Captopril ameliorates toxicity induced by paraquat in mitochondria isolated from the rat liver

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Abstract

The aim of the present study was to show the abilities of captopril as a thiol ACEi (angiotensin converting enzyme inhibitor), on mitochondria toxicity due to paraquat. Mitochondrial isolation from rat liver was divided into 4 groups. Group 1 was considered as control, group 2 received paraquat (5 mM), group 3 received captopril (0.08 mM) and group 4 received paraquat (5 mM) + captopril (0.08 mM). Lipid peroxidation, catalase activity, GSH (reduced glutathione) and GSSG (oxidized glutathione) concentrations were determined in isolated rat liver mitochondria. Simultaneous treatment of mitochondria with captopril (0.08 mM) + paraquat (5 mM) significantly ameliorate the mitochondria toxicity induced by paraquat (5 mM) alone. The results confirm antioxidant effect of captopril. This effect appears to be attributable to the Sulfhydryl Groups (SH) in the compound which may be due to captopril abilities to scavenge reactive oxygen species. The results indicate that captopril may prevent oxidative stress induced by paraquat.

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1. Introduction

Mitochondria are candidate targets of paraquat (N,N'-dimethyl 4,4'-bipyridium) toxicity in animal tissues and plants (Taylor et al., 2002). Many cases of acute poisoning and deaths have been reported over the past decade (Brundayckx et al., 2002). Mitochondria are considered to be the major source of reactive oxygen species in cells (Cadenas and Davies, 2000; Ramasarma, 1982; Lenaz, 1998). Components of the electron transport chain (e.g. flavoproteins, ubisemiquinone) are known to undergo auto-oxidation and generate reactive oxygen species in mitochondria (Turrens and Boveris, 1980; Cadenas et al., 1977). Inhibition of the electron transport chain can increase the steady-state levels of these auto-oxidizable components and consequently increase reactive oxygen species generation by mitochondria (Han et al., 2001; Boveris, 1977).

Paraquat is very toxic, with putative toxicity mechanisms associated to mitochondrial redox systems (Lambert and Bondy, 1989). The mechanisms of paraquat toxicity are frequently related to the generation of the superoxide anion, which can lead to the formation of more toxic reactive oxygen species, e.g., superoxide anion, often taken as the main toxicant (Farrington et al., 1973).

Angiotensin-converting enzyme (ACE) inhibitors, which have both an antihypertensive and a cardioprotective action, are commonly used in the treatment of hypertension and most forms of heart failure (Brunner et al., 1979; Kiowski et al., 1991; Konstam et al., 1992). The beneficial effects of ACE inhibitors were thought to be primarily due to the inhibition of angiotensin II formation. A number of studies have shown that ACE inhibitors improve oxidant stress and fibrosis. Treatment with captopril showed to increase antioxidant enzymes and nonenzymatic antioxidant defenses in several mouse tissues (De Cavanagh et al., 1997). Some
in vitro studies, indicate that, sulfhydryl containing (i.e. captopril) can scavenge free radicals (Ghazi-Khansari et al., 2005). In the present study, the inhibitory effect of captopril, on the mitochondrial toxicity of PQ have been assessed in isolated rat liver mitochondria.

2. Materials and methods

2.1. Chemicals

Paraquat dichloride salt, MTT, were purchased from the Sigma Chemical Co. (St. Louis, Mo). Janus green was obtained from Merck (Germany). All other chemicals were obtained from the Sigma Chemical Co.

2.2. Animals

Male Wistar rats (180–220 g) were housed environmentally (t= 25°C) and air humidity controlled room (60%) and kept on standard laboratory diet and were maintained on a 12 h light–dark cycle. Animals were fasted overnight before mitochondrial isolation. The Animal Ethics Committee of the Tehran University of Medical Sciences, School of Medicine, Education Section of Basic Sciences approved the experiment protocol (132/11737, March 18, 2006).

