Experimental research

The role of L-carnitine in acetyl salicylic acid-induced acute gastric mucosal injury in rats

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Abstract

Introduction: The aim of this study was to determine the protective effects of L-carnitine on acetyl salicylic acid (ASA)-induced acute gastric mucosal injury through oxidant/antioxidant parameters and histopathological alterations in rat gastric tissues.
Material and methods: Forty-two rats were randomly assigned to six groups: The control group received 1 mg/kg distilled water, while the other groups were pretreated with L-carnitine 50 mg/kg/day (LC), pantoprazole 40 mg/kg/day (PPI), ASA + LC (50 mg/kg/day), and ASA + PPI (40 mg/kg/day), for 21 days, respectively. On day 23, gastric mucosal injury was induced by a single intragastric administration of 600 mg/kg aspirin in ASA, ASA + LC, and ASA + PPI groups. The animals were killed 60 min after the administration of aspirin. The stomach of each animal was removed. Gastric mucosal injury was scored histopathologically (ulcer score). Tissue catalase (CAT), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) activities, and malondialdehyde (MDA) and nitric oxide (NO) levels were also measured.
Results: The ulcer score increased significantly in the ASA group, but this increase was not significantly inhibited by the administration of L-carnitine (2.71 ±1.0 vs. 2.57 ±0.5, $p = 0.965$). The CAT and GSH-Px activities were significantly reduced, whereas MDA and NO levels were significantly increased in the ASA group. Pretreatment with L-carnitine did not alter CAT or GSH-Px activities, but reduced MDA and NO levels insignificantly ($p = 0.204$ and $p = 0.277$, respectively).
Conclusions: Long-term administration of L-carnitine did not improve the oxidative and histological parameters of acute gastric mucosal injury induced by ASA.

Key words: lipid peroxidation, L-carnitine, oxidative injury, acetyl salicylic acid, anti-oxidant.

Introduction

Acetyl salicylic acid (ASA) is a potent non-steroidal anti-inflammatory drug (NSAID) used for the treatment of rheumatological disorders and more recently primary and secondary prevention of cardiovascular

events. The main concern regarding the use of aspirin is the potential for developing serious gastrointestinal (GI) events, such as GI bleeding [1]. Accumulating information indicates that reactive oxygen species (ROS) and oxidative injury might be possible underlying factors in the pathogenesis of mucosal injury [2]. Upper GI injury from ethanol [3], ischemia/reperfusion [4], *Helicobacter pylori* [5], and stress [6] can all be linked to an ROS-producing mechanism. The GI tract has the capability to produce large amounts of ROS via mucosal oxidases found in resident leukocytes of the lamina propria [7]. Physiologic antioxidant systems against these detrimental factors include antioxidant enzymes, such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px), and free-radical scavengers. A few, such as carnosine, glutathione, and uric acid, are synthesized by living cells, whereas most others are derived from food [8]. Therefore, the imbalance between the detrimental and defensive factors appears to be critical in the development of mucosal lesions in the GI tract.

Carnitine is a hydrophilic molecule that plays an essential role in the transfer of long-chain fatty acids across the inner mitochondrial membrane in which β-oxidation occurs via the citric acid cycle [9], and therefore it has been used to treat organic acidemias [10]. It was first characterized in muscle extracts in 1905 and named from the Latin *carnis* (flesh) [11]. The chemical structure later was shown to be 3-hydroxy-4-(*N*-trimethylammonio) butanoate [12], and in 1962, the biologically active form of carnitine was identified as the L(–) stereoisomer, or levocarnitine [13]. L-carnitine is also endogenously synthesized from trimethyllysine (using the essential amino acids lysine and methionine) in humans at a rate of 1–2 mol/kg/day [13, 14]. The enzyme responsible for the hydroxylation required in the last step of synthesis is only present in human kidney, liver, and brain [9]. Given its essential role in metabolism, L-carnitine plasma and tissue levels are maintained within a narrow homeostatic range that is controlled by GI absorption, endogenous biosynthesis, renal tubular reabsorption, and compartmentalization through carrier-mediated transport between plasma and tissues [14, 15].

Though the therapeutic benefits of L-carnitine and its ester congeners have yet to be clarified, they have exhibited many pharmacological effects. Carnitine therapy has been used to treat patients with heart failure; typical benefits include improved exercise capacity and peak oxygen consumption, reduced fatigue, and improved muscle conditioning [16]. Carnitine also shows potential benefits in the management of other cardiovascular diseases, including peripheral vascular disease, congestive heart failure, angina, cardiac arrhythmias, and anthracycline-induced cardiotoxicity [17, 18]. Studies also reported beneficial effects of L-carnitine on skeletal muscle functions, including reduced muscle cramps, improved exercise capacity, increased muscle strength and mass, decreased asthenia and dyspnea, and increased peak oxygen consumption [19].

