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Protective role of carnitine esters against alcohol-induced gastric lesions in rats

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Abstract

We have investigated in the current study the possible protective effects of two carnitine esters known to have powerful anti-oxidant potential namely, propionyl L-carnitine (PLC) and acetyl L-carnitine (AC) against alcohol-induced gastric lesions in rats. Both drugs were administered as a single oral dose of 200 mg kg^{-1} body weight 1 h before alcohol intake. Both carnitine esters could protect the gastric mucosa against the injurious effect of absolute alcohol and promote ulcer healing as evidenced from the ulcer index (UI) values. Propionyl L-carnitine prevented alcohol-induced increase in thiobarbituric acid-reactive substances (TBARS), an index of lipid peroxidation. The propionyl carnitine ester also increased the gastric content of reduced glutathione (GSH), besides it increased the enzymatic activities of gastric superoxide dismutase (SOD) and glutathione-*S*-transferase (GST). Likewise, AC did protect against the ulcerating effect of alcohol and mitigate most of the biochemical adverse effects induced by alcohol in gastric mucosa, but to a lesser extent than PLC. Neither PLC nor AC did affect catalase activity in gastric tissue. Based on these observations, one could conclude that carnitine esters, particularly PLC could partly protect gastric mucosa from alcohol-induced acute mucosal injury, and these gastroprotective effects might be probably induced, at least partly, through anti-oxidant mechanisms.

Keywords: Carnitine; Alcohol-induced gastric lesions; Anti-radical effects

1. Introduction

Carnitine is a natural substance that acts as a carrier for fatty acids across the inner mitochondrial membrane for subsequent beta-oxidation. L-Carnitine and its esters propionyl L-carnitine (PLC) and acetyl L-carnitine (AC) are endogenously synthesised in man and also found in diet [1]. Carnitines are essential cofactors of several enzymes necessary for the transformation of long-chain fatty acids, and act also as scavengers of oxygen free radicals in mammalian tissues [2]. The transport of AC and PLC occurs more easily than L-carnitine through the lipid component of the intestinal barrier suggesting possible better oral bioavailability of the esters than L-carnitine [3]. That is why we administered the carnitine esters in the current study by gavage.

Though the therapeutic benefits of L-carnitine and its ester congeners had yet to be clarified, they have exhibited many pharmacological effects. In rat cardiac microsomes, AC had a protective action on NADPH-induced lipid peroxidation via its anti-oxidant effect [4]. Acetyl L-carnitine has also shown neuroprotective effects in rats [5,6] and anti-apoptotic activity in primary murine cultured neurons [7]. Propionyl L-carnitine exhibited cardioprotective effect after ischemia–reperfusion injury through its anti-radical effect [8,9]. Besides, PLC has proven efficacy in the treatment of congestive heart failure. The rationale for its use resides on its effects on cardiac and skeletal muscles [10]. Moreover, the propionyl ester was able to protect erythrocytes and low-density lipoproteins against peroxidation induced by oxygen reactive species [11]. Cell and tissue lipoperoxidation of the kidney induced by cyclosporine in rats through the release of reactive oxygen species has been shown to be mitigated by PLC [12]. Sayed-Ahmed et al. [13] have reported that PLC therapy completely protected against adriamycin-induced lipid peroxidation of cardiac membranes in rats through its powerful anti-oxidant effects. Propionyl L-carnitine has also shown anti-inflammatory effects in some models of vascular inflammation in rodents [14].

Intragastric application of absolute ethanol has long been used as a reproducible method to induce gastric

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lesions in experimental animals [15]. The protective effect against various irritants applied to the gastric mucosa has been called cytoprotective activity [16]. Pathogenesis of ethanol-induced gastric lesions is complex. Depletion of non-protein sulfhydryls concentration [15], modulation of nitric oxide system [17], reduction of gastric mucosal blood flow [18] are among the mechanisms underlying the development of gastric lesions. Oxidative stress and depletion of anti-oxidants have also been considered a crucial step in alcohol-induced mucosal damage [19,20].

