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Biochemical, oxidative and histological changes caused by sub-acute oral exposure of some synthetic cyanogens in rats: Ameliorative effect of α-ketoglutarate

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ABSTRACT

Time-dependent cyanide generation and acute toxicity of six different cyanogens were reported earlier, out of which malononitrile (MCN), propionitrile (PCN), and sodium nitroprusside (SNP) were found to be very toxic. We report here 14 d sub-acute toxicity of MCN, PCN, and SNP (oral; 1/10 LD_{50} daily) in female rats, and its amelioration by α-ketoglutarate (α-KG; oral; 5.26 mmol/kg; +5 min), a potential cyanide antidote. Significant decrease in white blood cells (PCN, SNP), platelets count (PCN), and blood glucose levels (MCN, PCN, SNP) was accompanied by elevated levels of alanine aminotransferase, lactate dehydrogenase (MCN, PCN, SNP), and aspartate aminotransferase (PCN, SNP). Oxidative damage was evidenced by diminished total antioxidant status in plasma and enhanced malondialdehyde levels in liver and kidney. This was accompanied by diminished levels of reduced glutathione, glutathione peroxidase, glutathione reductase, superoxide dismutase, and catalase in the brain, liver and kidney. We also observed increased levels of blood cyanide and thiocyanate, together with inhibition of cytochrome *c* oxidase and thiosulfate-sulfur transferase activities in total brain and liver homogenate, respectively. Cyanogens also produced several histological changes in all the organs studied. Post-treatment with α-KG significantly abrogated the toxicity of cyanogens, indicating its utility as an antidote for long-term cyanogen exposure.

1. Introduction

Cyanogens usually include highly reactive and complex nitrile-containing compounds, which undergo hepatic metabolism to generate toxic levels of cyanide. The non-nitrile portion of cyanogens may also exert toxic effects, which are independent of cyanide toxicity (Bhattacharya et al., 2009). Aliphatic nitriles are extensively used as solvents and synthetic intermediates in polymers, plastics, synthetic fibers, resins, dyestuffs, pharmaceuticals, and vitamin industries (Enongene et al., 2000; Saillenfait and Sabate, 2000; Wu et al., 2009). Due to occupational exposures, many such compounds have been reported to cause pronounced toxicity following generation of cyanide ions (Hadri et al., 2005). Cyanide impairs cellular respiration by inhibiting cytochrome *c* oxidase (CYTOX), an end chain enzyme of cellular respiration (Bhattacharya and Flora, 2009). Perturbations in cellular energy metabolism leads to alterations in several vital intracellular processes, resulting in generation of reactive oxygen species (ROS), lipid peroxidation and oxidative stress (Tulsawani et al., 2005; Bhattacharya et al., 2009; Hariharakrishnan et al., 2009). Sub-lethal doses of cyanide are usually enzymatically detoxified to thiocyanate, which is eliminated through urine. However, lethal doses of cyanide are known to inhibit thiosulfate sulfur transferase (TST), an enzyme involved in cyanide detoxification (Singh et al., 2013). Also, prolonged exposure to cyanide may inhibit other enzymatic activities, resulting in various

Abbreviations: α-KG, α-ketoglutarate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ATCN, acetonitrile; ACN, acrylonitrile; CA, catalase; CNS, central nervous system; CYP, cytochrome P450; CYTOX, cytochrome *c* oxidase; DTT, dithiothreitol; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; HCT, hematocrit; LD_{50}, median lethal dose; LDH, lactate dehydrogenase; MCN, malononitrile; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; MDA, malondialdehyde; OBI, organ-to-body weight index; PCN, propionitrile; RBC, red blood cells; SNP, sodium nitroprusside; SCN, succinonitrile; SOD, superoxide dismutase; STS, sodium thiosulfate; TAS, total antioxidant status; TST, thiosulfate sulfurtranferase; TCA, trichloroacetic acid; TDW, triple distilled water; WBC, white blood cells.

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biochemical and pathological lesions (Tulsawani et al., 2005; Hariharakrishnan et al., 2009).

