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Ascorbic acid and α-tocopherol as potent modulators on arsenic induced toxicity in mitochondria

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Abstract

Arsenic exists ubiquitously in our environment and various forms of arsenic circulate in air, water, soil and living organisms. Since arsenic compounds have shown to exert their toxicity chiefly by generating reactive oxygen species, we have evaluated the effect of antioxidants ascorbic acid and α-tocopherol on lipid peroxidation, antioxidants and mitochondrial enzymes in liver and kidney of arsenic exposed rats. A significant increase in the level of lipid peroxidation and decrease in the levels of antioxidants and in the activities of mitochondrial enzymes were observed in arsenic intoxicated rats. Co-administration of arsenic treated rats with ascorbic acid and α-tocopherol showed significant reduction in the level of lipid peroxidation and elevation in the levels of ascorbic acid, α-tocopherol, glutathione and total sulfhydryls and in the activities of isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase, NADH-dehydrogenase and cytochrome c oxidase. From our results, we conclude that ascorbic acid and α-tocopherol alleviate arsenic-induced alterations in mitochondria.

1. Introduction

The environment in which humans live is generally considered safe. However, hazardous substances are found as contaminants and pollutants from different sources in many occasions. The contamination of arsenic in drinking water is a major health problem in certain areas including parts of Bangladesh, United States, Taiwan, Mexico, Japan and India where the arsenic concentration exceeds the WHO's drinking water provisional guideline value 10μg/L [1]. Arsenic exposure either due to geochemical enrichment or due to industrial process, is accompanied by many severe biochemical and pathological problems such as Blackfoot disease [2], diabetes mellitus [3], hypertension [4] and cancers of liver, lung, bladder and kidney in humans [5].

Arsenite, a trivalent form of arsenic, reacts with cellular thiols to exert its toxicity [6]. Alternatively, arsenic compounds generate reactive oxygen species during their metabolism in cells to cause tissue damage [7]. Mitochondria are the major site of utilization of oxygen and many of the mitochondrial enzymes contain essential sulfhydryl groups. In addition, the inner and outer mitochondrial membranes contain unsaturated lipids. Therefore, mitochondria are more susceptible to arsenic attack as well as by the free radicals produced by it than other organelles.

Antioxidants play an important role in preventing free radical mediated damages by directly scavenging them. α-Tocopherol is one of the most widely distributed, naturally occurring and biologically active antioxidant in the biological system. It protects against lipid peroxidation most efficiently through its chain-breaking antioxidant action [8]. Intracellularly, it is associated with lipid-rich membranes such as mitochondria and microsomes. In contrast to α-tocopherol, ascorbic acid is hydrophilic and functions better in aqueous environment than does α-tocopherol [9]. Moreover, it can restore the antioxidant property of oxidized tocopherol, suggesting that a major function of ascorbic acid is to recycle the tocopheroxyl radical [10].

The present study was undertaken to delineate the efficacy of α-tocopherol and ascorbic acid on the arsenic- altered activities of mitochondrial enzymes in rat liver and kidney.

2. Materials and methods

Sodium arsenite, ascorbic acid and α-tocopherol were purchased from Sigma chemical company (St Louis , USA). All other chemicals were of reagent grade.

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2.1. Experimental design

Male albino rats of Wistar strain weighing approximately 120-130 g were used. The animals were divided into five groups namely:

Group I	Rats, received vehicles alone, served as control
Group II	Rats received arsenic as sodium arsenite in drinking water at a concentration of 100 ppm
Group III	Rats treated with arsenic along with ascorbic acid (200 mg/kg body wt. dissolved in water) by oral gavage once per day
Group IV	Rats given arsenic along with α-tocopherol (400 mg/kg body wt. dissolved in mineral oil) by oral gavage once per day
Group V	Rats administered arsenic along with ascorbic acid (200 mg/kg body wt. dissolved in water) and α-tocopherol (400 mg/kg body wt. dissolved in mineral oil) by oral gavage once per day

The animals were maintained on commercial rat feed. Each group consisted of six animals and had access to food and water *ad libitum.* On completion of 30 days of experimental period the animals were killed by cervical decapitation. Liver and kidney were excised immediately and immersed in physiological saline.

2.2. Isolation of mitochondria

The mitochondria were isolated by the method of Johnson and Lardy [11]. A 10% (w/v) homogenate was prepared in 0.05M Tris-HCl buffer, pH 7.4 containing 0.25M sucrose and centrifuged at 600 x g for 10 min. The supernatant fraction was decanted and centrifuged at 15,000 x g for 5 min. The resultant mitochondrial pellet was then washed and resuspended in the same buffer.