The concept that carnitines have shown powerful anti-oxidant properties in a host of studies has prompted us to investigate in the current study the possible gastroprotective effects of two carnitine esters namely, propionyl L-carnitine and acetyl L-carnitine against alcohol-induced mucosal damage. Ulcer index (UI) and various anti-oxidants; enzymatic and non-enzymatic, were among the investigated biological endpoints.

2. Materials and methods

2.1. Chemicals and drugs

Acetyl L-carnitine and propionyl L-carnitine were a kind gift from Dr. Menotti Calvani (Sigma-Tau Pharmaceuticals, Pomezia, Italy). Absolute alcohol was obtained from Aldrich (Germany). Reduced GSH, superoxide dismutase (SOD), glutathione-*S*-transferase (GST), catalase (CAT), H_2O_2, Ellman's reagent [5,5-dithio-bis(2-nitrobenzoic acid)], 2-thiobarbituric acid (TBA) and 1,1,3,3-tetraethoxy-propane were all purchased from Sigma (St. Louis, MO, USA). Pyrogallol and 1-chloro-2,6-dinitrobenzene were obtained from Merck (Darmstadt, Germany). All other chemicals were of the commercial analytical grade.

2.2. Animals

Male Swiss albino rats weighing 200–225 g were obtained from the Egyptian Organisation for Biological Products and Vaccines (VACSERA, Giza, Egypt). The animals were acclimatised in the animal facility of the Faculty of Pharmacy, Al-Azhar University for 2 weeks before the experiment. The animals were fed a standard diet (El-Nasr Company, Abou-Zaabal, Cairo, Egypt) and tap water ad libitum, and kept under controlled conditions of room temperature (21±1 °C), relative humidity (55±5%) and a 12-h light/12-h dark cycle. Animals were fasted overnight (18 h) prior to the experiment, but were allowed free access to water.

2.3. Experimental design

A total of 24 rats were divided into 4 groups (6 animals per group). One group received an oral dose of absolute alcohol (1 ml per rat). A second group was administered a single dose of propionyl L-carnitine (200 mg kg^{-1} body weight p.o.) [21] followed by an oral dose of alcohol 1 h thereafter. A third group was given acetyl L-carnitine (200 mg kg^{-1} body weight p.o.) [6] followed by a single intragastric dose of alcohol 1 h later. A fourth group received distilled water orally (5 ml kg^{-1} body weight) as the vehicle and served as control. One hour after ethanol administration, the animals were euthanised by cervical dislocation, the stomachs were excised, cut along the greater curvature, and gently rinsed under tap water. The stomachs were stretched on a piece of cork with mucosal surface up and then examined macroscopically.

2.4. Determination of the ulcer index

The gastric lesions were evaluated according to the method of Yamamoto et al. [22]. Subjective scores of the lesions were recorded according to the severity of lesions between 1 (ulcerated area with an area of 1–6 mm^2) and 5 (ulcerated area >24 mm^2 or perforation). The mean scores of lesions in each group were summed. A zero score denoted no ulcer.

2.5. Determination of gastric anti-oxidants

Stomachs were cut into small pieces and then homogenised in ice-cold 0.15 M KCl using Heidolph Diax 900, type 595 (Germany) to give 20% homogenate. The homogenate was then made into aliquots and used for the assessment of anti-oxidant parameters.

2.5.1. Determination of total thiobarbituric acid-reactive substances (TBARS)

Total thiobarbituric acid-reactive substances (TBARS) were determined according to the method of Uchiyama and Mihara [23]. In brief, the adducts formed following boiling tissue homogenate with thiobarbituric acid is extracted with *n*-butanol. The difference in optical density at two distinct wavelengths; 535 and 525 nm is a measure of the gastric malondialdehyde (MDA) content as a measure of TBARS, which is undertaken as an index of lipid peroxidation.

2.5.2. Determination of reduced glutathione (GSH)

Reduced glutathione (GSH) was determined according to the method early described by Ellman [24]. The procedure is based on the reduction of Ellman's reagent by SH groups to form 2-nitro-5-mercaptobenzoic acid, which has an intense yellow colour that is measured spectrophotometrically at 412 nm using Shimadzu Spectrophotometer UV 1201 (Japan). The GSH concentration (μmol g^{-1}) was computed from a standard curve constructed using different concentrations of standard GSH.