It is known that L-carnitine and its derivatives prevent the formation of ROS, scavenge free radicals, and protect cells from peroxidative stress [20–24]. The beneficial effects of L-carnitine on ethanol- and stress-induced gastric mucosal injury in rats have been shown by several experimental studies [21, 25]. Among NSAIDs, L-carnitine was used in an indomethacin-induced gastric injury preliminary rat model and found to be effective [26]. However, no study has been reported for ASA-induced gastric mucosal injury despite its worldwide usage.

Therefore, it was reasonable to hypothesize that L-carnitine might provide protection against ASA-induced gastric mucosal injury. The aim of the present study was to assess the possible gastroprotective effect(s) of L-carnitine in a rat model of ASA-induced gastric mucosal injury and to elicit the potential underlying mechanisms. For this purpose, we studied the role of L-carnitine in oxidative stress by measuring alterations in CAT, GSH-Px and SOD activities. We also evaluated malondialdehyde (MDA) level as a marker of lipid peroxidation and nitric oxide (NO) level for endothelial functions. In addition, gastric mucosal injury was scored histopathologically for each sample (histological ulcer score).

Material and methods

Chemicals and drugs

L-carnitine (SIC0283-5G, L-carnitine hydrochloride, synthetic, ≥ 98%), β-nicotinamide adenine dinucleotide phosphate, and glutathione reductase were purchased from Sigma Chemical (St Louis, MO, USA). Chloroform, ethanol absolute, trichloroacetic acid, hydrochloric acid, sulfanilamide, Folin-Ciocalteu's phenol reagent, glutathione reduced and 2,4-dinitrophenylhydrazine were all purchased from Merck (Darmstadt, Germany). Pantoprazole (Pantpas 40 mg) was obtained from Bayer, and ASA (Asinpirine 300 mg) was obtained from Ibrahim Etem Ulagay.

Animals

The study protocol was approved by the Local Animal Care and Use Committee with the project number P-53010801-1. All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1978). Forty-two male Wistar albino rats,

each weighing about 250–280 g, were used. The animals were housed at 22 ±1°C on a 12-h day-night regimen and received a standard diet and water ad libitum.

Experimental design

The rats were randomly allocated to six groups, each consisting of 7 rats, with different pretreatments as follows: group 1 (control), 1 ml distilled water was given orally to control rats by intragastric gavage for 21 days; group 2 (ASA) received a standard diet and water ad libitum for 21 days (similar as in all study groups), and subsequently received ASA 600 mg/kg by intragastric gavage after 36-hour fasting (on day 23); group 3 (LC), L-carnitine 50 mg/kg/day was suspended with 1 ml of 0.9% NaCl and was administered by intragastric gavage to the rats for 21 days; group 4 (PPI), rats were treated with pantoprazole 40 mg/kg/day by intragastric gavage for 21 days; group 5 (ASA + LC), the rats were pretreated with L-carnitine 50 mg/kg/day for 21 days, followed by a single intragastric 600 mg/kg ASA administration after 36-hour fasting (in day 23); and finally group 6 (ASA + PPI), pantoprazole 40 mg/kg/day was administered for 21 days, and subsequently the animals were given ASA 600 mg/kg by intragastric gavage after 36-hour fasting (on day 23).

On day 22, all animals were deprived of food for 36 h prior to the experiments, but were allowed free access to water. Before the administration of ASA, the rats in groups 3 and 5 were treated with a single high dose of L-carnitine (375 mg/kg), and groups 4 and 6 were treated with pantoprazole 40 mg/kg. ASA at a single dose of 600 mg/kg was administered by intragastric gavage to group 2. The rats in groups 5 and 6 were subjected to ASA in the same manner as in group 2. At the end of the experimental period, and 60 min after the administration of ASA, all animals were anesthetized intraperitoneally with ketamine hydrochloride (75 mg/kg) and xylazine (8 mg/kg) and their abdomens were opened by a midline incision. The stomachs were excised rapidly, and cut along the greater curvature. The gastric tissues were gently rinsed under tap water to remove gastric contents and/or blood concomitant (if so); otherwise they were washed in a physiological saline bath. The stomachs were divided into two sections. One part was separated, immediately frozen in liquid nitrogen, and then stored at –80°C until the analysis time used for further enzymatic analysis, whereas the other part was stored in 10% formalin for histopathological examination.