Acute oral toxicity of several cyanogens, viz., acetonitrile (ATCN), acrylonitrile (ACN), malononitrile (MCN), propionitrile (PCN), sodium nitroprusside (SNP), and succinonitrile (SCN) has been reported earlier in rats (Bhattacharya et al., 2009). We further reported the acute toxicity of these cyanogens by different routes of administration in rats, and time-dependent cyanide generation and CYTOX inhibition in soft tissues after sub-lethal intoxication (Rao et al., 2013). Among six cyanogens tested, MCN, PCN, and SNP were found to be very toxic, and exhibited narrow window of cyanogenesis compared to ATCN ACN and SCN. MCN is a highly toxic compound by all the routes of administration. It is estimated that annually about 1200 workers are occupationally exposed to MCN, predominantly by dermal contact (HSDB, 2003; NAC, 2007). Systemic toxicity of MCN is attributed to metabolic release of cyanide, and the onset of symptoms is typically delayed for up to several hours (HSDB, 2003). On the other hand, PCN is a moderate to highly toxic compound and an eye irritant. It causes toxicity by inhalation, ingestion, and absorption through skin. The target organs of PCN include kidney, liver, lungs, eyes, and the central nervous system (CNS). PCN has also been shown to produce teratogenic effects in hamsters (Patnaik, 2007). In several cases, fatal PCN toxicity was preceded by symptoms like headache, nausea, seizures, and dizziness, which were usually accompanied by elevated blood cyanide levels (Scolnick et al., 1993; NAC, 2007). Sodium penta-cyanonitrosylferrate(II) or SNP is an inorganic compound, which is also considered as a potent cyanogen. SNP is comprised of a ferrous ion complexed with five cyanide moieties and a nitrosyl group. SNP interacts with oxyhemoglobin in the blood to produce methemoglobin, releasing cyanide ion and nitric oxide (Lockwood et al., 2010). Nitric oxide has strong vasodilatory properties and for this reason SNP has been used for the management of myocardial infarction (Cole and Vessey, 1988; Vaughan and Delanty, 2000; Elliott, 2004). However, the Federal Drug Administration (FDA) believes that any level of SNP above 3.5 mg/kg/min can be potentially lethal, and recommended a total dose of SNP as low as 1.5 mg/kg for therapeutic purposes (Vesey and Cole, 1985; Sipe et al., 2001). Severe cases of cyanide poisoning, including deaths have been reported following SNP administration in patients (Broderick et al., 2007; Lockwood et al., 2010).

Acute toxicity of MCN, PCN, and SNP has been adequately addressed earlier (Bhattacharya et al., 2009; Rao et al., 2013). However, there is a dearth of information on their toxicity following repeated exposure. Long-term occupational and environmental exposures of such cyanogens are of potential consequence to human health (Rongzhu et al., 2005; Patnaik, 2007). Therefore, there was a need to study their toxicity following repeated exposure. The present study reports the sub-acute toxicity of MCN, PCN and SNP following 14 d oral (po) administration in rats. The study mainly focused on biochemical, oxidative and histological changes, particularly in the brain, liver and kidney. Additionally, the ameliorative effect of α-ketoglutarate (α-KG), a potential cyanide antidote was assessed. It has been shown that α-KG alleviates cyanide toxicity after acute (Bhattacharya and Vijayraghavan, 2002), sub-acute (Tulsawani et al., 2005; Hariharakrishnan et al., 2009) and sub-chronic (Mathangi et al., 2011) exposures in experimental animals. The present study reinforces the protective efficacy of α-KG in treating cyanogen intoxication after repeated exposures.

2. Materials and methods

2.1. Animals

Female Wistar rats (150–200 g) bred in the animal facility of the Defence Research and Development Establishment (DRDE), Gwalior, were used in this study. The care and maintenance of animals were as per the approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Govt. of India, New Delhi, India. The animals were kept in controlled environmental conditions of ambient temperature (22 ± 2 °C) and relative humidity of 40–60%, in a 12:12 light/dark cycle. All the animals were fed standard pellet diet (Ashirwad Brand, Chandigarh, India) and water ad libitum. The animals were acclimatized for 7 d and fasted over night prior to the experiment. The experimental protocol was approved by the Institutional Ethical Committee (TOX-25/50/RB dated 16 July 2012) approved by CPCSEA.