2.5.3. Assessment of enzymatic activities

Stomach homogenate (20%) was centrifuged at 10000 rpm for 10 min at 4 °C for 30 min (Beckman XL-70, USA). Following centrifugation, the supernatant (cytosolic fraction) was carefully removed from the pellet and used directly

for the assay of the enzymatic activities of SOD, GST and CAT.

Gastric activity of SOD was assessed according to the method of Marklund [25]. It simply resides on computing the difference between auto-oxidation of pyrogallol alone and in presence of the cytosolic fraction that contains the enzyme. Changes in the absorbance at 420 nm were recorded at 1-min interval for 5 min. Enzymatic activity was expressed as U g^{-1} tissue.

Stomach GST activity was determined according to the method of Habig et al. [26]. In brief, the GST activity toward 1-chloro-2,4-dinitrobenzene in presence of glutathione as a co-substrate was measured spectrophotometrically at 25 °C. The enzyme activity was determined by monitoring the changes in absorbance at 340 nm for 4 min over 1-min intervals. The enzymatic activity was expressed as nmol min^{-1} g^{-1} tissue.

CAT activity was determined according to the method of Clairborne [27]. In short, the supernatant (50 μl) was added to a quartz cuvette containing 2.95 ml of 19 mmol l^{-1} H_2O_2 solution prepared in potassium phosphate buffer (0.1 M, pH 7.4). The change in absorbance was monitored at 240 nm over a 5-min period using a spectrophotometer (Shimadzu UV-1201, Japan). Commercially available CAT was used as the standard. CAT activity was expressed as U g^{-1} tissue.

3. Results

The effects of alcohol intake alone and following administration of PLC or AC on the ulcer index, contents of MDA and GSH, as well as the enzyme activities of SOD, GST and CAT in rat gastric tissue are shown in Fig. 1 and Table 1.

Intake of absolute alcohol induced severe and extensive macroscopic gastric mucosal damage characterised by elongated haemorrhagic lesions confined mainly to the gastric corpus and running parallel to the long axis of the stomach that had the highest ulcer scoring rate (Fig. 1).

Pre-treatment of rats with a single oral dose of PLC did reduce the ulcer index by 40% compared to the animals

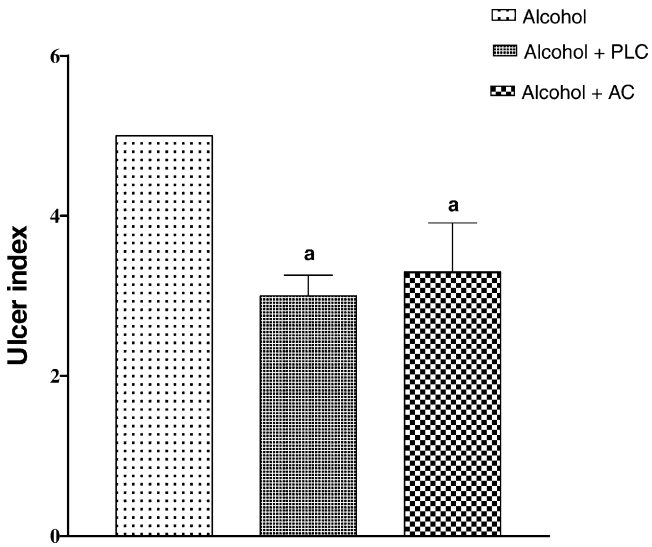

Fig. 1. Effects of alcohol intake alone and after administration of either propionyl L-carnitine (200 mg kg^{-1} body weight) or acetyl L-carnitine (200 mg kg^{-1} body weight) on the ulcer index in rats. (a) Significantly different from alcohol at $P < 0.05$ using one way ANOVA followed by Tukey–Kramer as post-ANOVA test.

that received alcohol alone. Similarly, prior administration of AC could protect the animals against the ulcerating effect of alcohol, as evidenced by a decrease in the ulcer index amounted to 34%.