Histopathological evaluations

After fixation in 10% formalin solution, samples were processed, embedded in paraffin and sectioned at 4 µm by a microtome. Then, sections were deparaffinized, rehydrated and stained with hematoxylin-eosin (HE). The lesions were counted and evaluated with a light microscope by an observer who was blinded to the treatment. Gastric damage was scored for each histological section on a 0–6 scale as follows [27]: score 0 – no lesion; score 1 – diffuse hyperemia; score 2 – one or two hemorrhagic lesions; score 3 – three to five hemorrhagic lesions or erosions; score 4 – more than five hemorrhagic lesions or erosions; score 5 – 20 to 40% of total gastric surface with hemorrhagic lesions or multiple erosions; score 6 – more than 40% of total gastric surface with hemorrhagic lesions or multiple erosions.

Assessment of enzymatic activities

After weighing the sample tissues, they were homogenized in ten volumes of ice-cold tris-HCl buffer (0.2 mM, pH 7.4); homogenization (homogenizer: IKA Ultra-Turrax t 25 Basic, Germany) was carried out for 2 min at 13 000 rpm. All procedures were performed at 4°C. Homogenate, supernatant and extracted samples were prepared and the following determinations were made on the samples.

Measurement of SOD activity

Total (Cu, Zn, Mn) SOD activities were determined according to the method of Sun *et al.* [28]. The principle of the method is based on the inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine–xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the supernatant after 1.0 ml of ethanol–chloroform mixture (5 : 3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD was defined as the amount causing 50% inhibition of the NBT reduction rate. The SOD activity is expressed as U/mg protein.

Measurement of CAT activity

The CAT activity was determined according to Aebi's method [29]. The principle of the method is based on the determination of the rate constant (s^{-1}, k) of the H_2O_2 decomposition rate at 240 nm. Results are expressed as k g^{-1} protein.

Measurement of GSH-Px activity

The GSH-Px activity was measured by the method of Paglia and Valentine [30]. The enzymatic reaction in the tube containing NADPH, reduced glutathione (GSH), sodium azide and glutathione reductase was initiated by addition of H_2O_2, and the change in absorbance at 340 nm was monitored by a spectrophotometer (Shimadzu UV-1700, Japan). Activity is expressed as U/g protein.

Determination of MDA

The MDA levels were determined by the double heating method of Draper and Hadley [31]. The principle of the method is the spectrophotometric measurement of the color generated by the reaction of thiobarbituric acid (TBA) with MDA. For this purpose, 2.5 ml of 100 g/l trichloroacetic acid solution was added to 0.5 ml of homogenate in each centrifuge tube and the tubes were placed in a boiling water bath for 15 min. After cooling in tap water, the tubes were centrifuged at 1000x*g* for 10 min, 2 ml of the supernatant was added to 1 ml of 6.7 g/l TBA solution in a test tube, and the tube was placed in a boiling water bath for 15 min. The solution was then cooled in tap water and its absorbance was measured using a spectrophotometer at 532 nm. The levels of MDA were calculated by the absorbance co-efficient of the MDA-TBA complex (absorbance co-efficient = 1.56×10^5 cm^{-1} M^{-1}) and are expressed as nanomoles per gram (nmol/g) of protein. Protein concentration was determined with the Lowry method [32].

Measurement of NO level of gastric mucosa

Measurement of NO is very difficult in biological specimens; therefore tissue nitrite (NO_2^-) and nitrate (NO_3^-) were estimated as an index of NO production. Samples were initially deproteinized with Somogi reagent. Total nitrite (nitrite + nitrate) was measured after conversion of nitrate to nitrite by copperized cadmium granules by a spectrophotometer at 545 nm. A standard curve was established with a set of serial dilutions (10^{-8}–10^{-3} mol/l) of sodium nitrite. Linear regression was carried out using the peak area from the nitrite standard. The resulting equation was then used to calculate the unknown sample concentrations. Results were expressed as micromoles per gram (µmol/g) of protein [33].

Statistical analysis

Data analysis was carried out with SPSS (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, United States) 11.5 Windows program package. The Shapiro-Wilk test was used to evaluate whether the distribution of continuous variables was normal or not. Descriptive statistics were presented as the mean ± standard error of mean or median ± interquartile range for continuous variables, and presented as number of cases and (%) percentage for nominal variables. The one-way analysis of variance (one-way ANOVA) was used to assess statistical significance of differences between groups for normally distributed continuous variables, and the Kruskal-Wallis test was used in order to assess for non-normally distributed continuous variables. Post-hoc Tukey or Kruskal-Wallis multiple comparison tests were used respectively to determine the group/groups which cause significant differences with ANOVA or Kruskal-Wallis analyses. A *p*-value of less than 0.05 was considered to be statistically significant.

