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Acute toxicity of some synthetic cyanogens in rats: Time-dependent cyanide generation and cytochrome oxidase inhibition in soft tissues after sub-lethal oral intoxication

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ABSTRACT

Cyanogens include complex nitrile-containing compounds that can generate free cyanide of toxicological significance. Acute toxicity, time-dependent cyanide generation and cytochrome oxidase (CYTOX) inhibition in soft tissues, and urinary thiocyanate levels were measured after acute cyanogen intoxication in rats. Order of cyanogens in terms of LD_{50} was: malononitrile (MCN) > propionitrile (PCN) ≈ sodium nitroprusside (SNP) > acrylonitrile (ACN) > succinonitrile (SCN) > acetonitrile (ATCN) for oral, and SNP > MCN > ACN > PCN > SCN > ATCN for intraperitoneal and subcutaneous routes. MCN was most toxic by oral (LD_{50} = 66.4 mg/kg) and SNP by intraperitoneal (LD_{50} = 16.7 mg/kg) and subcutaneous (LD_{50} = 11.9 mg/kg) routes. Minimum survival time (25 min) was recorded after 4.0 LD_{50} ATCN. Order of cyanogens (0.75 LD_{50}; oral) on the basis of maximum blood cyanide and time of peak cyanide generation were: ATCN > SNP > SCN > PCN > MCN > ACN, and MCN (30 min) < SNP (1 h) < PCN ≈ ACN (8 h) < SCN (24 h) < ATCN (72 h), respectively. In most cases, time profile of cyanide generation correlated with corresponding CYTOX inhibition and urinary thiocyanate levels. With the understanding of time-dependent toxicity of different cyanogens, suitable therapeutic windows can be designed for their management.

1. Introduction

Cyanogens usually refer to complex nitrile-containing materials that undergo hepatic metabolism to release toxic levels of cyanide ions. These compounds exhibit versatile physical and chemical properties (Wu et al., 2009). Cyanogens include aliphatic nitriles (R-CN); their structure–toxicity relationship was first elucidated in 1984 (Tanii and Hashimoto, 1984a). Occupational and environmental exposure of synthetic nitriles is of potential relevance to human health (Rongzhu et al., 2005). These highly reactive compounds are used in numerous industrial, domestic and medical settings (Akers et al., 1999; Hadri et al., 2005). They are used as solvents and synthetic intermediates in polymers, plastics, synthetic fibers, resins, dyestuffs, pharmaceuticals, vitamin industries, etc. (Wu et al., 2009; Enongene et al., 2000; Saillenfait and Sabate, 2000). Globally, approximately 26000 workers are potentially exposed to these compounds each day, which predominantly occurs through dermal absorption or inhalation of vapor and aerosols (Hadri et al., 2005; Patnaik, 2007). Such exposures adversely affect the central nervous system (CNS), hepatic, cardiovascular, renal, and gastrointestinal systems (Farooqui and Mumtaz, 1991). Also, there are several reports on genotoxic potentials of such cyanogens (Ballantyne, 1987; Fan et al., 2006; Patnaik, 2007; Wu et al., 2009). Worldwide, acetonitrile (ATCN) concentration in air is reported to be 0.20–42 μg/m^3. The levels causing toxicity in man are unknown but are probably in excess of 840 mg/m^3 (500 ppm) in air (WHO, 2000; Patnaik, 2007). In a case of fatal ATCN poisoning, blood concentrations of 45, 20, and 42 μg/ml of ATCN, cyanide and formic acid, respectively were reported (Nowicka et al., 2010). Typical workplace air concentration of acrylonitrile (ACN) is reported to range from 0.1 to 4 mg/m^3 (ATSDR, 2006). The human ceiling is 21.7 mg/m^3 or 10 ppm/15 min (Patnaik, 2007). Workmen exposed to ACN concentrations varying from 35–220 mg/m^3 for 20–45 min reported severe sickness (WHO, 2000). Significant amount of ATCN and ACN have been reported in cigarette smoke, with corresponding increase in blood cyanide levels (ATSDR, 2006). Malononitrile (MCN) is a highly toxic compound by all the routes. Its TLV-TWA is reported to be 8 mg/m^3 or 3 ppm (Patnaik, 2007). In an accident involving propionitrile (PCN), its work site concentration was found to be 77.5 mg/m^3 in the air, and blood

Abbreviations: ATCN, acetonitrile; ACN, acrylonitrile; CNS, central nervous system; CYTOX, cytochrome oxidase; CYP, cytochrome P450; MCN, malononitrile; PCN, propionitrile; SCN, succinonitrile; SNP, sodium nitroprusside.

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Table 1
Route-specific LD_{50} values of various cyanogens in rats.

Treatments	LD_{50} (mg/kg)		
	Oral	Intraperitoneal	Subcutaneous
ATCN	>5000	2388.1 (2053.0–2777.9)	>5000
ACN	95.1 (81.7–110.6)	95.1 (81.7–110.6)	95.1 (81.7–110.6)
MCN	66.4 (57.1–77.2)	41.9 (35.9–48.7)	47.6 (40.9–55.4)
PCN	83.6 (71.8–97.2)	189.7 (163.1–220.7)	150.7 (129.5–175.3)
SNP	83.6 (71.8–97.2)	16.7 (14.3–19.4)	11.9 (10.3–13.9)
SCN	378.5 (325.4–440.3)	835.5 (718.3–971.9)	755.2 (649.2–878.4)

Acute 24 h LD_{50} of acetonitrile (ATCN), acrylonitrile (ACN), malononitrile (MCN), propionitrile (PCN), sodium nitroprusside (SNP), and succinonitrile (SCN) was determined in female rats by Dixon's up and down method (Dixon, 1965). Values in parentheses are fiducial limits at 95% confidence interval.