Alcohol administration markedly stimulated lipid peroxidation in gastric tissues, and the MDA content was elevated by about 125.5% compared to control animals (Table 1). Alcohol also decreased the gastric level of GSH by about 52%. Except for CAT, the enzyme activities in gastric tissue have also been altered following alcohol administration. The SOD activity was decreased by 65%, while that of GST was reduced by about 58.5% compared to the control values (Table 1). The CAT activity was unchanged after alcohol intake.

Pre-treatment with PLC significantly reduced the MDA content in rat stomach by 67% compared to animals given alcohol alone. Acetyl L-carnitine, did also decrease

Table 1
Effects of alcohol intake alone and after administration of either propionyl L-carnitine or acetyl L-carnitine on the contents of MDA and GSH and activities of SOD, GST and CAT in rat stomach

Parameters	Control ($n = 6$)	Alcohol[#] ($n = 6$)	Alc + PLC[$] ($n = 6$)	Alc + AC[@] ($n = 6$)
MDA (nmol g^{-1} tissue)	138.23 ± 12.24	311.68 ± 18.28a	102.30 ± 21.09b	191.51 ± 32.03b
GSH (μmol g^{-1} tissue)	0.80 ± 0.03	0.39 ± 0.041a	0.51 ± 0.02b	0.43 ± 0.02
SOD (U g^{-1} tissue)	46.67 ± 4.75	16.37 ± 2.68a	55.97 ± 3.24b	49.07 ± 3.80b
GST (nmol min^{-1} g^{-1})	4.67 ± 0.33	1.94 ± 0.44a	4.24 ± 0.42b	4.25 ± 0.26b
CAT (U g^{-1} tissue)	55.56 ± 3.40	58.8 ± 8.60	54.93 ± 7.40	53.45 ± 11.20

Data are presented as means ± S.E.M. ($n = 6$). Multiple comparisons were done using one way ANOVA followed by Tukey–Kramer as post-ANOVA test. Gastric lesions were measured, summed and scored from one to five.
([#]) Absolute alcohol was given by gavage (1 ml per rat).
([$]) Propionyl L-carnitine (200 mg kg^{-1} body weight) was given orally 1 h before alcohol.
([@]) Acetyl L-carnitine (200 mg kg^{-1} body weight) was given orally 1 h before alcohol.
(a) Significantly different from control group at $P < 0.05$, (b) significantly different from alcohol group at $P < 0.05$.

the gastric MDA content, but to a lesser extent amounted to 39%.

Administration of PLC to rats ahead of alcohol intake increased the gastric GSH content by 32%. However, there was no significant change in gastric GSH observed following AC administration compared to animals received alcohol alone (Table 1).

Prior administration of PLC to rats markedly increased the gastric activity of SOD by about 242%. Likewise, AC increased the enzymatic activity of SOD by 200% compared to animals that received alcohol alone.

Pre-treatment of rats with either PLC or AC exhibited the same increase in the enzyme activity of gastric GST, which was amounted to about 119% (Table 1).

Neither PLC nor AC administered before alcohol had any effect on the CAT activity of gastric mucosa.

4. Discussion

Alcohol intake has been shown to be associated with marked oxidative damage to gastric mucosa. The cytoprotective role of anti-oxidants in the prevention and healing of gastric lesions has been widely investigated in a number of studies [28–30]. The notion that carnitines have shown a powerful anti-oxidant potential in various toxicity models [5,12,13] has warranted the attention to address the possible protective effects of two carnitine esters, namely propionyl L-carnitine and acetyl L-carnitine against alcohol-induced gastric lesions in rats.

Application of absolute alcohol by gastric gavage induced marked damage to the gastric mucosa that was obvious by macroscopic examination. The lesions were elongated haemorrhagic and were confined to the glandular portion with the highest subjective ulcer-scoring rate.

It was also apparent that alcohol caused severe oxidative stress in gastric tissue manifested as stimulated lipid peroxidation via increasing MDA content and reduction of gastric GSH content. These findings are coping with many previous reports that demonstrated an increase in MDA and decrease in GSH contents in rat gastric tissues following exposure to intragastric alcohol [28,30,31].

