EFFECTS OF PROLONGED ORAL EXPOSURE TO CYANIDE ON SOME OXIDATIVE STRESS BIOMARKERS IN BLOOD OF RATS

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Pathological effects of long-term cyanide exposure on various organs have been reported. Cyanide-induced oxidative stress contributes to the harmful effects of cyanide poisoning on various tissues. The present study was planned to investigate the effects of prolonged oral exposure to cyanide on some oxidative stress biomarkers in erythrocytes and plasma of rats. Two groups of rats received for 6 weeks either tap water or water containing 200 mg L⁻¹ inorganic cyanide in the form of potassium cyanide. The activity of glutathione peroxidase was significantly (P<0.05) higher, whereas that of superoxide dismutase was significantly (P<0.01) lower in the erythrocytes of cyanide exposed animals, in comparison with the control values. Malondialdehyde concentration was significantly increased (P<0.01) in the erythrocyte haemolysate following cyanide exposure. However, cyanide exposure had no significant effect on erythrocyte glutathione concentrations. Indeed, the effects of cyanide exposure on the plasma levels of malondialdehyde, ferric reducing ability of plasma (FRAP) and protein carbonyls were not significant as compared to the control group. It can be concluded that prolonged cyanide poisoning significantly influenced some circulating oxidative stress biomarkers in rats. These studies may provide help for both additional explanations of the pathophysiological aspects of cyanide poisoning and supportive treatment of prolonged cyanide toxicity.

Key words: Cyanide poisoning, Oxidant/antioxidant parameters

INTRODUCTION

Cyanide is one of the most potent cytotoxic poisons known to humans and animals. Besides acute cyanide poisoning, its chronic intoxication has often been documented and it has been suggested that most complications due to cyanide toxicity are attributed to prolonged exposure to dietary, industrial and environmental
sources of this toxic compound (1). Experimental studies on some animal species have revealed that chronic cyanide ingestion causes impaired body growth, neurological and thyroid disturbances and also some pathologic effects on different tissues (2-6). However, the exact mechanism by which cyanide exerts a damaging action on tissues is not clear, although some researchers have proposed that oxidative stress may be implicated in the harmful effects of cyanide poisoning (7, 8). Cyanide has been previously shown to induce oxidative stress and damage in a number of biological systems. Cyanide-induced oxidative stress may be due to increases in reactive oxygen species (ROS) and nitric oxide (9, 10) as well as to inhibition of antioxidant systems (11) and mitochondrial function (12).

There are numerous works in the literature on the effects of prolonged cyanide exposure on various biochemical, oxidant/antioxidant and histopathological indices in some organs of animal species (2, 4, 5, 7, 8, 13, 14, 15, 16), but to our knowledge, there is only one available report (14) on the blood oxidant/antioxidant profile following prolonged cyanide exposure. Thus, the present investigation was planned to find out whether prolonged oral sublethal cyanide exposure could affect the levels of various oxidative stress related parameters in erythrocytes and plasma of rats. These studies may provide help for additional explanation of the pathophysiological aspects of cyanide poisoning and for supportive treatment of prolonged cyanide toxicity.

MATERIAL AND METHODS

CHEMICALS

Potassium cyanide (KCN) was obtained from Merck (Darmstadt, Germany). Commercial enzyme kits for superoxide dismutase (Ransod, RANDOX/SD-125) and glutathione peroxidase (Ransel, RANDOX/RS-505) were achieved from Randox Laboratories (UK). 2, 4-dinitrophenylhydrazine (DNPH), 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB), 2, 4, 6-tripyridyl-s-triazine (TPTZ) and 2-thiobarbituric acid (TBA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The rest of the applied chemicals were of analytical grade and were purchased from Sigma (St Lewis, MO, USA) or Merck (Darmstadt, Germany).

EXPERIMENTAL DESIGN AND SAMPLING

Twelve male Wistar rats weighing nearly 180 g were randomly divided into two equal groups. Rats were kept in clean cages at room temperature (22-25 °C) and a photoperiod of 12 h light and 12 h dark per day. Rats were given standard laboratory balanced commercial diet ad libitum. Animals in the control group were
received tap water while the rats of KCN group were given tap water containing 200 mgL\(^{-1}\) inorganic cyanide, achieved by dissolving 500 mg KCN in 1000 ml drinking water.

At the end of the study (6 weeks), the animals were fasted overnight for 12h before blood sampling. Rats were ether-anaesthetized and blood samples were collected by cardiac puncture into EDTA-containing vials. Following plasma separation by centrifugation at 750 g for 20 min, erythrocytes were washed three times with physiological saline. The washed centrifuged erythrocytes were lysed by adding the same volume of ice-cold redistilled water. Plasma and erythrocyte haemolysate was pipetted into different aliquots and stored at -70°C until analysis.

