Original Article

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Mitochondrial Impairment Induced by Chenodeoxycholic Acid: The Protective Effect of Taurine and Carnosine Supplementation

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Abstract

The cholestatic liver disease ensues with a hepatic accumulation of cytotoxic molecules. Several hydrophobic bile acids are known as cytotoxic agents accumulated in the liver during cholestasis. Chenodeoxycholic acid (CDCA) is a toxic hydrophobic bile acid. Oxidative stress and mitochondrial dysfunction are well-known mechanisms of bile acid cytotoxicity. In the current study, CDCA effect on isolated liver mitochondria was monitored by analyzing the changes in mitochondrial dehydrogenases activity, mitochondrial permeabilization, and mitochondrial membrane potential. On the other hand, taurine (1 mM) and carnosine (1 mM) were added as potential protective agents against CDCA-induced mitochondrial dysfunction. Increasing the concentrations of CDCA (100 µM-1000 µM) impaired mitochondrial membrane potential, decreased mitochondrial dehydrogenases activity, and enhanced mitochondrial permeabilization and swelling. It was found that taurine and carnosine supplementation preserved mitochondrial function in the presence of CDCA. The results mention that toxicologically relevant concentrations of CDCA impaired mitochondrial function. On the other hand, taurine and carnosine might be applicable as protective agents against bile acids-induced mitochondrial impairment and toxicity.

Keywords: Amino acids, Cholestasis, Hepatotoxicity, Hepatoprotection, Liver fibrosis, Organ Injury.

1. Introduction

Bile acid synthesis is a vital function of hepatocytes. On the other hand, any defect in the bile acid transportation from hepatocytes to the gastrointestinal (GI) tract could ensue with deleterious consequences (1-3). Most bile acids are hydrophobic molecules with detergent properties (4, 5). These chemicals are well-known biomembrane disruptors and protein degrading agents (4, 5). Oxidative stress and its following events are the proposed mechanisms involved in the bile acid cytotoxicity (4, 6-13). Chenodeoxycholic acid (CDCA) (Figure 1) is a hydrophobic cytotoxic bile acid, which accumulates in hepatocytes during cholestasis.

At the cellular level, mitochondria are critical targets for bile acid toxicity (6, 10, 12, 14-19). It has been well-characterized that bile acids deteriorate the mitochondrial function (6, 10, 12, 14-19). The collapse of mitochondrial membrane potential, mitochondrial permeabilization and swelling, impaired mitochondrial ATP biosynthesis, and release of cell death mediators are attributed to bile acid cytotoxicity (6, 10, 12, 14-19). These events could finally lead to the energy crisis,

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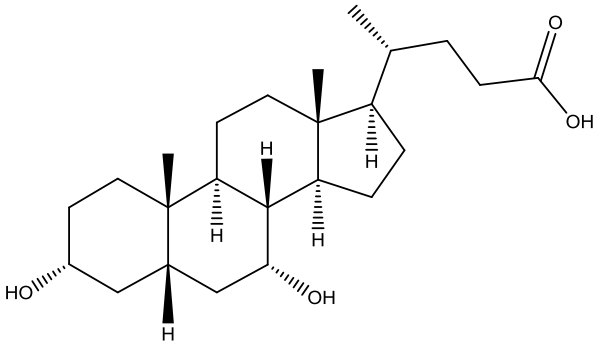

Figure 1. Chemical structure of chenodeoxycholic acid as a hydrophobic cytotoxic bile acid.

cell death, and organ injury.

Taurine is the most abundant free amino acid in the body (20). Several biological roles have been attributed to this amino acid (21, 22). The protective properties of taurine have repeatedly been mentioned in different experimental models (23-32). Interestingly, taurine is a good modulator of cellular mitochondrial function (32-35). It has been found that this amino acid efficiently mitigated mitochondria-mediated cytotoxicity of xenobiotics (32, 36-39). In the current study, the potential protective properties of taurine have been evaluated in the isolated liver mitochondria exposed to toxic CDCA concentrations.

Carnosine is an endogenous dipeptide with a wide range of pharmacological properties (40-46). It has been found that carnosine efficiently interacts with reactive species (e.g., cytotoxic aldehydes) and ameliorates oxidative stress in different experimental models (40-45, 47-50). On the other hand, the positive effects of this peptide on cellular mitochondria has been also mentioned in the previous studies (41, 51-53). In the current study, carnosine has been applied to preserve mitochondrial function in the presence of CDCA.

