Mitigation of cataractogenic potential of cyanide by antioxidant vitamin administration

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ABSTRACT

The effect of antioxidant vitamin (AOV) administration on cyanide-induced ocular damage was investigated in New Zealand White rabbits maintained for 30 days on either pure growers mash or mash + 400ppm cyanide with or without oral AOVs. Cyanide caused significant decreases in superoxide dismutase, catalase and Na^+-K^+ ATPase; and significant increases in malondialdehyde levels in the lens ($p < 0.05$). AOVs reversed the elevation in malondialdehyde but had no effect on the enzymes. The results suggest that the damaging effects of oxidative stress imposed by cyanide on the lens can be mitigated by AOVs, probably through enhancement of its antioxidant status rather than directly reversing the inhibition of SOD and catalase. This underscores the need for AOV supplements especially by individuals routinely exposed to habitual, dietary and occupational cyanide.

INTRODUCTION

The toxicity of cyanide in aerobic organisms arises from inhibition of cytochrome oxidase, the terminal electron acceptor in the respiratory chain. Thus, cyanide blocks electron transport, mitochondrial oxygen utilisation and cellular respiration.[1,2] Generally, two forms of toxicity have been recognised, namely, acute and chronic. Acute exposure to cyanide results in fatalities as a consequence of the high susceptibility of the nerve cells of the respiratory centre to hypoxia.[3] On the other hand chronic cyanide intoxication has been linked to the pathogenesis of goitre[4]; tropical ataxic neuropathy[5] and spastic paraparesis[6]. Routes of human exposure to cyanide may be either dietary or environmental.[7] Smoke inhalation appears to be a major environmental source of cyanide.[8-10] It has been suggested that tobacco amblyopia, the sudden dimness of vision in smokers, might be due to the toxic influence of cyanide on ocular tissues.[11] To date, no studies have been carried out to elucidate the biochemical mechanisms through which cyanide impairs visual acuity. In an attempt to unravel this, we recently demonstrated that cyanide imposes oxidative stress on ocular tissues of rabbits by

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inhibition of superoxide dismutase, SOD and catalase, leading to opacification of the rabbit lenses.[12] Several workers have demonstrated that oxidative stress may be overcome by enhancement of antioxidant status via supplementation with AOVs.[13-16]

The present study was aimed at investigating the influence of AOV administration on cyanide-induced oxidative stress in rabbit lenses.

MATERIALS AND METHODS

Animals and feeding

Three groups of New Zealand White rabbits (six per group) aged about three months were used for this experiment. They were housed in clean disinfected metal hutches and initially acclimatised on growers mash (Bendel Feed & Flour Mills Ltd., Ewu, Nigeria) for two weeks. Subsequently members of each group were housed singly. One group received growers mash only, while another group was fed growers mash containing 400ppm inorganic cyanide (sodium cyanide). Members of the third group received, in addition to mash and 400ppm cyanide, daily oral dose of vitamin A, C and E mixture. The vitamin cocktail was prepared by adding 1000U of vitamin A and 500U of vitamin E (Mason Natural, Miami, Florida) to 5ml solution containing 40mg ascorbic acid. The mixture was thoroughly blended at room temperature ($\approx 27°C$) and the resultant jelly-like syrup was administered orally once daily to the rabbits through sterile disposable syringes. Prior to presentation, the feeds were mixed with water in the ratio of 10:1 (w/v) to attain an acceptable texture. Each rabbit was fed at the rate of 65g feed/day. Clean drinking water was liberally provided while stale feed remnants were daily weighed and discarded prior to fresh feed presentations. All animals were weighed weekly. After two weeks, feeding was terminated and blood samples were drawn from the rabbit ear veins into citrate vials using sterile disposable 21-guage syringes.

The animals were then weighed and sacrificed painlessly by rapid cervical dislocation. The rabbit lenses were quickly dissected out, rinsed in cold physiological saline and used immediately for the assay of SOD, catalase, Na^+-K^+-ATPase and malondialdehyde. Thiocyanate measurements were carried out in plasma.

Enzyme and metabolite assays

Na^+-K^+-ATPase was assayed colorimetrically by measuring the amount of inorganic phosphate released following incubation of the lens extracts with disodium ATP.[17] Tissue homogenates were prepared by grinding the lenses in a pre-chilled mortar (with acid-washed sand) in ice cold 20mM tris HCl buffer pH 7.4 containing 0.25mM sucrose and 0.5mM EDTA. Enzyme assay was carried out at room temperature ($\approx 27°C$) in a medium containing 0.05M tris buffer, pH 7.4, 1mM EDTA, 1.5mM NaCL, 0.1M KCL, 30mM $MgCl_2$ and 0.2M disodium ATP[17].

