of 25% trichloroacetic acid. Afterwards, the precipitate was centrifuged at 4200 rpm for 40 min at 4 °C temperature. Supernatant was preferred in order to identify the level of GSH. 1500 µL of measurement buffer (200 mmol/L Tris–HCl buffer containing 0.2 mmol/L EDTA at pH 7.5), 500 µL supernatant, 100 µL DTNB (10 mmol/L) and 7900 µL methanol were mixed and then vortexed. The mixture was exposed to incubation for 30 minutes in 37°C. 5.5-Dithiobis (2-nitrobenzoic acid) (DTNB) was used as a chromogen. The color was yellow with sulfhydryl groups. The measurement of absorbance was carried out at 412 nm by means of a spectrophotometer (Beckman DU 500, USA). Finally, a standardized curve was found by means of decreased glutathione.

2.5.3 ***Superoxide dismutase (SOD) analysis***

The method of Sun et al was used for all of the measurements[13]. Xanthine is converted into uric acid using xanthine oxidase. When nitro blue tetrazolium (NBT) was mixed into the reaction, SOD reacted with NBT. The color of the formazan dye became purple. The weight measurement was carried out and the mixture was homogenized in 2 ml of 20 mmol/L phosphate buffer containing 10 mmol/L EDTA at pH 7.8. Then centrifugation was performed at 6000 rpm for 10 minutes. The supernatant was preferred as the sample of assay. The sample containing 2450 µL mixture (0.3 mmol/L xanthine, 0.6 mmol/L EDTA, 150µmol/L NBT, 0.4 mol/L Na_2CO_3, 1 g/l bovine serum albumin), 500 µL supernatant and 50 µL xanthine oxidase (167 U/l) was vortexed.

Afterwards the incubation was carried out for 10 minutes. Formazan was formed as the result of the reaction. The measurement of absorbance was made on the formazan in purple colorat 560 nm. If there were more enzymes, there would be a decrease in $O2^{-}$ radical reacting with NBT.

2.6. Statistical Analysis

The results were expressed as "mean ± standard error of mean" (x ± SD). The significance of difference between the groups was determined using one-way analysis of variance (ANOVA) test followed by post-hoc Bonferroni test. All statistical analyses were performed with "SPSS statistical software" (Version 18, IBM Corporation, Armonk, Y, USA) and p values < 0.05 were considered significant.

2.7. Biochemical Findings

2.7.1 *MDA, tGSH and SOD analysis results*

According to Table 1 the amount of MDA in the IR-treated gastric tissue increased significantly compared to the sham and ALA-treated groups (p<0.001). However, the amount of MDA in the gastric tissue of the animals treated with ALA was similar to the sham group (p=0.046).

Table 1 Effects of ALA on oxidative biochemical parameters in the gastric tissue of experimental groups

Parameters	SG (mean ± SD)	GIR (mean ± SD)	AGIR (mean ± SD)
MDA (µmol/g protein)	1,22±0,15	3,43±0,28*	1,57±0,22
tGSH (nmol/g protein)	3,28±0,54	1,17±0,16*	2,32±0,23
SOD (Umg protein)	7,33±0,56	3,67±0,54*	6,65±0,44**

MDA: Malondialdehyde; tGSH: Glutathione peroxidase; SOD: Superoxide dismutase. N=6 for all groups andP value of ANOVA test, *p<0.001 compared to SG and AGIR groups, ** p>0,05 compared to SG group.

Similarly, IR procedure led to a fall in tGSH in gastric tissue. The amount of MDA in the IR-treated gastric tissue increased significantly compared to the sham and ALA-treated groups (p<0.0001).

IR procedure resulted in decreased SOD activity in gastric tissue. However, ALA significantly prevented the decrease of SOD activity in the gastric tissue of animals treated with IR (p<0.001). The difference in SOD activity between the group treated with ALA and the sham group was found to be statistically insignificant (p> 0.05).

2.8 Histopathological findings

According to Figure 1, no histopathological findings were found in the gastric tissue of the sham group.