Results

Effect of L-carnitine on ASA-induced acute gastric mucosal injury

In contrast to the normal color and appearance of the gastric mucosa in control, PPI and LC group rats (Figure 1 A–C), the mucosa of rats exposed to ASA lost its integrity, and petechial bleeding and erosions were detected (Figure 1 D). The histopathological gastric injury score (ulcer score) increased significantly 1 h after the administration of ASA (Table I). This increase was not significantly inhibited by the treatment with PPI (Table I and Figure 1 E), nor was it significantly reduced by L-carnitine treatment (Table I and Figure 1 F).

The histopathological ulcer scores of the study groups are depicted in Table I. Ulcer scores of control, PPI and LC groups were similar. The ulcer score of the ASA group was significantly higher than control, LC and PPI groups ($p < 0.001$, $p < 0.001$, and $p < 0.001$, respectively). The ulcer score of the ASA + PPI group was lower than that of the ASA group, but the difference was not significant ($p = 0.081$). Pretreatment of rats with L-carnitine (ASA + LC group) did not reduce the ulcer index compared to the animals that received ASA alone (ASA group) ($p = 0.965$).

Activity of gastric antioxidant enzymes

Catalase activity

Catalase activity was significantly reduced after the administration of acetyl salicylic acid in the ASA group compared to controls ($p = 0.028$). A borderline decline in catalase activity was detected in LC and PPI groups compared to the control group ($p = 0.057$, $p = 0.051$, respectively). Pretreatment with PPI (ASA + PPI) or L-carnitine (ASA + LC) did not alter the catalase activity compared to the ASA group ($p = 0.927$, $p = 1.00$, respectively) (Table II and Figure 2).

SOD activity

There was no significant difference between study groups in terms of SOD activity (Table II).

GSH-Px activity

The GSH-Px activity was significantly reduced after the administration of ASA and L-carnitine ($p = 0.001$, $p = 0.028$, respectively), but PPI administration did not alter GSH-Px activity significantly ($p = 0.143$) compared to controls. Pretreatment

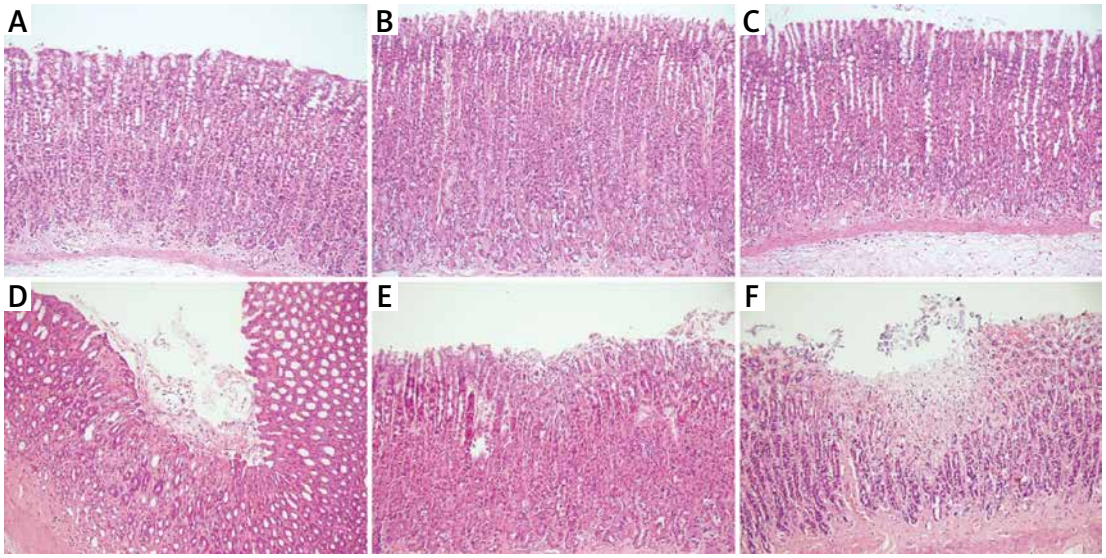

Figure 1. A – Normal appearance of gastric mucosa in control group of rats, HE 200×. **B –** Normal appearance of gastric mucosa in proton-pump inhibitor (PPI) group of rats, HE 200×. **C –** Normal appearance of gastric mucosa in L-carnitine (LC) group of rats, HE 200×. **D –** Hemorrhagic mucosal erosions and inflammatory cell infiltrations developed in the glandular stomach of rats 1 h after the administration of acetyl salicylic acid (ASA), HE 200×. **E** – The administration of PPI at a dose of 40 mg/kg macroscopically reduced these hemorrhagic erosions and edematous lesions (ASA + PPI), HE 200×. **F** – The administration of L-carnitine did not reduce the hemorrhagic erosions and edematous lesions (ASA + LC), HE 200×

with PPI (ASA + PPI) and pretreatment with L-carnitine (ASA + LC) did not alter GSH-Px activity significantly compared to the animals that received only ASA (p = 0.279, p = 0.995, respectively) (Table II and Figure 3).