2.2. Chemicals

α-Ketoglutaric acid disodium salt (α-ketoglutarate; α-KG), cytochrome *c* (from bovine heart), trizma base, dithiothreitol (DTT), pyridine, sodium thiosulfate (STS), chloramine T, pyridine, trichloroacetic acid (TCA), ferric nitrate, sodium thiocyanate, nitric acid, potassium cyanide (KCN) and other analytical grade chemicals or reagents were purchased from Sigma–Aldrich, St. Louis, MO, USA. 3-Methyl-1phenyl-2pyrazolin-5-on and bispyrazolone were procured from Fluka AG, Chemische Fabrik, Germany and BDH Laboratory, London, UK, respectively. Malononitrile (MCN 99%+) and propionitrile (PCN 99%+) were purchased from Acros Organics, New Jersey, USA, and sodium nitroprusside (SNP 99%+) was procured from Merck, Darmstadt, Germany.

2.3. Treatment

All the solutions of cyanogens and α-KG were prepared fresh in triple distilled water (TDW), and administered (po) in a volume <10 ml/kg body weight. A 16 gauge animal feeding needle (HSE-Harvard, March-Hugstetten, Germany) was used for the administration of cyanogens and α-KG for 14 d. One hundred twenty rats were divided into two groups of 48 and 72 rats each. Each group was further divided into eight sub-groups as follows: (1) Control (saline), (2) α-KG (5.26 mmol/kg), (3) MCN, (4) PCN, (5) SNP, (6) MCN + α-KG, (7) PCN + α-KG, and (8) SNP + α-KG. Each sub-group of group 1 contained six rats each, while that of group 2 contained nine rats each. The doses of cyanogens were equivalent to respective 1/10 LD_{50} (po) values (Rao et al., 2013), while the dose of α-KG and its time of administration (+5 min) were based on our previous report (Tulsawani et al., 2005). Animals of group 1 and 2 were sacrificed 7 d and 14 d post-exposure, respectively. Animal body weight was recorded daily till the termination of experiment. After 7 and 14 d of treatments, six animals from each sub-group were randomly selected for various hematological and biochemical estimations. Blood was drawn from the retro-orbital plexus of each animal under ether anesthesia. Soon after, animals were killed by cervical dislocation, and the brain, heart, lung, liver, kidney and spleen were excised quickly. The organs were rinsed in 0.9% saline, blotted and weighed to determine organ-to-body weight index (OBI). The OBI is defined as the relative organ weight or organ weight expressed as a percentage of body weight, and is calculated as the ratio of organ weight × 100 and the animal body weight. Various biochemical estimations were performed in blood and tissue (brain, liver and kidney) homogenate. The remaining three animals from each sub-group of group 2 were used for histopathological observations after 14 d.

2.4. Hematological variables

Various hematological variables, viz., white blood cells (WBC), red blood cells (RBC), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), mean cell hemoglobin (MCH), platelets and hemoglobin were measured by automated blood analyzer (Beckman-Coulter Inc., CA, USA).

2.5. Biochemical variables

2.5.1. Clinical biochemistry

Blood glucose (mg/dl), plasma creatinine (mg/dl), alanine aminotransferase (ALT; U/L), aspartate aminotransferase (AST; U/L), lactate dehydrogenase (LDH; U/L) and urea (mg/dl) were measured on a UV visible spectrophotometer (Thermo Electron Corpn., Madison, USA), using Ecoline diagnostic kit (Merck Ltd., Mumbai, India). Plasma levels of Na^+ and K^+ ions (meq/L) were measured by a 128 flame photometer (Systronics, Ahmedabad, India).

2.5.2. Assay of oxidative stress markers

Reduced glutathione (GSH; μmol/g) and oxidized glutathione (GSSG; μmol/g) were measured in tissue homogenate (Hissin and Hilf, 1976). Lipid peroxidation was characterized by malondialdehyde (MDA; nmol/g) estimation (Okhawa et al., 1979). Glutathione peroxidase (GPx; nmol/min/g), glutathione reductase (GR; nmol/min/g), catalase (CA; nmol/min/g), and superoxide dismutase (SOD; U/g) were also measured in tissue homogenate, and total antioxidant status (TAS; mM) was estimated in plasma employing the diagnostic kits of Calbiochem-Merck, Darmstadt, Germany.

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