Indian J Physiol Pharmacol 2002; 46 (3) : 343–348

EFFECT OF URSODEOXYCHOLIC ACID ON HYDROGEN PEROXIDE INDUCED LIPID PEROXIDATION IN SHEEP LIVER MITOCHONDRIA

ARUMUGAM GEETHA* AND SOMASUNDARAM PARAMESWARI

P.G. Department of Biochemistry,
Bharathi Women's College,
North Chennai – 600 108

(Received on January 15, 2002)

Abstract : The effect of various concentrations of ursodeoxycholic acid (UDCA), a potent hepatoprotective agent on hydrogen peroxide-induced mitochondrial swelling was evaluated *in virto* to find out the mechanism of action of the drug. Aliquots of sheep liver mitochondria were pre-incubated with various concentrations of UDCA [0–600 µg] and swelling was induced by hydrogen peroxide [1 mM]. Swelling was assessed at various time intervals and lipid peroxide, reduced glutathione status were also evaluated simultaneously. UDCA minimized hydrogen peroxide-induced swelling in a dose-dependent manner. Time-dependent elevation in the level of lipid peroxides was noted in mitochondria treated with hydrogen peroxide and this elevation was minimized in UDCA pre-treatment. UDCA also maintains the reduced glutathione level in mitochondria. UDCA acts against the oxidative stress imposed in liver mitochondria. It reduces lipid peroxidation-induced abnormalities such as swelling and thiol group depletion and the anti lipid peroxidative efficacy of the drug may be related to its hydrophilic nature which might protect the hydrophobic regions of the mitochondrial membranes which are prone for free radical-mediated reactions.

Key words : hydrogen peroxide, mitochondrial swelling, ursodeoxycholic acid, lipid peroxide, sulphydryl groups

INTRODUCTION

Ursodeoxycholicacid (UDCA), a beta isomer of chenodeoxycholic acid is a potent drug for the treatment of gall stone diseases, primary biliary cirrhosis and primary sclerosing cholangitis (1–3). UDCA is a hydrophilic bile salt, protects directly against the toxicity of hydrophobic endogenous bile salts such as chenodeoxycholic acid and deoxycholic acid (4).

Liver mitochondrial abnormalities have been reported in cholestatic liver diseases. Respiratory functions of liver mitochondria was reported to be disturbed markedly in

*Corresponding Author

hypotension and the degree of which correlated with the time of biliary obstruction (5). The degradation of liver mitochondrial phospholipids by bile acids has been shown to be responsible for the early phase of liver dysfunction in obstructive jaundice (6).

Clinical and experimental studies have been conducted to prove the role of lipid peroxidation in the pathogenesis of the above mentioned disorders (7–8) and the beneficial effects of UDCA as a therapeutic agent (9–10).

The aim of the present study is to find out the role of UDCA against the lipid peroxidation related abnormalities in liver mitochondria and thereby the mechanism of action of the drug.

METHODS

Isolation of mitochondria

Fresh sheep liver samples were obtained from slaughter house, in ice cold containers. After homogenisation, mitochondria were isolated in a sucrose based medium containing 0.1 mM EDTA, 3 mM KH_2PO_4, 300 mM sucrose, 1 mM MgCl2, pH 7.2 (11). Protein content was estimated by a modified Biuret method (12). The protein content of the mitochondrial stock was found to be 20–23 mg/ml.

Preparation of drug solution

UDCA was finely powdered and dissolved in 0.9% saline to get a concentration of 1 mg/ml.

Measurement of mitochondrial swelling

Aliquots of mitochondrial preparations (2 mg protein) were incubated at 37°C for 1 hour with various concs of UDCA (0-600 µg) in a total volume of 2 ml. 1.0 ml of swelling medium (13) was added followed by 1.0 mM hydrogen peroxide. Swelling was assessed by change in optical density at 540 nm at different time intervals.

Measurement of lipid peroxides

Mitochondrial preparations were incubated with UDCA as described previously and 1.0 mM of H_2O_2 was added. Aliquots were withdrawn at different time intervals and treated with 10% TCA (2.0 ml). Lipid peroxidation was monitored by measuring the thiobarbituric acid reacting substances at 533 nm (14).

Measurement of protein sulphydryl groups

Aliquots were withdrawn similarly as described for lipid peroxide determination at different time intervals and protein sulphydryl groups were determined (15) after removal of non protein sulphydryl groups by washing the aliquots with 6.5% ice cold TCA. The precipitate was suspended in 2 ml of 0.3 mM Na_2HPO_4. 100 µl of 5-5' dithio bis nitro benzoic acid (4.0 mg/ml) was added and the absorbance measured at 412 nm.