SOD was assayed by monitoring the inhibitory effect of the enzyme on the auto-oxidation of epinephrine.[18] Catalase was estimated by calculating the first order rate constant for the decomposition of H_2O_2 by the tissue extracts.[19] The measurement of malondialdehyde was done colorimetrically using thiobarbituric acid[20] while protein was estimated colorimetrically using the folin phenol reagent.[21] Thiocyanate was assayed in de-proteinised plasma using the Sorbo reagent.[22]

Statistics

The means $\pm$ SEM of values for the various parameters were compared for statistically significant differences using analysis of variance. P values < 0.05 were indicative of significance.

RESULTS

Data in respect of feed consumption, weight changes and plasma thiocyanate levels for rabbits in the three groups are set out in Table

1. The cyanide-fed rabbits had significantly higher feed intake, higher plasma thiocyanate and lower weight gains when compared to rabbits in the control group (p < 0.05). However, the rabbits given AOVs gained significantly more weight than those given cyanide alone. Cyanide ingestion led to significant decreases in activities of SOD, catalase and Na^+-K^+-ATPase in the lenses of the rabbits (Table 2). Administration of AOVs did not relieve these inhibitions.

DISCUSSION

The lens contains a battery of antioxidants and antioxidant enzymes (including SOD and catalase) which function to protect it from oxidative damage by reactive oxygen species.[23,24] The inhibition of lens SOD and catalase by cyanide strongly suggests that cyanide imposes oxidative stress on this tissue. A number of studies have indicated a direct causal relationship between oxidative stress and cataract formation.[25-28] The cyanide-induced oxidative stress is consistent with the significantly raised malondialdehyde levels in the lens. This is so because malondialdehyde is a reliable index of free radical induced lipid peroxidation in tissues.[29,30] The inhibition of SOD and catalase was not reversed by AOV administration. However, the significantly lowered malondialdehyde levels in the lens of rabbits that received cyanide + AOVs relative to those given cyanide without AOV clearly shows that these vitamins mitigated the oxidative stress imposed on the lens by cyanide; most probably through enhancement of its antioxidant status. This protective role,

Table 1 **Feed intake, weight gains and plasma thiocyanate values for rabbits in the three groups***

Group/feed	Control mash	1 Mash + cyanide	2 Mash + cyanide + AOVs
Feed consumption (g/rabbit/day)	44.0 ± 1.6^a	52.3 ± 2.3^b	59.8 ± 3.1^b
Weight gain (g/rabbit)	288 ± 7^a	202 ± 11^b	274 ± 13^a
Feed efficiency (g body wt/g feed)	6.5	4.0	4.6
Plasma thiocyanate (mg/100ml)	1.23 ± 0.50^a	5.84 ± 0.82^b	6.13 ± 0.37^b

Values are Mean ± SEM. For each parameter, those having different superscripts across differ significantly (p < 0.05)

Table 2 **SOD, catalase, Na^+–K^+- ATPase and malondialdehyde levels in the lenses of rabbits in the 3 groups**

Group/feed	Control mash	1 Mash + cyanide	2 Mash + cyanide + AOVs
SOD (U/g fresh wt)	8.35 ± 0.44^a	5.10 ± 0.80^b	4.75 ± 0.92^b
Catalase ($min^{-1}K$)	3.67 ± 0.39^a	2.10 ± 0.61^b	2.43 ± 0.32^b
Na^+-K^+-ATPase (ìgP$_i$/min/mg protein)	1.05 ± 0.04^a	0.63 ± 0.09^b	0.51 ± 0.10^b
Malondialdehyde (nmol/g fresh weight)	1.80 ± 0.1^a	6.3 ± 1.3^b	2.30 ± 0.80^a

Values are Mean ± SEM. For each parameter, values having different superscripts across differ significantly (p < 0.05; n = 6)

which was certainly not confined to the lens alone may be responsible for the superior weight gains of the animals that received AOVs over and above those that were fed cyanide and mash only. Several investigators have reported inverse relationships between AOVs and disease.[23,31,32] Moreover, studies have shown that free radical mediated lipid peroxidation in cigarette smokers can be mitigated by AOV supplementation.[14,33] These reports are in remarkable agreement with the protective roles of AOVs seen in the present study, for cigarette smoke contains not only free radicals[34] but also cyanide gas[8,10].

The inhibition of lens Na^+-K^+-ATPase by cyanide is in agreement with previous reports on the inhibitory effect of cyanide on the enzyme.[35,36] Na^+-K^+-ATPase is vital for maintenance of electrolyte and water balance in the lens. Cortical cataracts is always associated with impairment of electrolyte and water balance, while nuclear cataracts is mostly linked to oxidative stress-catalysed modification of lens proteins.[37] Thus, it appears that cyanide exposure may be involved in the aetiology of both cortical and nuclear cataracts, the latter of which may be relieved by AOV supplementation.

This study has demonstrated that cyanide-induced oxidative stress can be mitigated by AOV administration. If animal-to-man extrapolation is permissible, these findings are considered vital, for they underscore the importance of AOV supplements especially amongst populations routinely exposed to cyanide through habits, diets and occupations.

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