2.3. Preparation of mitochondria

The liver was removed with small scissor and minced in a cold manitol solution containing 0.225 M d-manitol, 75 mM sucrose and 0.2 mM ethylenediaminetetraacetic acid (EDTA). Approximately 30 g of minced liver was gently homogenized in a glass homogenizer with a Teflon pestle and then centrifuged at 700 g for 10 min at 4°C to remove nuclei, unbroken cells and other non-subcellular tissues. The supernatants were centrifuged at 7000 g for 20 min. These second supernatant were pooled as the crude microsomal fraction and the pale loose upper layer, which was rich in swollen or broken mitochondria, lysosomes and some microsomes, of sediments was washed away. The dark packed lower layer (heavy mitochondrial fraction) was resuspended in the manitol solution and recentrifuged twice at 7000 g for 20 min. The heavy mitochondrial sediments were suspended in Tris solution containing 0.05 M Tris–HCl buffer (pH 7.4) 0.25 M sucrose, 20 mM KCl, 2.0 mM MgCl₂ and 1.0 mM Na₂HPO₄ at 4°C before assay.

2.4. Experimental design

In order to determine the LC₅₀ of paraquat and the submaximal concentration of captopril (the concentration without any effect on mitochondrial viability), mitochondrial viability was investigated by 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide (MTT) assay. Different concentrations of paraquat (1, 5, 10, 50 and 100 mM) was used and the submaximal concentration was determined to be 0.08 mM (Fig. 1). Mitochondria suspensions were divided into 4 groups of six duplicate samples. Group 1 was considered as control, group 2 received paraquat (5 mM), group 3 received captopril (0.08 mM) and group 4 received paraquat (5 mM) + captopril (0.08 mM). Mitochondria suspensions were incubated in vitro for 1 h at 37°C.

2.5. MTT assay

This assay is a quantitative colorimetric method to determine cell viability. It utilizes the yellow tetrazolium salt (MTT) which is metabolized by mitochondrial dehydrogenase enzyme from viable cells to yield a purple formazan reaction product which was determined spectrophotometrically at wavelength of 570 nm (Mosmann, 1983).

2.6. Lipid peroxidation

Lipid peroxidation was evaluated as thiobarbituric acid reactive products. Measurement of TBA reactive compounds was performed after mixing 1 ml of the mitochondrial suspension with 2 ml of TBA reagent containing 0.5 M HCl, TCA15%, TBA 0.3%. This mixture was heated at 95°C for 20 min, after cooling in tap water, 2 ml of n-butanol were added and the mixture shaken vigorously, then a centrifugation at 3500 rpm for 15 min was performed, the n-butanol layer was taken for spectrophotometric measurement. The amount of reactive products formed was calculated by using an extinction coefficient of 165 mM⁻¹cm⁻¹ at 530 nm (Babincova et al., 2002).

2.7. Determination of GSH and GSSG

Aliquots of isolated mitochondria homogenates were deproteinized with 20% (w/v) trichloroacetic acid and centrifuged at 10,000g for 20 min. The supernatant was analyzed for reduced glutathione (GSH) by the 5,5′-dithiobis-2-
nitrobenzoic acid (DTNB) recycling procedure (Tietze, 1969). GSSG (oxidized glutathione)+ GSH were determined in supernatant after mixing with 1 mL of 5% sodium borohydride (NaBH₄), a reducing agent, and then incubated at 45°C for 60 min. The mixture was neutralized with 0.5 mL of 2.7 N HCl and the resulting sulphydryl groups of GSH were assayed as described above. The absorbance at 412 nm was measured immediately after mixing and the GSH values were determined by extrapolation from a standard curve (Zacharias et al., 1992) and GSSG expressed as GSH equivalents.

2.8. Catalase assay

The Cayman Chemical Catalase assay Kit utilizes the peroxidation of CAT for determination of enzyme activity. The method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H₂O₂. The catalase activity was measured spectrophotometrically with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald) as the chromogen (Johansson and Borg, 1988; Wheeler et al., 1990).

2.9. Protein concentration

Mitochondrial protein concentrations were determined using the method developed by Bradford (1979).

2.10. Statistical analysis

All values were expressed as mean ± standard error (SEM) of 6 samples. Analysis of variance (ANOVA) followed by student Newmans–Keuls test was used to evaluate the significance of the results obtained. All computations were performed using SPSS software.