RESULTS

Hydrogen peroxide treatment resulted in a time dependent increase in the rate of swelling in the absence of UDCA which was

measured as a change in O.D at 540 nm (Fig. 1). Time-dependent elevation in the level of "TBA Reactants" was observed in hydrogen peroxide treated mitochondria with/without UDCA (Fig. 2). UDCA pre-treatment was found to decrease the rate of swelling induced by hydrogen peroxide in a dose-dependent manner. The decrease was significant ($P<0.001$) at the UDCA concentration of 300 µg. Lipid peroxide level was found to be low in UDCA treated mitochondria. A highly significant inhibition was observed ($P<0.001$) at the concentration of 300 µg UDCA. Levels of protein sulphydryl groups were found to be depleted in H_2O_2 treatment without UDCA.

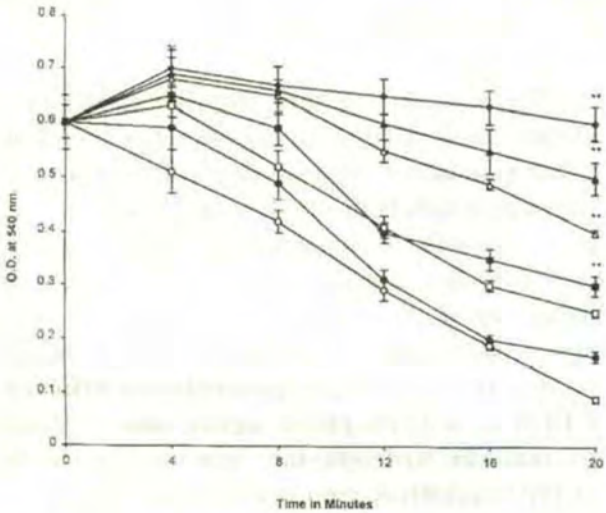

Fig. 1: The Rate of swelling as a function of time in sheep liver mitochondria. Aliquots of mitochondrial preparations (2 mg protein) were incubated at 37°C for 1 hour with various concentrations of UDCA (0–o, 100 µg – •, 200 µg – □, 300 µg – ■, 400 µg – Δ, 500 µg – ▲, 600 µg – ♦) in a total volume of 2ml. 1.0 ml of swelling medium was added followed by 1.0 mM hydrogen peroxide. Swelling was assessed by measuring change in optical density at 540 nm at different time intervals. Values are mean±SD from 6 different mitochondrial preparations. Statistically significant variations are expressed at **P<0.001.

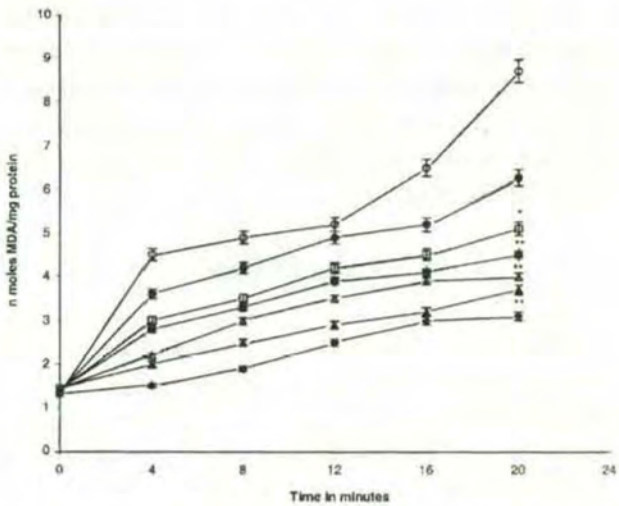

Fig. 2: Effect of UDCA on H_2O_2 induced lipid peroxidation. Mitochondrial preparations were treated similarly as explained in Fig. 1. Aliquots were withdrawn at different time intervals after the addition of hydrogen peroxide. Lipid peroxidation products were measured with "TBA Test" as described in the methods. Values are mean±SD from 6 different mitochondrial preparations. Statistically significant variations are expressed as *P<0.01, **P<0.001.