2. Materials and methods

2.1. Chemicals

Carnosine and taurine were purchased from Sigma (St. Louis, MO, USA).4,2 Hydroxyethyl,1-piperazineethanesulfonic acid (HEPES), fatty acid free bovine serum albumin (BSA; Fraction V), 3-(N-morpholino)propane sulfonic acid (MOPS), dimethyl sulfoxide (DMSO), D-mannitol, thiobarbituric acid (TBA), ethylene glycol-bis (2-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), chenodeoxycholic acid (CDCA), trichloroacetic acid (TCA), 3-[4,5dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), rhodamine123, coomassie brilliant blue, hydroxymethyl aminomethane hydrochloride (Tris-HCl), sodium succinate, and ethylenediaminetetraacetic acid (EDTA) were purchased from Merck (Darmstadt, Germany). All salts for preparing buffer solutions (analytical grade) were obtained from Merck (Darmstadt, Germany).

2.2. Animals

Male BALB/c (20-25 g) were obtained from Animal Breeding Center of Shiraz University of Medical Sciences, Shiraz Iran. Mice were housed in cages with wood-chip bedding at a temperature of 23±1 °C and relative humidity of ≈40%. Animals had free access to a standard rodents chow diet (Behparvar®, Tehran, Iran) and tap water. Mice were fasted 24 h before the mitochondria isolation procedure. Animals were handled in compliance with the guidelines of the laboratory animals care and use approved by an institutional ethics committee in Shiraz University of Medical Sciences, Shiraz, Iran (15115/14883).

2.3. Liver tissue mitochondria isolation and experimental setup

Mice liver mitochondria were isolated based on the differential centrifugation method (54-56). Animals were anesthetized (Thiopental, 80 mg/kg, i.p) and the liver was excised, washed, and minced with an ice-cooled saline solution (Sodium chloride 0.9%) (54, 57, 58). Then, the liver tissue was homogenized in a buffer containing 75 mM mannitol, 220 mM sucrose, 0.5 mM EGTA, 2 mM HEPES, 0.1% essentially fatty acid-free bovine serum albumin and pH=7.4 at a 10:1

buffer to tissue (v:w) ratio (54). Afterwards, tissue homogenate was centrifuged to remove intact cells and nuclei (1000 g for 10 min at 4 °C). The supernatant was further centrifuged at 10,000 g (4 °C for 10 min) to precipitate the heavy membrane fractions (mitochondria). This step was repeated three times using fresh buffer medium to increase the mitochondrial yield. As mentioned, all manipulations for mitochondrial isolation were performed at 4 °C or on ice to preserve mitochondrial intactness (54).

In all experiments using taurine and carnosine as the protecting agents, isolated liver mitochondria were pre-incubated with these chemicals 15 min before CDCA exposure. The isolated liver mitochondria were exposed to CDCA for 30 min, then mitochondrial function was assessed. Taurine and carnosine were dissolved in mitochondria medium. CDCA was dissolved in DMSO. The maximum volume of DMSO added to the mitochondria preparations was 5 µl/10 ml incubation media.

2.4. Mitochondrial dehydrogenases activity (MTT assay)

The methyl tetrazolium (MTT) assay was applied as a colorimetric method for determination of mitochondrial dehydrogenases activity in the isolated liver mitochondria (59-61). Briefly, mitochondrial suspension (1 mg protein/ml) in a buffer containing 0.32 M sucrose, 1 mM EDTA, and 10 mM Tris-HCl, pH=7.4, was incubated with 40 µl of MTT (0.4% w:v) at 37 °C (30 min, in the dark) (56). Samples were centrifuged (10,000 g, 15 min) and the product of the purple formazan crystals was dissolved in DMSO (1 ml). Then, 100 µl of the dissolved formazan was added to a 96 well plate, and the optical density (OD) at λ=570 nm was assessed with an Epoch plate reader (BioTek® Instruments, Highland Park, USA) (58, 62).

2.5. Mitochondrial depolarization

The mitochondrial ability to uptake the cationic fluorescent dye, rhodamine 123, was used as an index of mitochondrial depolarization (54, 63-67). For this purpose, the mitochondrial fractions (0.5 mg protein/mL) were incubated with rhodamine 123 (final concentration of 10 µM) in a buffer containing 65 mM KCl, 125 mM sucrose, 5 mM sodium succinate, 20 µM Ca^{2+}, and 10 mM HEPES, pH=7.2 (20 min, 37 ºC) (61, 68). Samples were centrifuged (10,000 *g*, 5 min, 4 ºC) and the fluorescence intensity of the supernatant was monitored using a FLUOstar Omega® multifunctional microplate reader (LABTECH, Germany) at the excitation and emission wavelengths of λ=485 nm and λ=525 nm, respectively (54).

2.6. Mitochondrial permeabilization and swelling assay

Mitochondrial swelling was assessed by the light scattering method as previously described (54). Briefly, the isolated liver mitochondria preparations (0.5 mg protein/mL) were suspended in swelling buffer (125 mM sucrose, 65 mM KCl, 10 mM HEPES-KOH, 20 µM Ca^{2+}, pH=7.2). The light absorbance at λ=540 nm was monitored during 30 min of incubation at a constant temperature of 30 °C (54). A decreased light absorbance is consistent with an increase in mitochondrial volume (54). Hence, as mitochondria are more swelled, the differences between light absorbance of two-time points are higher. The differences between the absorbance of samples at two time points (10 and 30 min) were assessed and reported as maximal mitochondrial swelling amplitude (ΔOD540 nm) (54).