2.3. Biochemical analysis

The level of lipid peroxidation (LPO) was assayed by the method of Ohkawa et al [12], in which the malondialdehyde released served as the index of LPO. The method of Omaye [13] was followed for the estimation of ascorbic acid. α-Tocopherol was estimated by the method of Desai [14]. Glutathione (GSH) was assayed by the method of Moron et al [15], based on the reaction of 5,5′-dithiobis-(2- nitrobenzoic acid). The total sulfhydryls (TSH) were done according to the method of Sedlack and Lindsay [16].

The activity of isocitrate dehydrogenase was assayed by the method of King [17]. α-Ketoglutarate dehydrogenase activity was assayed according to the colorimetric determination ferrocyanide produced by the decarboxylation of α-ketoglutarate with ferricyanide as electron acceptor [18]. Succinate dehydrogenase activity was assayed by the method of Slater and Bonner [19], in which the rate of reduction of potassium ferricyanide was measured in the presence of potassium cyanide. The method of Minakami et al [20] was followed for the determination of reduced nicotinamide adenine dinucleotide (NADH)- dehydrogenase activity. The activity of cytochrome c oxidase was assayed by the method of Warton and Tzagoloff [21]. Protein was estimated by the method of Lowry et al [22].

Values are mean $\pm$ SD for six rats in each group, and significance of the differences between mean values were determined by one-way analysis of variance (ANOVA) followed by the Duncan test for multiple comparison. Values of $P < 0.05$ were considered to be significant.

3. Results

Table 1 shows the levels of lipid peroxidation, non-enzymatic antioxidants ascorbic acid, α-tocopherol, GSH and TSH and enzyme activities in the liver mitochondria of arsenic exposed and vitamin supplemented rats. Observation of arsenic treated rats have shown increase in the concentration of LPO (55%) and decrease in the concentration of ascorbic acid (51%), α-tocopherol (48%), GSH (52%) and TSH (48%). The activities of enzymes were found to be significantly lowered in arsenic treated rats. The decrease being 42% for isocitrate dehydrogenase, 52% for α-ketoglutarate dehydrogenase, 49% for succinate dehydrogenase, 41% for NADH-dehydrogenase and 39% for cytochrome c oxidase. Co-administration of arsenic along with ascorbic acid and α-tocopherol reversed these changes as comparable to control rats.

Arsenic intoxication imposes deleterious effects on the kidney mitochondria as evidenced by increase in the levels of lipid peroxidation ($p < 0.05$) and decrease in the levels of ascorbic acid, α-tocopherol, GSH and TSH and in the activities of the citric acid cycle enzymes and NADH-dehydrogenase and cytochrome c oxidase ($p < 0.05$), the components of the electron transport chain. Simultaneous administration of ascorbic acid and α-tocopherol to arsenic treated rats restored these changes to near normal. The combination of both these antioxidants had greater effect rather than given individually (Table 2).

4. Discussion

Mitochondria are the energy reservoir of the cell and the damage inflicted in mitochondria would ultimately result in the reduction of energy production and thereby leads to cell death. Subcellular membrane and associated thiol bearing enzymes represent sensitive sites for arsenic, causing perturbation of cellular function [23]. Reactive oxygen species can themselves reduce the activities of enzymes [24].

The lipid peroxidation of arsenic exposed mitochondria demonstrated a significant elevation and reduction in the concentration of ascorbic acid, α-tocopherol, GSH and TSH compared to the control. Arsenite can be assumed to suppress the mitochondrial formation of reducing equivalents by inhibiting nicotinamide nucleotide transhydrogenase

Table 1
Status of liver mitochondrial lipid peroxidation, antioxidants and enzymes in control, arsenic exposed and ascorbic acid and α-tocopherol treated rats