cyanide concentration in victims was 3.5 μg/ml (Patnaik, 2007). Pharmacological preparations of sodium nitroprusside (SNP; an inorganic cyanogen) and succinonitrile (SCN) have been involved in several cases of human cyanide poisoning (Baskin et al., 2008). Federal Drug Administration (FDA) believes that any level of SNP above 3.5 mg/kg/min could 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). Patients administered SNP for 26–160 h with doses ranging from 1.8 to 12 mg/kg showed toxic levels (>500 μg/L) of cyanide (Lockwood et al., 2010). Regarding SCN, it has been estimated that approximately 60% of the compound administered to rats is transformed to cyanide, and subsequently excreted as thiocyanate (Contessa and Santi, 1973).

Metabolism of aliphatic nitriles may occur via two competing pathways: (1) direct conjugation with reduced glutathione (GSH) and (2) epoxidation by cytochrome *P*450s (CYPs) leading to formation of epoxide intermediates. Epoxide hydrolase mediated metabolism of epoxide intermediates lead to cyanide generation (Chanas et al., 2003; Hadri et al., 2005). Cyanide is rapidly distributed to a volume of approximately 40% of total body weight and inhibits cytochrome c oxidase (CYTOX), a terminal enzyme of mitochondrial respiratory chain (Bismuth et al., 1987). Cyanide is enzymatically metabolized to thiocyanate, which is excreted through urine

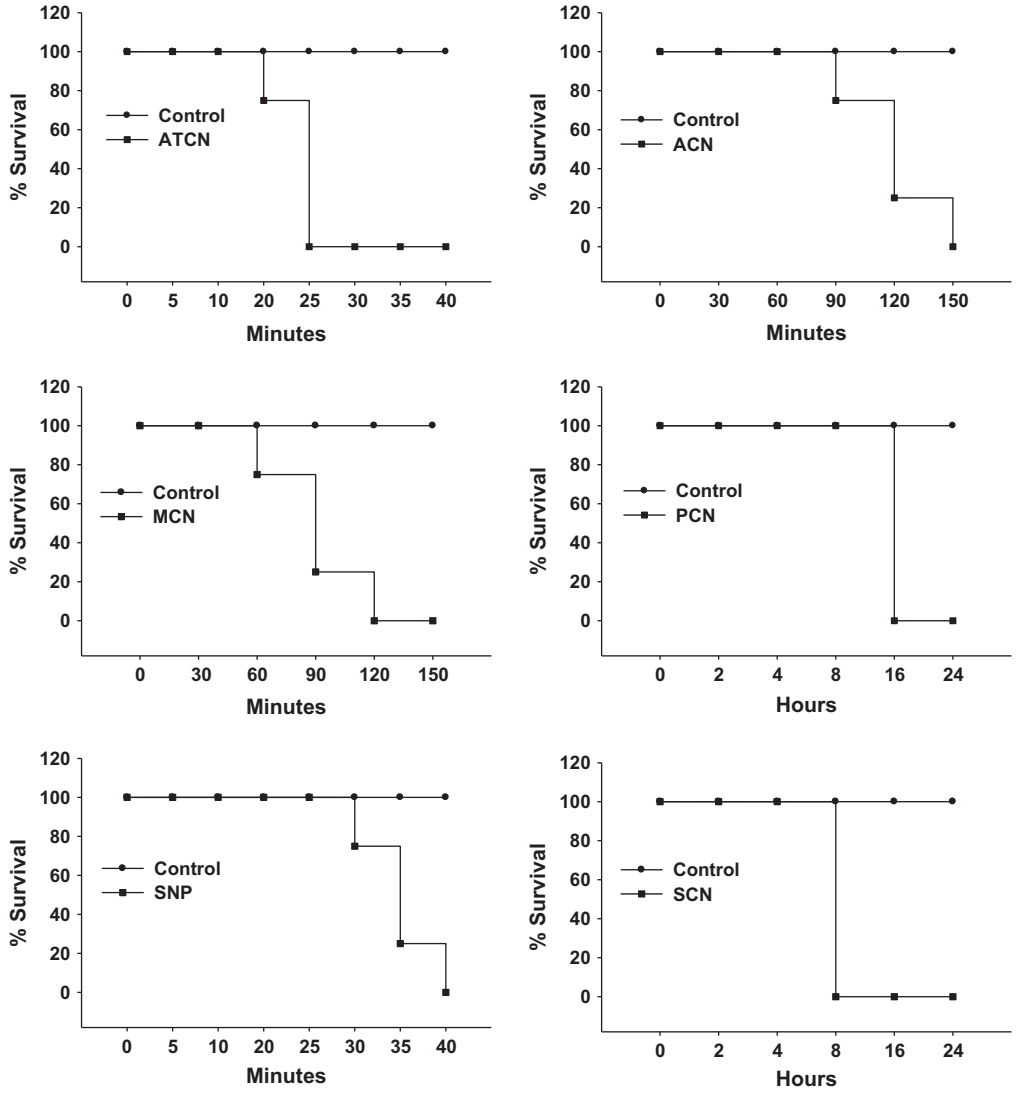

Fig. 1. Rats received oral treatment of 4.0 LD_{50} of acetonitrile (ATCN), acrylonitrile (ACN), malononitrile (MCN), propionitrile (PCN), sodium nitroprusside (SNP), and succinonitrile (SCN). Percent survival rate was determined at various time intervals. Survival Kaplan–Meier method was used for data analysis ($n = 4$).