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Effect of sub-acute oral cyanide administration in rats: Protective efficacy of alpha-ketoglutarate and sodium thiosulfate

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Abstract

Chronic toxicity of cyanide in humans and animals has been previously described. Alpha-ketoglutarate (alpha-KG) and sodium thiosulfate (STS) are known to confer remarkable protection against acute cyanide poisoning in rodents. Their efficacy against sub-acute or chronic cyanide exposure is not known. The objective of the present study was to assess the sub-acute toxicity of potassium cyanide (KCN) in female rats following oral administration of 7.0 mg/kg (0.5 LD_{50}) for 14 d. The effect of alpha-KG (oral; 1.0 g/kg) and/or STS (intraperitoneal, 1.0 g/kg) on cyanide toxicity was also evaluated. Various hematological and biochemical indices were determined after 7 d of treatment and additional parameters like organ-body weight index (OBI) and histology of brain, heart, lung, liver, kidney and spleen were performed after 14 and 21 d (recovery group) of cyanide exposure. Sub-acute exposure of KCN did not produce any significant change in body weight of the animals, OBI, hematology and the levels of blood urea, creatinine, aspartate aminotransferase, triiodothyronine (T3) and tetraiodothyronine (T4). The levels of temporal glutathione disulfide (GSSG) and hepatic malondialdehyde (MDA), reduced glutathione (GSH) and GSSG were unaffected. However, in KCN treated animals elevated levels of blood glucose and reduced levels of alanine aminotransferase were observed. Activities of cytochrome c oxidase in the brain and rhodanese in the liver were diminished. Reduced levels of GSH and enhanced levels of MDA in brain were observed. Increased levels of blood thiocyanate were observed in all the treatments of KCN. Additionally, KCN also produced various histological changes in the brain, heart, liver and kidney. Although, treatment of alpha-KG and STS alone significantly blunted the toxicity of KCN, concomitant use of both interventions afforded to maximum protection. This study indicates a promising role of alpha-KG and STS for the treatment of prolonged cyanide exposures.

Keywords: Cyanide; Sub-acute effects; Alpha-ketoglutarate; Sodium thiosulfate; Protection

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1. Introduction

The risks of occupational sub-acute or chronic exposure to cyanide in industries are enormous and wide spread [1–3]. Tobacco smoking or chronic dietary intake of cyanogenic foods may also account for severe sub-acute or chronic toxicity like tobacco amblyopia, tropical ataxic neuropathy and tropical goiter [4,5]. Toxic levels of cyanide in the body have also been generated after administration of certain drugs like laetrile and sodium nitroprusside [6–8]. There have been a number of fatalities or chronic toxic syndromes following intensive use of these drugs. Sublethal doses of cyanide are normally converted rapidly to the less toxic thiocyanate (SCN) but continuous exposure may cause inhibition of certain enzymatic activities resulting in various biochemical and pathological lesions [9,10]. Sub-acute or chronic cyanide poisoning is characterized by prolonged energy deficit, loss of ionic homeostasis and oxidative stress leading to CNS pathology [11]. Previous studies have shown a variety of toxic effects in experimental animals after chronic administration of cyanide through diet or water [12–14]. No treatment strategies were considered in these studies. Although, treatment of acute cyanide poisoning has been widely addressed, detoxification following sub-acute cyanide exposure has not been extensively investigated.

Treatments for cyanide poisoning are numerous but sodium nitrite (SN) and sodium thiosulfate (STS) are the most widely used antidotes [15]. Parenteral or oral treatment of alpha-ketoglutarate (alpha-KG), alone or with SN and/or STS has also been shown to confer significant protection against acute cyanide poisoning in rodents [16–19]. Cyanide antagonism by alpha-KG has been ascribed to formation of cyanohydrin, a complex formed by the interaction of CN^- and the carbonyl group of alpha-KG [20]. Oral treatment of alpha-KG is particularly envisaged for fire fighters, victims of smoke inhalation, chronic occupational or dietary poisoning, accidental or deliberate ingestion of cyanide or as an oral pretreatment for personnel engaged in evacuation/decontamination, apprehending cyanide exposure. The purpose of this study was to characterize cyanide toxicity after sub-acute oral (gavage) administration in rats, and evaluate the protective efficacy of alpha-KG and STS.

2. Experimental

2.1. Chemicals and reagents

Alpha-KG disodium salt, cytochrome c and rhodanese were purchased from Sigma, St. Louis, USA and potassium cyanide (KCN) was from Ferrack (Germany). Other chemicals including STS were from E. Merck (India). Urine analysis was performed by Multistix® from Bayer Diagnostics (India). Diagnostic kits for blood glucose, urea, protein and creatinine were from Ranbaxy Laboratories (India) and those for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were from E. Merck (India). Plasma concentrations of thyroid hormones viz. total triiodothyronine (T3) and tetraiodothyronine (T4) were measured using commercial ELISA kits of Jenix Technology Inc. (Canada).

2.2. Animals

Female Wistar rats (120–150 g) bred in the animal facility of Defense Research and Development Establishment (DRDE), Gwalior were maintained on a bedding of rice husk in polypropylene cages. The animals had access to water and rodent pellet feed (Amrut Feeds, Pranav Agro, Delhi, India) ad libitum. All the animals were fasted overnight before the experiment and were given food 1 h after treatment. The study had the approval of the Institutional ethical committee on animal experimentations.

2.3. Treatment

All the solutions were prepared fresh in triple distilled water (TDW) and were administered in a volume <10 ml/kg body weight. TDW, alpha-KG (1.0 g/kg) or KCN (7.0 mg/kg equivalent to 0.50 LD_{50}) was administered orally using a 16 gauge oral feeding cannula (HSE-Harvard, Germany) and STS (1.0 g/kg) was given intraperitoneally. Control animals received equivalent amount of TDW. Sixty three rats were divided into seven groups of nine animals each. Various treatment groups were: (1) Control, (2) alpha-KG, (3) STS, (4) KCN, (5) KCN + alpha-KG, (6) KCN + STS and (7) KCN + alpha-KG + STS. All the animals were treated once daily for 14 d. Animals in group five, six and seven received α-KG and/or STS after 5 min of