**BIOCHEMICAL ASSAYS AND ANALYSIS**

The activity of glutathione peroxidase (GPx) in erythrocyte haemolysate was measured using RANDOX-Ransel enzyme kit, and the results were expressed as units/g haemoglobin. Hemoglobin concentration was determined by cyamethaeamoglobin method.

The activity of superoxide dismutase (SOD) in erythrocyte haemolysate was assayed by a modified method of iodophenyl nitrophenol phenyltetrazolium chloride applying the RANDOX-Ransod enzyme kit, and the results were given as units/g haemoglobin.

Glutathione (GSH) concentration was measured in erythrocyte haemolysate by the method formerly described by Ellman (17). In this method, 5, 5′-dithiobis (2-nitrobenzoic acid) is reduced to 2-nitro-5′-mercaptobenzoic acid by GSH. The intensity of the yellow color formed was determined at 412 nm and the calculated results were given as µmoles/g haemoglobin.

Malondialdehyde (MDA) measurement in plasma and erythrocyte haemolysate was based on spectrophotometric analysis of the pink colored product of thiobarbituric acid reactive substances, as described by Latha and Pari (18). Concentration of MDA was determined using a molar absorptivity value of 156,000 M\(^{-1}\) cm\(^{-1}\).

The FRAP (ferric reducing ability of plasma) was determined as previously described by Benzie and Strain (19). This method is based on the reduction of a ferric tripyridyltriazine reagent to the ferrous form by antioxidants in the sample. This reaction produces an intense blue color that can be determined spectrophotometrically at 593 nm. Using a calibration curve of Fe\(^{3+}\), FRAP values were computed and expressed in µmol Fe\(^{2+}\) formed per L of plasma.

Carbonyl groups of plasma proteins were assayed by reaction with 2,4-dinitrophenylhydrazine, which leads to the formation of a stable 2,4-dinitrophenyl hydrazone product (20). The amount of 2,4-dinitrophenylhydrazones produced was determined spectrophotometrically at 370 nm applying a molar absorptivity value of 22,000 M\(^{-1}\) cm\(^{-1}\).
STATISTICAL ANALYSIS

All measured parameters have been shown as mean ± standard error of mean (SEM). The acquired data were analysed using Student’s t test. The level of significance was considered to be P<0.05 or P<0.01 as indicated. All analyses were done using SPSS/PC software.

RESULTS

The effects of KCN exposure on the values (mean ± SEM) of the measured oxidant/antioxidant parameters in plasma and erythrocyte haemolysate of rats are presented in Tables 1 and 2. The activity of GPx was significantly (P<0.05) higher, whereas that of SOD was significantly (P<0.01) lower in the cyanide exposed animals in comparison with control values (Table 1). Malondialdehyde concentration was increased significantly (P<0.01) in the erythrocyte haemolysate following cyanide exposure. However, cyanide exposure had no significant effect on erythrocyte glutathione concentrations (Table 1). Indeed, the effects of cyanide exposure on the plasma levels of malondialdehyde, FRAP and protein carbonyls were not significant as compared to control group (Table 2).

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group</th>
<th>Cyanide treated group</th>
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</thead>
<tbody>
<tr>
<td>GPx (U/g Hb)</td>
<td>24.30±6.10</td>
<td>65.42±12.53*</td>
</tr>
<tr>
<td>SOD (U/g Hb)</td>
<td>2186.06±313.2</td>
<td>884.12±131.39**</td>
</tr>
<tr>
<td>GSH (nmol/g Hb)</td>
<td>1.22±0.11</td>
<td>1.10±0.11</td>
</tr>
<tr>
<td>MDA (nmol/g Hb)</td>
<td>20.10±3.08</td>
<td>35.88±3.13**</td>
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</tbody>
</table>

Each value represents mean ± SEM. n = 6 in each group; *P < 0.05; **P < 0.01.

Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group</th>
<th>Cyanide treated group</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRAP (nmol/g Hb)</td>
<td>114.47±19.83</td>
<td>76.80±6.21</td>
</tr>
<tr>
<td>Plasma MDA (nmol/ml)</td>
<td>4.89±0.73</td>
<td>5.46±0.54</td>
</tr>
<tr>
<td>Carbonyl contents (nmol/mg protein)</td>
<td>1.33±0.21</td>
<td>2.43±0.59</td>
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Each value represents mean ± SEM. n = 6 in each group.
DISCUSSION

There are numerous evidences that oxidative stress has been implicated in diverse pathological conditions. In this study on experimental cyanide poisoning, our results display that prolonged cyanide poisoning caused considerable changes in some oxidative stress biomarkers.