Parameters	Group I (Control)	Group II (Arsenic)	Group III (Arsenic + ascorbic acid)	Group IV (Arsenic + α-tocopherol)	Group V (Arsenic + ascorbic acid + α-tocopherol)
LPO	1.68 ± 0.15	2.60 ± 0.31[a]	1.81 ± 0.20[b]	1.77 ± 0.19[b]	1.70 ± 0.16[b]
Ascorbic acid	55.2 ± 4.94	27.0 ± 2.89[a]	55.0 ± 3.07[b]	50.7 ± 4.03[b]	55.1 ± 5.05[b]
α-Tocopherol	9.29 ± 0.68	4.82 ± 0.38[a]	9.03 ± 0.51[b]	9.16 ± 0.68[b]	9.24 ± 0.66[b]
GSH	39.1 ± 4.26	18.7 ± 1.98[a]	34.9 ± 2.96[b]	36.6 ± 3.45[b]	38.6 ± 3.61[b]
TSH	87.3 ± 8.23	46.2 ± 4.95[a]	70.4 ± 6.43[a,b]	74.3 ± 4.26[a,b]	85.1 ± 8.82[b,c,d]
Isocitrate dehydrogenase	10.5 ± 1.21	6.11 ± 0.73[a]	8.48 ± 0.82[a,b]	9.12 ± 0.95[a,b]	10.3 ± 0.98[b,c,d]
α-Ketoglutarate dehydrogenase	7.83 ± 0.67	3.76 ± 0.41[a]	6.95 ± 0.69[a,b]	7.24 ± 0.74[b]	7.56 ± 0.79[b]
Succinate dehydrogenase	33.1 ± 3.12	16.9 ± 2.08[a]	25.6 ± 2.85[a,b]	27.0 ± 3.11[a,b]	32.6 ± 2.94[b,c,d]
NADH-dehydrogenase	29.6 ± 3.15	17.5 ± 2.37[a]	22.4 ± 2.48[a,b]	24.1 ± 2.61[a,b]	27.8 ± 2.91[b,c,d]
Cytochrome c oxidase	6.91 ± 0.63	4.21 ± 0.45[a]	5.38 ± 0.59[a,b]	6.05 ± 0.64[a,b]	6.73 ± 0.71[b,c]

Each value is expressed as mean ± SD for six rats in each group.

(LPO - nmoles of malondialdehyde released/mg protein; ascorbic acid, α-tocopherol, GSH and TSH - nmoles/mg protein; isocitrate dehydrogenase - nmoles of α-ketoglutarate formed/min/mg protein; α-ketoglutarate dehydrogenase - μmoles of potassium ferrocyanide liberated/min/mg protein; succinate dehydrogenase - μmoles of succinate oxidized/min/mg protein; NADH-dehydrogenase - μmoles of NADH oxidized/min/mg protein and cytochrome c oxidase-O.D. × 10^{-2} /min/mg protein)

[a, b, c, d] represent $p < 0.05$

[a] As compared with group I

[b] As compared with group II

[c] As compared with group III

[d] As compared with group IV

[25]. The shortage of reduced nicotinamide adenine dinucleotide phosphate (NADPH) suppresses the reduction of oxidized glutathione by glutathione reductase and subsequently decreases glutathione content by promoting the increase in the concentration of hydroperoxides and lipid peroxides, which are derived from free radicals [26]. Moreover, the decrease of thiol groups by binding with arsenite was shown to favor lipid peroxidation [23]. Our present study revealed a substantial reduction in lipid peroxidation products and elevation in non-enzymatic antioxidant status in ascorbic acid and α-tocopherol administered arsenic exposed rats. Ascorbic acid has been shown to scavenge superoxide, hydroxyl and peroxyl radicals efficiently. α-Tocopherol reacts with lipid peroxyl radicals at high rates and

Table 2
Status of kidney mitochondrial lipid peroxidation, antioxidants and enzymes in control, arsenic exposed and ascorbic acid and α-tocopherol treated rats

Parameters	Group I (Control)	Group II (Arsenic)	Group III (Arsenic + ascorbic acid)	Group IV (Arsenic + α- tocopherol)	Group V (Arsenic + ascorbic acid + α-tocopherol)
LPO	1.46 ± 0.15	2.03 ± 0.22[a]	1.61 ± 0.15[b]	1.59 ± 0.18[b]	1.48 ± 0.13[b]
Ascorbic acid	46.6 ± 4.14	23.8 ± 2.90[a]	46.3 ± 3.58[b]	44.8 ± 3.46[b]	46.6 ± 4.59[b]
α-Tocopherol	8.79 ± 0.89	4.66 ± 0.59[a]	7.90 ± 0.74[b]	8.64 ± 0.72[b]	8.74 ± 0.87[b]
GSH	33.25 ± 3.61	16.3 ± 2.02[a]	29.9 ± 2.38[b]	31.7 ± 2.64[b]	32.9 ± 3.12[b]
TSH	76.9 ± 7.84	42.3 ± 4.62[a]	54.2 ± 6.01[a,b]	58.9 ± 7.54[a,b]	74.4 ± 6.64[b,c,d]
Isocitrate dehydrogenase	7.24 ± 0.71	4.34 ± 0.45[a]	5.87 ± 0.56[a,b]	6.02 ± 0.55[a,b]	7.12 ± 0.72[b,c,d]
α-Ketoglutarate dehydrogenase	4.48 ± 0.41	2.28 ± 0.31	3.81 ± 0.35[a,b]	3.92 ± 0.41[a,b]	4.37 ± 0.42[b,c]
Succinate dehydrogenase	27.1 ± 2.63	15.5 ± 1.85[a]	23.4 ± 2.01[a,b]	24.4 ± 2.53[b]	26.5 ± 2.42[b,c]
NADH-dehydrogenase	18.8 ± 2.12	11.7 ± 1.93[a]	15.4 ± 2.03[a,b]	17.2 ± 2.11[b]	18.0 ± 1.98[b,c]
Cytochrome c oxidase	6.35 ± 0.62	4.18 ± 0.48[a]	5.21 ± 0.50[a,b]	5.44 ± 0.62[a,b]	6.12 ± 0.60[b,c]