Lipid peroxidation in gastric tissue

MDA levels

The MDA levels were significantly increased in rats that received ASA alone compared to controls (p = 0.003). Administering L-carnitine alone did not alter the MDA levels (p = 0.967). Pretreatment with PPI (ASA + PPI) significantly reduced MDA levels (p < 0.001), whereas pretreatment with L-carnitine (ASA + LC) reduced MDA levels insignificant-

Table I. Histopathological scoring of the study groups

Group	*N*	Ulcerated animal	Ulcer score
Control	7	0/7	0.14 ±0.4**
PPI	7	0/7	0.14 ±0.4**
LC	7	0/7	0.29 ±0.5**
ASA	7	7/7	2.71 ±1.0*
ASA + PPI	7	5/7	1.57 ±0.8*
ASA + LC	7	7/7	2.57 ±0.5*

*N – number of rats in each study group, PPI – proton pump inhibitor, LC – L-carnitine, ASA – acetyl salicylic acid. Values are expressed as mean ± standard deviation. *P < 0.05 compared with the control group, **p < 0.001 compared with the group given only acetyl salicylic acid.*

Table II. Antioxidant enzyme activities and lipid peroxidation levels of the study groups

Groups	CAT [kg/g protein]	SOD [U/mg protein]	GSH-Px [U/g protein]	MDA [nmol/g protein]	NO [µmol/g protein]
Control	0.090 ±0.02	0.020 ±0.004	1.694 ±0.37[b]	4.387 ±1.15[d]	0.176 ±0.03[f]
PPI	0.056 ±0.02	0.020 ±0.002	1.315 ±0.23[b]	4.606 ±1.20[d]	0.220 ±0.04[f]
LC	0.057 ±0.03	0.021 ±0.005	1.176 ±0.32[c]	3.973 ±0.65[d]	0.255 ±0.06
ASA	0.053 ±0.02[a]	0.017 ±0.002	0.827 ±0.30[c]	6.546 ±1.04[e]	0.343 ±0.08[g]
ASA + PPI	0.064 ±0.02	0.022 ±0.004	1.152 ±0.23[c]	3.945 ±0.50[d]	0.251 ±0.06[f]
ASA + LC	0.056 ±0.02	0.017 ±0.004	0.748 ±0.18[c]	5.318 ±1.12	0.279 ±0.06[g]

CAT – catalase, SOD – superoxide dismutase, MDA – malondialdehyde, GSH-Px – glutathione peroxidase, NO – nitric oxide, ASA – acetyl salicylic acid group, ASA + PPI – acetyl salicylic acid and proton pump inhibitor group, LC – L-carnitine group, ASA + LC – acetyl salicylic acid and L-carnitine group, PPI – proton pump inhibitor group. Values are expressed as arithmetic mean ± SD. [a]p < 0.05 vs. control group, [b]p < 0.05 vs. ASA group, [c]p < 0.05 vs. control group, [d]p < 0.05 vs. ASA group, [e]p < 0.05 vs. control group, [f]p < 0.05 vs. ASA group, [g]p < 0.05 vs. control group.

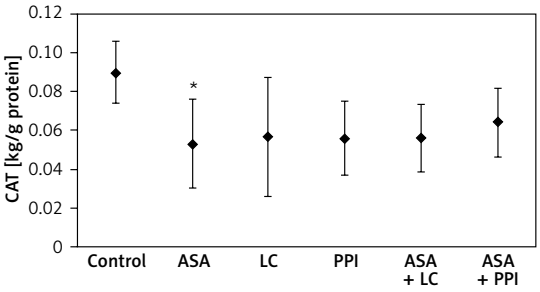

Figure 2. Gastric mucosal catalase activities of the study groups

**P < 0.05 when compared to control group (n = 7 in each group).*

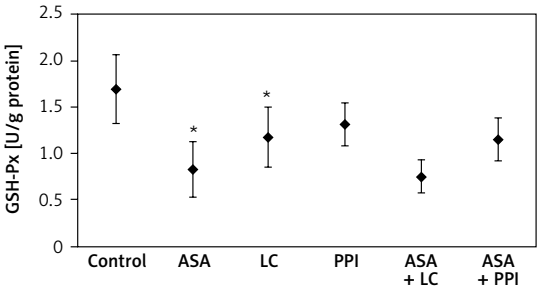

Figure 3. Gastric mucosal glutathione peroxidase activities of the study groups. Administration of acetyl salicylic acid and L-carnitine significantly reduced the GSH-Px activity

**P < 0.05 when compared to the control group (n = 7 in each group).*

ly (p = 0.204), compared to rats that received ASA alone (Table II and Figure 4).