2.7. Statistical analysis

Data are given as the Mean±SD. Data comparison was conducted by the one-way analysis of variance (ANOVA) with Tukey's multiple comparison tests as the *post hoc*. Differences were considered statistically significant when $P<0.05$.

3. Results

The effect of CDCA on mitochondrial dehydrogenases activity (MTT test) has been shown in Figure 2. It was found that concentrations of CDCA ranging from 100 µM-1000 µM caused a significant decrease in mitochondrial dehydrogenases activity as compared with the control group (Figure 2). On the other hand, pre-incubation of liver mitochondria with taurine (1 mM) and/or carnosine (1 mM) prevented CDCA-induced decrease in mitochondrial dehydrogenases activity (Figure 2).

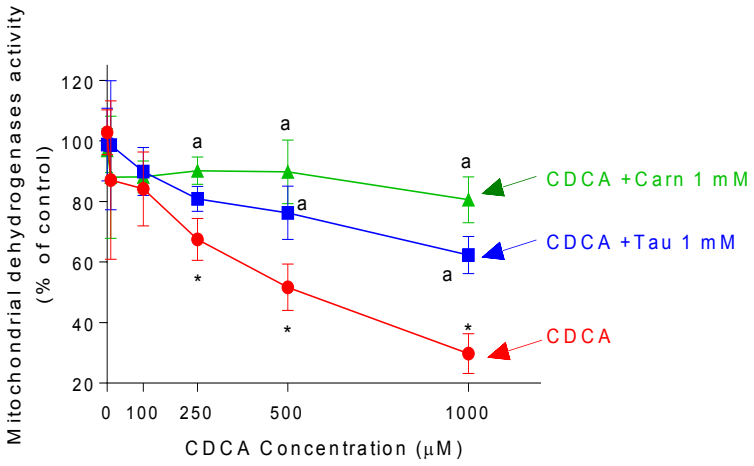

Figure 2. Mitochondrial dehydrogenases activity in the presence of the cytotoxic bile acid chenodeoxycholic acid (CDCA), taurine (Tau), and carnosine (Carn).
Data are given as Mean±SD (n=8).
*Indicates significantly different as compared with control (0 µM CDCA) (P<0.001).
[a]Indicates significantly different as compared with CDCA-treated group (P<0.001).

Incubation of the isolated mice liver mitochondria with different concentrations of CDCA increased mitochondrial permeabilization and swelling (Figure 3). It was found that taurine and carnosine treatment ameliorated CDCA-induced mitochondrial permeabilization and swelling (Figure 3).

Concentrations of 100-1000 µM of CDCA abolished mitochondrial capability of rhodamine 123 uptake as an index of mitochondrial membrane potential (Figure 4). On the other hand, it was found that taurine (1 mM) and carnosine (1 mM) supplementation prevented CDCA-induced mitochondrial depolarization (Figure 4).

4. Discussion

Cholestasis is the stoppage of bile flow from hepatocytes to the GI tract. Several pathological conditions, as well as a wide range of drugs

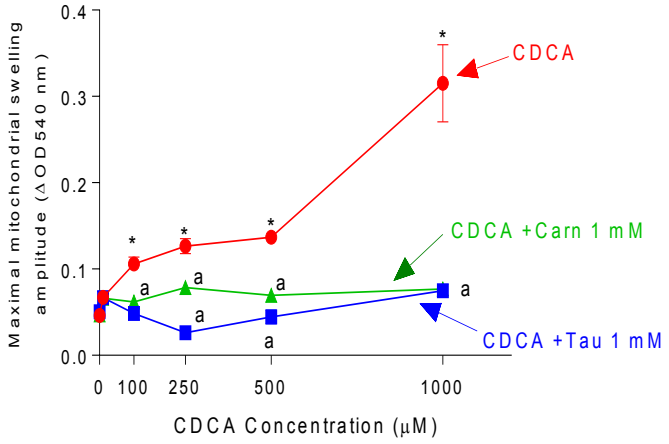

Figure 3. Mitochondrial permeabilization and swelling in the presence of chenodeoxycholic acid (CDCA), taurine (Tau), and carnosine (Carn).
Data are given as Mean±SD (n=8).
***Indicates significantly different as compared with control (0 µM CDCA) (P<0.001).
[a]Indicates significantly different as compared with CDCA-treated group (P<0.01).