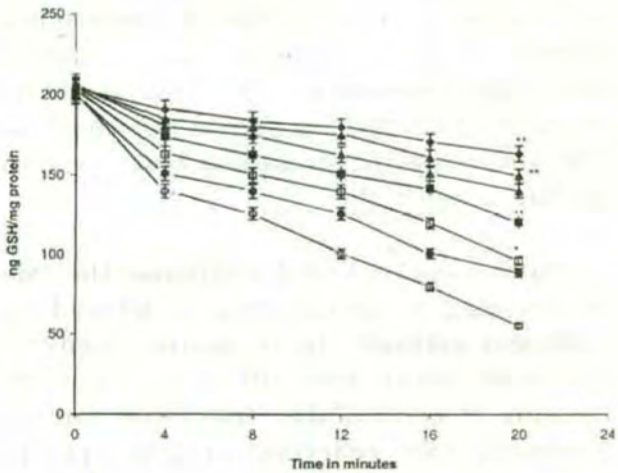

Fig. 3: Effect of UDCA on H_2O_2 induced changes in the level of protein sulphydryl groups. Aliquots were withdrawn from the experimental mixture (as described in Fig. 1) at different times intervals. Protein sulphydryl groups were measured with DTNB Test. Values are mean±SD from 6 different mitochondrial preparations. Statistically significant variations are expressed as *P<0.01, **P<0.001.

The effect of UDCA on protein sulphydryl groups was also found to be time and dose dependent. There was a significant ($P<0.001$) prevention of protein sulphydryl groups depletion at the concentration of 300 µg UDCA (Fig. 3)

DISCUSSION

Mitochondria have been recognised as a major physiological source of reactive oxygen species which arise as a consequence of oxygen reduction and formation of hydrogen peroxide and singlet oxygen. The ability of hydroperoxides to increase the ion permeability of the inner mitochondrial membrane and to induce mitochondrial swelling have been well established (16-18).

Swelling is a well known and one of the most common responses of the mitochondria to many unfavourable influences under both in vivo and in vitro experimental conditions. Mitochondrial swelling has been reported to be the consequence of the opening of permeability transition pore in mitochondria (19) and oxidative stress has been involved in this process (20).

In this study, UDCA minimises the rate of swelling in mitochondria induced by hydrogen peroxide. In an another study, it has been shown that UDCA prevents the opening of permeability transition pore by reducing the reactive oxygen species production (21). Ethanol induced liver mitochondrial injury has been found to be minimised in UDCA treatment (22). So, one of the mechanisms by which UDCA protects mitochondria from oxidant injury may be due to the inhibition of mitochondrial membrane depolarisation and channel formation (23).

Lipid peroxidation damages the structural integrity of mitochondria, large amplitude swelling, increased permeability to cations and decreased membrane potential (24). Protection against such oxidative damage is provided by antioxidants such as Vitamin E (25) and reduced glutathione (26).

Two mechanisms accounting for the hydrogen peroxide induced mitochondrial abnormalities have been reported. One of mechanisms is related to hydrogen peroxide decomposition, leading to formation of free radicals (27) and subsequent initiation of lipid peroxidation (28).

The results of this investigation have shown that UDCA decreases the level of "TBA reactants" in dose-dependent manner. Lipid peroxidation plays a major role in the pathogenesis of various liver disorders and gall bladder diseases for which UDCA is being used as therapeutic drug. So, the observed beneficial effect of UDCA might be due to its antilipid peroxidative efficacy. UDCA is a hydrophilic agent and it might protect the hydrophobic, lipid rich portions of mitochondrial membrane which are more prone to free radical damage. The hydrophilic nature of the drug has already been explained with respect to the modulation of mitochondrial membrane perturbation (21). Many hydrophilic agent have been proved to be potent antioxidants. Alpha - tocopherol acetate, a hydrophilic analogue of alpha - tocopherol has been shown to be more effective that alpha -

tocopherol in attenuating the effect H_2O_2 (29).

UDCA protects the protein sulphydryl groups significantly from oxidative damage. Hydrogen peroxide and a few other hydroperoxides have been reported to cause progressive decrease in the glutathione and reduced niacinamide coenzyme levels suggesting pro-oxidative changes (30). Oxidative damage to mitochondria have been reported to be more significant after glutathione depletion by chemical methods and glutathione supplementation has been shown to suppress lipid peroxidation induced changes in mitochondria (31).

Lipid peroxidation in membrane has been reported to result in the formation of free fatty acids (32), having protonophoric effect and act as inducers of mitochondrial swelling (13). UDCA due to its anti lipid peroxidative property might have inhibited the free fatty acid formation and thereby to some extent swelling.

The results presented in this study indicate that the protective effect of UDCA on mitochondria might be due to its antioxidative property. The hydrophilic nature of the drug might be responsible for the protection rendered to the hydrophobic, free radicals susceptible, lipid region in the mitochondrial membranes. The anti lipid peroxidative property might have resulted in the preservation of protein sulphydryl groups which are necessary for the normal functioning of the organelle. UDCA protects liver from oxidative stress perhaps by preserving mitochondrial functions.

ACKNOWLEDGEMENT

The author AG acknowledges the financial assistance given by University Grants Commission, New Delhi, India for this research.

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