Measurement of NO levels of gastric mucosa

The NO levels were significantly increased in rats that received ASA alone compared to control and PPI groups (p = 0.001, p = 0.002, respectively). Administering L-carnitine alone did not alter the NO levels (p = 0.098). Pretreatment with PPI (ASA + PPI) significantly reduced NO levels (p = 0.037), whereas pretreatment with L-carnitine (ASA + LC) reduced NO levels insignificantly (p = 0.277), compared to rats that received ASA alone (Table II and Figure 5).

Discussion

Salient findings of the present study are that (a); ASA impaired the gastric mucosal barrier as shown by histopathologic ulcer scores, by reducing the gastric antioxidant enzyme activities (including CAT and GSH-Px) and conversely increasing the products of lipid peroxidation (MDA) and NO levels, (b); the administration of L-carnitine did not prevent the occurrence of mucosal lesions; in addition, pretreatment with L-carnitine (ASA + LC) did not improve gastric antioxidant enzyme activities, lipid peroxidation products, or NO levels significantly compared to the ASA group, and (c); gastric tissue MDA and NO levels were significantly lower in the ASA + PPI group than in the ASA group.

The NSAIDs, such as ASA, are known to induce gastric mucosal injury in humans and animals via local and systemic effects [34]. The ASA causes a direct irritant effect by increasing the transport of H^+ ions, whereas it decreases mucin, surface active phospholipids, bicarbonate secretion, and mucosal proliferation. The ASA also causes damage by formation of free radicals [35]. Lipid peroxidation leads to loss of membrane fluidity and impairment of ion transport and membrane integrity on the surface of epithelial cells and helps to generate gastric lesions [36]. The mechanism by which it causes injury to the gastric mucosa is mainly due to the inhibition of cyclooxygenase and the sup-

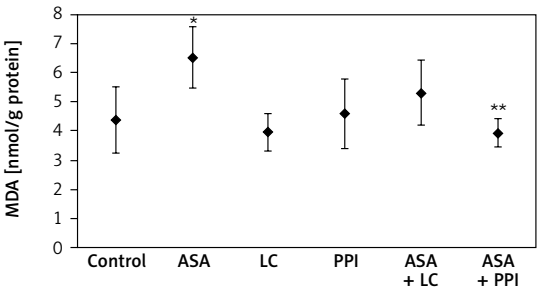

Figure 4. Gastric mucosal malondialdehyde levels of the study groups. Acetyl salicylic acid (ASA) significantly increased MDA levels compared to those in control rats. Pretreatment with a proton pump inhibitor (ASA + PPI) significantly improved MDA levels increased by ASA, but pretreatment with L-carnitine (ASA + LC) did not

**P < 0.05 when compared to control group and **p < 0.05 when compared to ASA group, respectively (n = 7 in each group).*

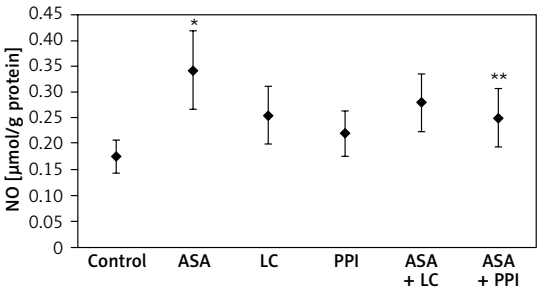

Figure 5. Gastric mucosal nitric oxide (NO) levels of the study groups. Acetyl salicylic acid (ASA) significantly increased NO levels compared to those in control rats. Pretreatment with a proton pump inhibitor (ASA + PPI) significantly improved NO levels increased by ASA, but pretreatment with L-carnitine (ASA + LC) did not

**P < 0.05 when compared to control group and **p < 0.05 when compared to ASA group, respectively (n = 7 in each group).*

pression of prostaglandin (PG)-mediated effects on mucosal protection, but the exact pathogenic mechanism remains to be elucidated [37].

The ROS plays a crucial role in the pathogenesis of oxidative damage. In the case of gastric injury, with inflammation and the recruitment of neutrophils by proinflammatory cytokines, the production of ROS is further increased. Gastric mucosal ischemia increases the generation of OH^- radicals, which subsequently facilitate the formation of gastric ulcer via cellular antioxidant depletion, lipid peroxidation, and inactivation of gastric peroxidation and protein oxidation [38]. The implication of ROS in gastric mucosal damage was strengthened by the fact that many free radical scavengers, such as ambrex [39], *Allophylus serratus* [40], and *Jasminum grandiflorum* [41], provide marked functional and histopathological protection against *in vivo* ASA-induced gastric mucosal damage. In the present study, pretreatment of rats with PPI partly reduced the histopathological ulcer score, but this was not statistically significant. However, pretreatment with LC had no protective effect on the histopathological ulcer score induced by ASA. Except for SOD, the enzyme activities in gastric tissue were also altered significantly following ASA administration. The CAT and GSH-Px activities were significantly reduced compared to the control values. The ASA administration markedly stimulated lipid peroxidation in gastric tissues, and the MDA content was elevated significantly compared to control rats. In addition, NO levels, an oxidative stress marker, were higher than the control values. These results are in line with the previous reports that demonstrated marked alterations in the enzymatic anti-oxidants following acute administration of ASA to rats [39, 41].