3. Results

3.1. GSH and GSSG concentration

As shown in Fig. 2, mitochondrial GSH levels of paraquat-treated rats were significantly lower than those of controls and PQ+captopril treated group (p < 0.001). Mitochondrial GSSG levels of paraquat-treated rats were significantly higher than those of controls and PQ+captopril treated p < 0.001 (Fig. 3). GSSG expressed as GSH equivalents p < 0.001 (Figs. 2 and 3).

3.2. Catalase activity

Data have shown that the paraquat treatment and the administration of captopril provided protection against paraquat-induced oxidative stress, p < 0.001 (Fig. 4).

3.3. Lipid peroxidation

The TBARs concentration was significantly greater in mitochondria suspension treated with paraquat alone compared to controls and PQ+captopril treated groups p < 0.001 (Fig. 5).

4. Discussion

Studies have demonstrated that oxidative stress plays an important role in the pathogenesis of diseases such as cancer, diabetes, cardiovascular diseases, Parkinson’s disease, schizophrenia, atherosclerosis, lung diseases, cataracts, etc.
Biological effects of ROS are controlled by a wide spectrum of enzymatic and non-enzymatic defense mechanisms such as catalase (CAT), glutathione peroxidase, and total-SH protein. GSH is the major low molecular weight antioxidant in cells and its levels are often decreased during oxidative stress (Halliwell and Gutteridge, 1999). Since GSH is known to play an important role in protecting mitochondria from oxidant-induced injury, levels of GSH as well as GSSG in the isolated mitochondria were measured in this study. Among the oxidative stress agents paraquat is a thiol-oxidizing agent resulting in fast oxidation of GSH to GSSG. GSH is considered the principal mitochondrial antioxidant and its depletion markedly enhances the sensitivity of the mitochondrial structure to the ROS-mediated injury the sensitivity of the mitochondrial structure to the ROS-mediated injury (Fernandez-Checa et al., 1998). Our data demonstrate that treatment with captopril, ameliorate the decreased total GSH and increased GSSG due to paraquat. Since both the GSH and GSSG represent specific marker of oxidant stress (Jaeschke, 1990), therefore captopril may be able to affect the glutathione cascade (Figs. 2 and 3).

Membranes are highly permeable to H$_2$O$_2$ (Chance et al., 1979). Paraquat toxicity has been assigned to H$_2$O$_2$ production (Farrington et al., 1973; Hassan and Fridovich, 1979). Catalase is known to protect paraquat toxicity as well. Our data indicate that captopril may interfere with the H$_2$O$_2$ production therefore decreased catalase activity due to paraquat (Fig. 4).

Lipid peroxidation has been implicated in a number of deleterious effects such as increased membrane rigidity, osmotic fragility, decreased cellular and subcellular components, reduced mitochondrial survival and lipid fluidity (Aydin et al., 2004). Our results suggest that captopril may exert its effect by inhibiting membrane lipid peroxidation mediated by superoxide anion (Fig. 5).

Captopril was effective in ameliorating mitochondrial toxicity by paraquat. Our pervious observation suggests that the presence of a thiol group in the ACEi structure may be determinant for the antioxidant properties (Ghazi-Khansari et al., 2005). Under normal physiological conditions, antioxidants protect against reactive oxygen species (ROS) generated in the mitochondria. Numerous studies have employed antioxidants, such as vitamin E (Garcia-Estrada et al., 2003; Yilmaz and Yilmaz, 2006) and melatonin, as treatments for excessive ROS production. The beneficial effect of captopril on paraquat toxicity appears to be through enhancement of the endogenous antioxidant system preventing the lung fibrosis (De Cavanagh et al., 2001). Captopril is a thiol compound which can react with superoxide anion radical acting as a scavenger, or with hydroxyl radical (Al-Shabanad et al., 1998; Aruoma et al., 1991; Bartoz et al., 1997; Benzie and Tommison, 1998). Antioxidant effect of captopril as shown by its effect on GSH, GSSG, lipid peroxidation and catalase activity suggest that exogenously administered antioxidants such as captopril may either make their way to mitochondria or improve the cells antioxidant status, thus protecting these organelle from oxidant damage. More studies are needed to elucidate the exact mechanism by which captopril ameliorate the paraquat mitochondrial toxicity.

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References