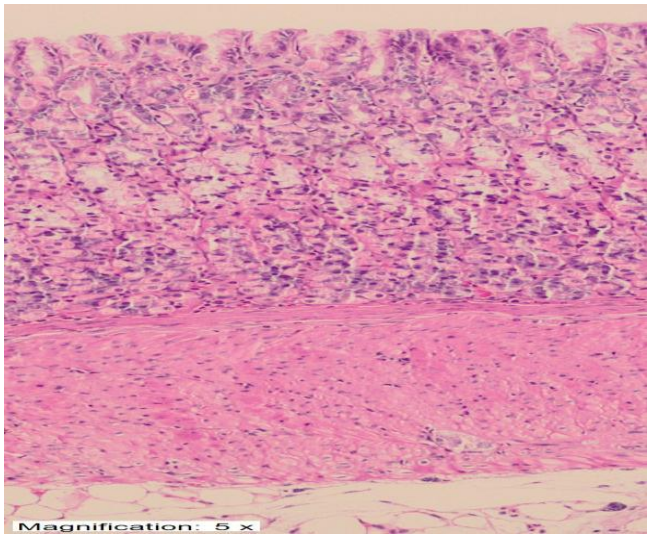

Figure 1 Gastric tissue section of sham (SG) group (HEX-100)

However, severe hemorrhage, PNL infiltration, edema and destruction were found in the gastric tissue of the GIR group treated with IR (Figure 2).

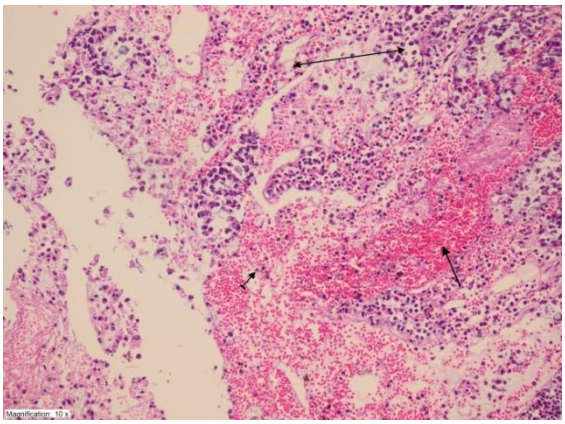

Figure 2 Section showing hemorrhage (straight arrow), PNL infiltration (striated arrow), edema and destruction (double arrow) in gastric tissue of IR induced GIR group (HEX-200)

Furthermore, severe dilated congested blood vessels were observed in the GIR group (Figure 3).

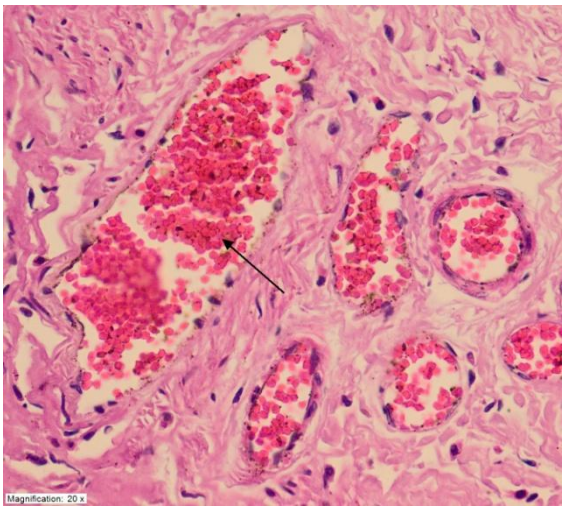

Figure 3 Section showing severe dilated congested blood vessels (straight arrow) in the IR induced GIR group (HEX-400)

However, there was no pathological finding in the gastric tissue of the ALA-treated AGIR group excluding mild dilated congested blood vessels (Figure 4).

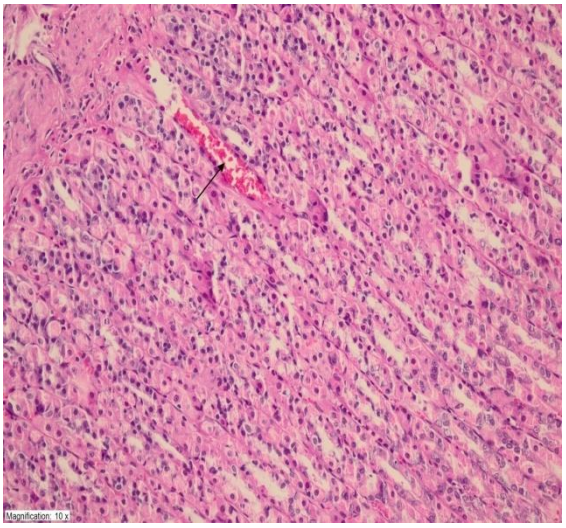

Figure 4 Section showing mild dilated congested blood vessels in the gastric mucosa tissue in the AGIR group (HEX-200)