L-carnitine and its derivatives prevent the formation of ROS, scavenge free radicals and protect cells from peroxidative stress [20–24]. Two different physiologic mechanisms of L-carnitine have been reported. Firstly, Ronca *et al.* suggested that L-carnitine inhibits OH^- radical production in the Fenton reaction system by chelating iron required for the generation of OH^- radicals [42]. Secondly, Di Giacomo *et al.* showed the preventive effect of L-carnitine on the formation of ROS via the xanthine/XO system [43]. Arafa and Sayed-Ahmed reported that carnitine esters, particularly propionyl L-carnitine, could protect the rat stomach against alcohol-induced injury possibly by its anti-oxidant property [44]. Conversely to its protective effects on gastric mucosa, Valoti *et al.* have previously reported that L-carnitine derivatives, acetyl L-carnitine and propionyl L-carnitine, stimulated the gastric acid secretion in a dose-dependent manner *in vitro* and *in vivo* [45].

Izgut-Uysal *et al.* suggested that the reducing effect of L-carnitine on gastric damage could be related to the increasing effect on CAT activity in rats following exposure to chronic restraint stress. Pretreatment with carnitine did not change SOD activity, but it increased CAT activity in stressed rats [46]. In our study, only GSH-Px activity was significantly reduced in rats treated with L-carnitine alone, compared to controls. Apart from this, neither PPI nor L-carnitine alone significantly changed the anti-oxidant enzyme activity, compared to control animals. Pretreatment with PPI (PPI + ASA group) or L-carnitine (LC + ASA group), similarly, did not alter the anti-oxidant enzyme status significantly when compared to the ASA group.

Gastric ulcers are also associated with increased lipid peroxidation [47–52]. It has been demonstrated that many pathologic conditions that caused elevation of MDA due to lipid peroxidation were prevented by carnitine and its derivatives, including ischemia/reperfusion injury [53–55], drug-induced cardiomyopathy [56–58], and myocardial infarction [59, 60]. Izgut-Uysal *et al.* investigated the gastroprotective effects of L-carnitine in a cold-restraint stress (CRS) rat model. They reported that pretreatment with L-carnitine 50 mg/kg per day for 10 days reduced the products of lipid peroxidation as well as increasing CAT activity in both blood and gastric mucosa [21]. More recently, Dokmeci *et al.* demonstrated that L-carnitine significantly reduced the area of mucosal lesions induced by ethanol starting at a dose of 50 mg/kg, with the maximum effect at a dose of 500 mg/kg. They also observed that pretreatment with high dose LC (500 mg/kg) not only inhibited thiobarbituric acid reactive substances in the plasma and gastric mucosa, but also prevented the ethanol-induced reduction of gastric GSH [25]. In our study, pretreatment with PPI significantly reduced the MDA levels in rat stomach compared to animals given ASA alone, whereas pretreatment with L-carnitine did not alter MDA levels in comparison to rats that received only ASA. We treated the rats with 50 mg/kg L-carnitine for 21 days. In addition, 1 h before the administration of ASA, they were treated with a single high dose of L-carnitine (375 mg/kg), similar to Dokmeci *et al.* [25]. Izgut-Uysal *et al.* also reported a protective effect of L-carnitine on CRS exposed rats at concentrations of more than 50 mg/kg per day. However, no significantly different effect was observed between 50 and 100 mg/kg per day of L-carnitine [21]. More recently, like in our study, Izgut-Uysal *et al.* used L-carnitine (50 mg/kg) for 21 days in Wistar rats with chronic restraint stress-induced gastric mucosal injury; the authors reported that L-carnitine treatment prevented the stress-induced increase in the lesion index and lipid peroxidation, and improved the PGE_2 and mucus content in gastric mucosa [46]. Therefore, we consider that the failure

of L-carnitine treatment in our study should not be attributed only to the L-carnitine dosage.