There are many documents in the literature on the effect of various xenobiotics on the antioxidant enzymes systems in different cells and tissues of animals. SOD catalyses the dismutation of superoxide radical to hydrogen peroxide, which is then converted to water by GPx or by catalase (21). Based on the present study results prolonged sublethal cyanide administration caused a significant increase in the mean value of erythrocytic GPx activity as compared to control group. By contrast, our results indicated a significant decline of SOD activity in red blood cells of cyanide treated rats as compared to control group. The reduction of SOD in the erythrocytes of the cyanide treated rats is somewhat similar with the previous reports indicating the reduction of SOD and catalase activities in some tissues of cyanide toxified rats (1) and rabbits (7, 22). It has been also shown that acute exposure of lethal dose of cyanide alters the levels of brain, but not liver, antioxidant enzymes in rats (16). However, the results of Mathangi et al. (14) showed that cyanide poisoning (KCN; oral; 1.4 mg/kg) for 90 days in rats caused no significant alteration in blood SOD and GPx activities.

The decreased SOD activity could be attributed to irreversible inactivation of this enzyme by its product, H$_2$O$_2$, due to cyanide-induced increase in superoxide anion generation (21, 23) or directly to its irreversible inhibition by cyanide (21). On the other hand, the higher activity of erythrocytic GPx in cyanide treated group might be as a compensatory response to enhanced ROS formation and may supply more protection against free radical-mediated oxidative damages.

GSH is requisite for the removal of hydrogen peroxide by the GPx-catalysed reaction (24). It has been reported that cyanide poisoning in rats significantly decreased reduced glutathione in the blood, liver and brain (14). Also, acute exposure of lethal dose of cyanide was found to decline GSH levels in the liver of rats (16). However, our results indicated no significant reduction in erythrocytic GSH concentration in cyanide toxified rats in comparison to controls.

In the current study, the total plasma antioxidant capacity was evaluated by applying the FRAP assay. FRAP levels have been shown to be proportional to the reducing power of the principally non-enzymatic antioxidants in the plasma (19, 25). When FRAP values were compared between controls and cyanide intoxicated rats, we found no significant reduction of plasma antioxidant capacity of cyanide intoxicated rats as compared to that of control individuals.

Oxidative shocks can cause severe damage to various cellular macromolecules. Some analytical methods have been applied to measure the oxidation products directly (e.g. carbonyl contents for oxidized proteins) or the end
degradation products (e.g., MDA as a marker of lipid peroxidation) (26). These oxidation products can be used as biomarkers in the tissue or plasma to monitor the irreversible consequences of oxidative stress in animals and humans (27, 28).

Based on the present results, significant enhancement of MDA values were observed in erythrocyte haemolysate of cyanide-intoxicated rats. In line with our findings, increased lipid peroxidation following cyanide exposure has been reported in the brain and kidney of mice (13) and in the lenses of rabbit (7). Indeed, the results of Mathangi et al. (14) showed that oral administration of cyanide to rats for 90 days caused increased lipid peroxidation in the brain and liver, but not in the kidney. Moreover, the results of Tul sawani et al. (6) showed a significant increase in the levels of brain MDA. The induction of lipid peroxidation by cyanide was also found in cultured neurons from chick embryo telencephalon and in mouse brain cortical slices incubated with cyanide in vitro (11, 29). On the other hand, based on the present study results, KCN administration caused no significant alteration on plasma MDA concentrations, which is otherwise than the results of Mathangi et al. (14), who reported significant elevation of plasma MDA concentration following sub-chronic cyanide poisoning in rats. Moreover, based on the present results, plasma protein carbonyl contents were increased nonsignificantly following cyanide administration.

The possible cause underlying the remarkable changes of oxidative status biomarkers in erythrocytes following cyanide intoxication, as observed in the present study, could mainly be attributed to the reality that the most of cyanide in circulation is sequestered in the erythrocytes and these cells contain most of the blood cyanide (30, 31). Moreover, red blood cells are notably susceptible to oxidative shocks because they are exposed to oxygen radicals that are continuously generated primarily due to the auto-oxidation of haemoglobin. Erythrocytes are also exposed to oxidative force from plasma (especially mediated by hydrogen peroxide and nitric oxide) (21).

On the basis of the current findings, it can be concluded that prolonged cyanide poisoning significantly influenced some circulating oxidative stress biomarkers in rats. This suggests that antioxidant therapy approaches may have beneficial effects on cyanide intoxication. However, further investigations are necessary to clarify the basic molecular mechanism of cyanide induced oxidative stress.

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REFERENCES