Each value is expressed as mean ± SD for six rats in each group.

(LPO - nmoles of malondialdehyde released/mg protein; ascorbic acid, α-tocopherol, GSH and TSH - nmoles/mg protein; isocitrate dehydrogenase - nmoles of α-ketoglutarate formed/min/mg protein; α-ketoglutarate dehydrogenase - μmoles of potassium ferrocyanide liberated/min/mg protein; succinate dehydrogenase - μmoles of succinate oxidized/min/mg protein; NADH-dehydrogenase - μmoles of NADH oxidized/min/mg protein and cytochrome c oxidase-O.D. × 10^{-2} /min/mg protein)

[a, b, c, d] represent $p < 0.05$

[a] As compared with group I

[b] As compared with group II

[c] As compared with group III

[d] As compared with group IV

interrupts the radical chain, thereby preventing further lipid peroxidation [27].

In addition, ascorbic acid and α-tocopherol improved the thiol status by recycling oxidized glutathione to glutathione [28]. The antioxidants GSH, ascorbic acid and α-tocopherol are interrelated by recycling processes. α-Tocopherol present in the cell membrane counteracts with free radicals and ultimately gets transformed into tocopheroxyl radicals. Recycling of tocopheroxyl radicals to α-tocopherol is achieved by reaction with ascorbic acid [29]. The dehydroascorbic acid formed in this reaction is reduced to ascorbic acid by non-enzymatic reaction with GSH [30].

In the present study, arsenic dosed rats showed a significant reduction in the activities of the citric acid cycle enzymes. Arsenite is an effective inhibitor of mitochondrial pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. This inhibition is mainly due to the reaction of arsenite with the vicinal thiol group of reduced lipoic acid [31]. The decrease in pyruvate dehydrogenase activity leads to the decrease in the formation of acetyl CoA and subsequently isocitrate. The low availability of the substrate, isocitrate, may be the reason for the decrease in the activity of isocitrate dehydrogenase. Similarly, the decrease in the activity of succinate dehydrogenase may be ascribed to the decrease in the level of succinyl CoA, which resulted in the inhibition of α-ketoglutarate dehydrogenase activity by arsenic. In the present study, supplementation of α-tocopherol and ascorbic acid increased the activities of these enzymes and this may be attributed to their capacity to increase the thiol status. Glutathione and lipoic acid serve as an important thiol reserve. Lipoate is also a sensitive component in the complex of mitochondrial branched chain ketoacid dehydrogenase. Therefore, the observed increase in thiol status may subsequently also enhance glutathione and lipoate content [32].

NADH-dehydrogenase, a flavin-linked dehydrogenase, constitute complex I of the electron transport chain, which passes electrons from NADH to coenzyme Q. Cytochrome c oxidase donates electrons directly to molecular oxygen and constitutes complex IV. The significant decline in the activities of the two enzymes on arsenic exposure would result in the inhibition of electron flow from NADH to oxygen. The decrease in the activity of NADH-dehydrogenase may be due to arsenic induced depletion of reducing equivalents NADH and NADPH, which are necessary for the formation of glutathione from oxidized glutathione. Thus a decline in the levels of reducing equivalents decreases mitochondrial glutathione content and thereby leads to loss of cytochrome c oxidase [33].

Increased lipid peroxidation along with decreased efficiency of electron transport contributes to loss of mitochondrial functions during arsenic exposure. From our experimental observations it can be concluded that ascorbic acid and α-tocopherol serve as an effective intervention against arsenic induced mitochondrial dysfunction through their capacity to maintain thiol status thereby preventing reactive oxygen species mediated damage.

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