The gastric activities of SOD and GST have been notably decreased following alcohol intake. The CAT activity, however, was unchanged. These results are in line with previous reports that demonstrated marked alterations in the enzymatic anti-oxidants following acute administration of alcohol to rats [31–34].

Indeed, the pathogenesis of ethanol-induced gastric lesions is not fully explored. Many postulates have been hypothesised. Depletion of non-protein sulfhydryls concentrations [15], modulation of nitric oxide system [35], reduction of mucosal blood flow [18], and autonomic nervous system regulation [36], have been suggested to be involved in the development of gastric lesions. One of the major mechanisms suggested to underlie the induction of gastric erosions by absolute alcohol is the oxidative damage with its dual events of lipid peroxidation and oxygen reactive species generation. Actually, oxygen-derived radicals have been implicated in the pathogenesis of gastric tissue damage and ulcerogenesis [19,37,38].

Pre-treatment of rats with a single oral dose of PLC could partly reduce the ulcer index and promote healing of gastric lesions induced by acute intake of alcohol.

The propionyl ester significantly decreased the gastric MDA content, while it increased the gastric level of GSH compared to animals received alcohol alone. The gastric activities of both SOD and GST were markedly elevated following administration of PLC, whereas the CAT activity was not altered. Likewise, prior administration of AC to animals could protect gastric mucosa and ameliorate most of the biochemical adverse effects induced by alcohol application, but to a lesser extent than PLC.

These data are in harmony with the results of Izgut-Uysal et al. [2]. The authors have investigated the gastroprotective effect of L-carnitine in rat gastric mucosa following exposure to cold-restraint immobilisation. They found that prior administration of L-carnitine could prevent the occurrence of mucosal lesions by interfering with lipid peroxidation that associates the oxidative damage in gastric tissue.

The concept that anti-radicals could protect against alcohol-induced gastric injury has been established in a number of studies. Ito et al. [39] have reported that the lipid-lowering agent probucol may partly protect against gastric mucosal injury and promote the healing of chronic gastric ulcers by its anti-oxidant activity. Similarly, quercetin, alpha-tochopherol, nifedipine and tetracycline were found to possess gastric cytoprotective and gastric ulcer healing-promoting activities that have been attributed to their radical scavenging activities [28]. Bilici et al. [30] have recently demonstrated that the free radical scavenger; melatonin prevented ethanol-induced gastric mucosal damage presumably due to its anti-oxidant property. By the same token, the gastroprotective effects of both carnitine esters observed in the current study could possibly be mediated through their well-known anti-oxidant potential. In this sense, many reports have demonstrated substantial anti-radical effects of the carnitines in different pathologic conditions. Reznick et al. [9] have reported that propionyl L-carnitine could protect the rat heart against ischemia–reperfusion injury possibly by its anti-radical effects. Similarly, the propionyl carnitine ester did protect erythrocytes and low-density lipoproteins against peroxidation induced by oxygen reactive species. Further, Longoni et al. [12] have reported that the protective role of propionyl L-carnitine against the lipid peroxidation induced by cyclosporin in rat kidney tissue is most probably via anti-oxidant mechanisms. More recently, Sayed-Ahmed et al. [13] have shown that PLC induced a powerful anti-oxidant defence mechanism against adriamycin-induced lipid peroxidation of cardiac membranes.

On the other hand, Schinetti et al. [4] have earlier documented that the protective action of AC on NADPH-induced lipid peroxidation of cardiac microsomes might be mediated through anti-oxidant mechanisms. Likewise, AC could inhibit lipoperoxidation and xanthine oxidase activity and their consequences in rat skeletal muscle [5]. Moreover, the neuroprotective effects of AC observed by Calvani and Arrigoni-Martelli [40] after ischemia and brain reperfusion have been attributed to its free radical scavenging property.

Based on these broad observations, one could conclude that pre-treatment of rats with carnitine esters; particularly PLC could partly protect the gastric mucosa against the injurious effect of absolute alcohol and promote ulcer healing. The carnitine congeners did also mitigate most of the biochemical adverse effects induced by alcohol instillation in gastric tissue. Taken together, one could conclude that the gastroprotective effects of both PLC and AC observed in the current study could possibly reside at least in part on their anti-radical effects.

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