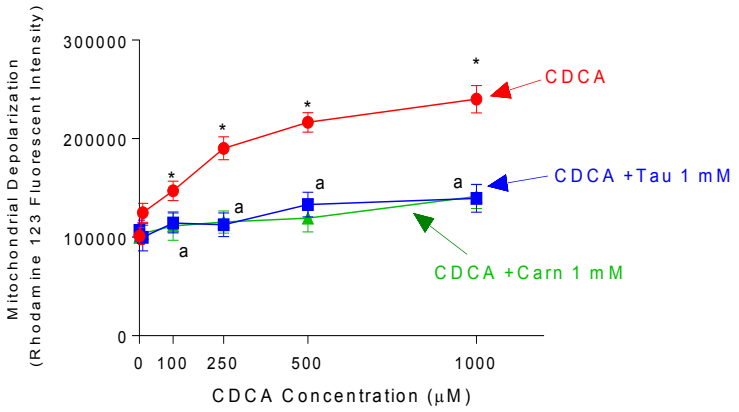

Figure 4. Chenodeoxycholic acid (CDCA)-induced mitochondrial depolarization.
Data are given as Mean±SD (n = 8). Tau: Taurine; Carn: Carnosine.
*Indicates significantly different as compared with control (0 µM CDCA) (P<0.001).
[a]Indicates significantly different as compared with CDCA-treated group (P<0.001).

and xenobiotics, are capable of inducing cholestasis (1-3, 69). Despite its etiology, accumulation of highly toxic substances such as bile acids is the common outcome of cholestasis. Chenodeoxycholic acid (CDCA) is one of the cytotoxic hydrophobic bile acids accumulated in hepatocytes during cholestasis (Figure 1).

The prominent role of mitochondria in cellular energy metabolism, as well as its role in cell death process and apoptosis, make it a critical target for xenobiotics toxicity (70, 71). Several investigations suggest that cellular mitochondria are the principal targets of bile acids-induced injury (6, 10, 12, 14-19). Although the exact mechanisms of mitochondrial impairment induced by toxic bile salts are unclear, the promotion of mitochondrial permeabilization, release of cell death mediators, dissipation of mitochondrial membrane potential, and impairment of mitochondrial energy metabolism are the well-characterized events associated with cholestasis-induced liver injury (6, 10, 12, 14-19). In the current study, our data indicate that mitochondrial impairment is involved in the mechanism of injury induced by CDCA as a toxic bile acid. On the other hand, we found that administration of taurine and/or carnosine could be a protective strategy against CDCA-induced mitochondrial dysfunction.

The cytoprotective properties of taurine have been repeatedly mentioned in previous studies (23-32). Taurine has been proposed to poses its protective properties through different mechanisms including regulation of oxidative stress, prevention of biomembrane lipid peroxidation, and modulation of mitochondrial function (21-32). Some studies also mentioned that the antioxidant properties of this amino acid might be directly associated with its effects on cellular mitochondria (72-74). Interestingly, taurine plays a vital role in the structure of mitochondrial tRNA (74, 75). Hence, a proper level of this amino acid in mitochondria could help in regulating mitochondrial protein synthesis machinery and efficient mitochondrial respiratory complexes function. On the other hand, it has been found that taurine might act as a regulator of mitochondrial matrix pH (34). Mitochondrial matrix pH is a critical factor for preservation of mitochondrial membrane potential ($\Delta\Psi$). All these data mention a role for taurine in the regulation of mitochondrial function.

In the current study, it was found that taurine supplementation preserved mitochondrial function upon interaction with the toxic bile acid CDCA. Hence, administration of this amino acid could serve as a protective strategy against bile acids-induced mitochondrial impairment and organ injury. Risk assessment studies revealed that taurine is safe even at high doses (76, 77). Therefore, this amino acid might be clinically applicable against cholestasis-induced organ injury.

Carnosine is a dipeptide widely investigated for its protective properties in different ex-

perimental models (40-45, 47-50). Carnosine is a well-known antioxidant and scavenger of different reactive species (46, 78-80). Hence, this peptide could efficiently protect biological targets against xenobiotics toxicity. The positive effects of carnosine on cellular mitochondria have also been mentioned in previous studies (51, 81). It has been found that carnosine preserved mitochondrial membrane potential, prevented mitochondrial permeabilization, and enhanced mitochondrial energy metabolism and ATP production (51, 82). In line with previous findings, the protective properties of carnosine were evident against CDCA toxicity in the current investigation. All these data might mention the potential protective properties of carnosine against cholestasis-induced liver injury. On the other hand, as carnosine is an endogenous molecule, it might be readily applicable in clinical situations.

Collectively, our data mention mitochondrial toxicity of the hydrophobic bile acid CDCA and the potential protective properties of taurine and carnosine supplementation. Indeed, more studies in different experimental models or human subjects of cholestasis could reveal the therapeutic significance of these data.

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Conflict of Interest

None declared.

5. References

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