Prostaglandins play an important role in the prevention of gastric mucosal injury. Among PGs, PGE_1, PGE_2, and $PGI_{2\alpha}$ have been shown to prevent gastric mucosal injury induced by various noxious stimuli [61, 62]. Especially, PGI_2 and PGE_2 are well-known protectors of the gastric mucosa. A reduction of these compounds leads to decreases in mucus synthesis and mucosal blood flow resulting in the susceptibility of gastric mucosa to gastric acid and noxious factors [63]. Izgut-Uysal *et al.* suggested that L-carnitine might have prevented gastric mucus depletion by increasing mucosal PGE_2 in the CRS group since PGE_2 is important in mucus production [21]. In our study, we were unable to detect the changes in acidic mucopolysaccharide or PGE_2 content of the gastric mucosa. However, the findings of Garrelds *et al.* where there was no detectable changes in PGE_2 content with short- (300 mg/kg) and long-term (50 mg/kg) feeding of L-carnitine and congeners on the production of eicosanoids from rat peritoneal leukocytes [64] may, at least partly, support our negative results with L-carnitine in rats exposed to ASA.

The NO released from vascular epithelium, epithelial cells of the gastrointestinal tract and sensory nerves can influence many of the same components of mucosal defense as do PGs. Synthetic analogues of lipoxins as well as the newer class of NSAIDs releasing NO may be used in the future as the therapeutic approach to counteract adverse effects in the stomach associated with NSAID ingestion [37]. In the present study, pretreatment with PPI significantly reduced the NO levels in rat stomach compared to animals given ASA alone. However, pretreatment with L-carnitine did not affect the NO levels in comparison with rats exposed to only ASA.

Neutrophil and oxygen radical-dependent microvascular injuries may also be important prime events that lead to mucosal injury induced by NSAIDs [65]. Lipid peroxidation mediated by oxygen radicals, especially hydroxyl radicals, plays a crucial role in the development of the gastric mucosal injury induced by indomethacin [66]. A single intragastric administration of L-carnitine at various doses (10, 50, 100 mg/kg) significantly prevented indomethacin-induced gastric mucosal injury in rats and decreased the ulcer index macroscopically and histopathologically [26]. In an ischemia-reperfusion injury model, L-carnitine 100 mg/kg significantly reduced both the gastric injury and myeloperoxidase activity, which is an important index of neutrophil accumulation [65].

Carnitine deficiency led to severe gut injury accompanied by a severe immune phenotype and pro-inflammatory status in OCTN2 (SLC22A5) carnitine transporter (–/–) newborn mice [67]. Very recently a significant impairment in the β-oxidation pathway was reported in mucosal biopsies of 26 patients with active ulcerative colitis. The administration of carnitine, either alone or in combination with ATP, did not improve Na-butyrate metabolism [68]. However, in a rat model of 2,4,6-trinitrobenzenesulfonic acid-induced acute and reactivated colitis, oral and intrarectal propionyl L-carnitine (but not L-carnitine or propionate) was shown to be effective in reducing intestinal mucosal inflammation, endothelial dysfunction, and oxidative stress induced by ROS [69]. And finally, coadministration of intraperitoneal L-carnitine played a major protective role in radiation-induced ileal (100 mg/kg/day was better than 200 mg/kg/day) and colonic (300 ml/kg) rat mucosal injury [70, 71].

The bioavailability of L-carnitine decreased from 100% to 42% with an increase in dose in a rat model of GI disposition. This dose-dependent alteration in the GI absorption of L-carnitine was attributed to its physiologic intestinal transport-based mechanism [72]. After oral administration, the small intestine is the major site of L-carnitine absorption without a first-pass degradation effect, whereas its absorption in the cecum and the colorectal sites is negligible [73]. Taken together, not only the dose intensity, but also the complex kinetic physiological mechanisms including absorption site, gastric emptying rate [72], and other intestinal transport dynamics may, at least partly, play a role in the gastroprotective roles of L-carnitine in gastric injury models. In our gastric injury model, the mucosal injury of the small intestine in addition to gastric tissue might have been related to the ineffectiveness of L-carnitine treatment.

In conclusion, taken together, gastroprotective effects of L-carnitine have been shown in some conditions via oxidative stress and lipid peroxidation. Although the anti-oxidant and/or free radical scavenging mechanism of L-carnitine remains unclear, the results of the present study indicate that L-carnitine did not prevent gastric mucosal injury induced by ASA histopathologically or in terms of oxidative reactions. Hence, further comprehensive studies may elucidate the other mechanisms involved in the anti-ulcer effect of L-carnitine treatment.

Acknowledgments

Chemical compounds studied in this article: L-carnitine hydrochloride, synthetic, ≥ 98% [(–)-beta-hydroxy-gamma-(trimethylammonio) butyrate] [PubChem CID: 656657 (6645-46-1)].

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Conflict of interest

The authors declare no conflict of interest.

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