3. Discussion

The impact of ALA on experimental oxidative gastric injury induced by IR was investigated biochemically and histopathologically on experimental animals in the study. Today, gastrointestinal (GIS) IR injury is a clinical issue related to high morbidity and mortality[14]. This is due to the fact that the stomach is one of the tissues most susceptible to ischemia and that the excessive FORs produced by reperfusion cause damage through severe LPO and neutrophil infiltration in the cell membrane[5]. In literature, it has been suggested that LPO and FORs were essential in the pathogenesis of acute gastric mucosal injury caused by IR[15,16]. According to the results obtained, pretreatment with antioxidants such as allopurinol, superoxide dismutase, catalase, dimethylsulfoxide and alpha-tocopherol has been shown to decrease IR-associated damage: this information confirms that FORs are the major component in the pathogenesis of gastric IR injury[17].

Being in consistency with the literature, our results have revealed that the amount of MDA in IR treated tissue significantly increased compared to healthy tissue and ALA treated tissue. ALA significantly prevented the MDA increase in gastric tissue caused by IR. MDA is an oxidant parameter that maintains and exacerbates the damaging effect of FOR on membrane lipids by causing cross-linking and polymerization of membrane components[18]. Studies on the antioxidant action mechanism of ALA in literature have revealed that the antioxidant activity of ALA is due to the dithiolene ring in its structure. It has been emphasized that this antioxidant effect was achieved through FOR capture, chelating with metals, and increasing the reusability of other antioxidants[19]. ALA has also been reported to purify FOR and nitrogen species such as hydrogen peroxide, hydroxyl radical, hypochloric acid, and peroxynitrite[20].

Moreover, another parameter showing that the IR process induced oxidative stress in gastric tissue and ALA suppressed oxidative stress was tGSH. It is used to determine the antioxidant activity of drugs used against gastric IR oxidative damage [21]. GSH is a tripeptide consisting of L-glutamate, L-cysteine, and glycine found in many cells. Catalyzed by the enzyme glutathione peroxidase (GPx) which contains selenium in its active zone, GSH reacts with H_2O_2 and organic peroxides and shows antioxidant activity by removing H_2O_2 from the cells. GSH chemically detoxifies H_2O_2 and

organic oxides and protects cells from FOR damage. There are no studies examining the effect of ALA on IR-induced gastric tGSH levels in literature. However, it has been reported that ALA protects liver tissue from oxidative damage of methotrexate by slowing down the depletion of GSH[22]. Our results showed that oxidative stress was suppressed in the gastric tissue of the ALA treated animal group and these results were consistent with the literature. Furthermore, we also found that SOD enzyme activity significantly decreased in the gastric tissue of the animals exposed to IR process compared to healthy and ALA treated animals. As is known, SOD is the enzyme catalyzing the conversion of superoxide to hydrogen peroxide and molecular oxygen. SOD is present in almost all living organisms and catalyzes the dismutation reaction of superoxide[23]. Various studies have noted that SOD decreased gastric IR oxidative damage[24] confirming the findings of the study were in compliance with the information obtained in relevant researches.

Biochemical test results obtained from IR applied gastric tissue in this study overlap with histopathological findings. In the IR applied gastric tissue, where MDA was found to increase, and tGSH and SOD were found to decrease, there was severe hemorrhage, PNL infiltration, edema, destruction, and severe dilated congested blood vessels. In a previous study, similar histopathological symptoms were reported in gastric tissue where oxidant parameters were high and antioxidant parameters were low[25]. Liu Y et al. reported that an antioxidant drug that suppressed the increase of IR-related MDA production in gastric tissue also improved the histopathological damage[26]. In addition, it has been shown histopathologically that the prevention of antioxidant loss protects the stomach from oxidative damage of IR[27]. No pathological findings were found in the gastric tissue of the ALA-treated group except for mildly dilated congested blood vessels. There are no previous studies investigating the effect of ALA on IR-associated gastric injury. However, there are studies showing that ALA suppresses IR damage in different tissues [28-29]. In conclusion, IR process has been biochemically and histopathologically shown to cause oxidative damage in stomach tissue. It was found that ALA prevented the oxidative damage caused by IR in the stomach. These results obtained from our experiment suggest that ALA may be beneficial l in the treatment of IR associated gastric injury.

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Conflicts of Interest: None

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