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CYANIDE INDUCED CHANGES IN DYNAMIC PULMONARY MECHANICS IN RATS

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Abstract: Effect of subcutaneously (s.c.) administered potassium cyanide (0.5 and 1.0 LD_{50}) and inhalation of hydrogen cyanide (55 ppm or 60.6 mg/m^3) for 30 minutes was studied on various physiological parameters related to dynamic pulmonary mechanics in anaesthetized rats. Total pulmonary phospholipid with its fractions were also estimated. Both s.c. (1.0 LD_{50}) and inhalation exposures increased air flow, transthoracic pressure and tidal volume accompanied by significant decrease in pulmonary phospholipids. Inhalation of hydrogen cyanide also exhibited direct effect on the pulmonary cells as evidenced by decreased compliance. The study suggests that inhalation of cyanide is more injurious compared to parenteral route.

Key words: cyanide toxicity pulmonary mechanics

INTRODUCTIONS

Cyanide is a notorious homicidal, genocidal and chemical warfare agent, inhalation of which (as hydrogen cyanide, HCN) is well established as a cause of death in fire victims (1). In such cases the lethality is preceded by respiratory cessation, which is ascribed to stimulation of chemoreceptors by acid metabolites (2). These pathophysiological changes are secondary to the predominant biochemical lesion i.e. inhibition of cytochrome oxidase of chemoreceptors by cyanide. These changes may further complicate the condition. In such cases respiratory stimulation as evidenced by tachypnoea and hyperpnoea observed in early phase of cyanide poisoning (2) may aggravate the toxic manifestations. This study was aimed to identify various physiological responses of dynamic pulmonary mechanics in rats, to assess the local effects of inhaled HCN on such parameters and compare it with that produced by cyanide administered parenterally. In addition, an attempt is made to correlate the changes with the level of pulmonary surfactants, which prevent complete collapse of the lung during expiration by decreasing the surface tension phenomenon at the air-liquid interface in the alveoli. Furthermore, the technique involved for generation and exposure of HCN to rats, for monitoring on-line changes in dynamic pulmonary mechanics is elucidated.

METHODS

Male Wistar rats (140 ± 10 g) bred in the animal facility of our establishment maintained on Lipton's pellet diet and water *ad libitum* were fasted overnight. Each treatment group comprised of six rats. Animals were injected normal saline subcutaneously (s.c.) or potassium cyanide (KCN) in a dose of 0.5 or 1.0 LD_{50}. The acute 24 hour LD_{50} of KCN was determined previously (3). Exposure of animals to HCN was performed at a mean concentration of 55 ppm (60.6 mg/m^3) for 30 minutes duration. This dose was well tolerated by the animals for the said duration.

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Generation of HCN : HCN was generated in a 21 litre capacity all glass static exposure assembly as described elsewhere (4). Minor modifications were incorporated to refine the system to facilitate on line dynamic exposure in tracheostomized rats. Briefly, the HCN generated, by reacting predetermined quantity of KCN with sulfuric acid was circulated through the exposure chamber and plethysmograph by employing a rodent ventilator (UGO BASILE, Model 7025) at a rate of 1 litre per minute. Silicone tubes were used for different connections. The chamber cyanide concentration was determined at various time intervals by drawing 50 ml of chamber air through high efficiency impinger containing 0.1 N NaOH. The cyanide thus trapped was estimated following the method of Epstein (5). The concentration decay of HCN in the chamber was found to be 0.18 ppm (0.19 mg/m^3) per minute at a concentration of 55 ppm (60.6 mg/m^3) for 30 minutes duration.

Physiological parameters : The animals were prepared for recording various physiological parameters related to dynamic pulmonary mechanics on Buxco Dynamic Lung Mechanics Analyzer (Model 6, Buxco Electronic Inc., Germany) employing programme 1, as detailed elsewhere (6). Control recordings were taken after stabilization (30 min) of the preparation followed by exposure to cyanide and observation at every 10 minute interval for 30 minutes.

Biochemical parameter : After 30 min post exposure the animals were removed from the plethysmograph and the lungs were excised quickly. Adhering extraneous materials were removed and washed with cold normal saline. Thereafter, the lungs were weighed and homogenized in anhydrous sodium sulfate. Lipids were extracted and fractionated for phosphatidylcholine (PC), phosphatidylethanolamine (PE) and sphingomyelin (Sphingo) by thin layer chromatography (7). Total phospholipid (TPL) and the fractions were estimated spectrophotometrically (8).

Statistics : The statistical analysis was done by Student's 't' test. The level of significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

The purpose of the current study was to evaluate the effect of cyanide on various physiological indices associated with dynamic pulmonary mechanics in rats. Identification of such effects would facilitate designing of treatment regimens that may relieve such secondary changes which otherwise could exacerbate the toxicity.

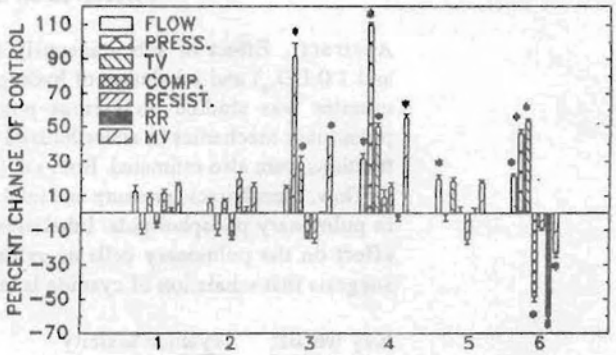

Fig. 1: Effect of cyanide on dynamic pulmonary mechanics in anaesthetized rats. Values are mean ± SE, n = 6, *P < 0.05. Control values : Air flow (Flow) 3.26 ± 0.19 ml/sec; Transthoracic pressure (Press) 5. 36± 0.68 cm H_2O; Tidal volume (TV) 0.76 ± 0.07 ml; Compliance (Comp) 0.49 ± 0.05 ml/cm H_2O; Resistance (Resist) 0.709 ± 0.109 cm H_2O/ml/sec; Respiratory rate (RR) 115.43±10.89; respiration/min; Minute volume (MV) 78.88±5.86 ml/min.

1. KCN 0.5 LD_{50} (10 min) 2. KCN 0.5 LD_{50} (30 min)

2. KCN 1.0 LD_{50} (10 min) 4. KCN 1.0 LD_{50} (30 min)

5. HCN 55 ppm (10 min) 6. HCN 55 ppm (30 min)

Fig. 1 shows the effect of cyanide on various parameters of dynamic pulmonary mechanics in rats, observed at 10, 20 and 30 minute intervals (data of 20 min interval not depicted in Fig 1). Potassium cyanide (s.c.) at 0.5 LD_{50} dose did not alter any of the parameters, while 1.0 LD_{50} dose produced significant increase in transthoracic pressure, minute volume (MV), the amount of air flowing through the respiratory passage per unit time (flow) and the tidal volume (TV). Inhalation of HCN (55 ppm) significantly increased the pressure, flow and TV, more conspicuously at 20 and 30 minute intervals, with a progressive decrease in compliance, respiratory rate (RR) and MV. No

change in air way resistance was observed in any of the treatments. Our results indicate that the changes observed in dynamic pulmonary mechanics are regardless of the route of exposure of cyanide, with the exception of MV and compliance. The magnitude of change was however, pronounced in inhalation group. The MV increased in 1.0 LD_{50} group and decreased in inhalation group. This result is opposed to the observation on primates, where inhalation of HCN (102-156 ppm) resulted in hyperventilation with significant increase in respiratory MV (9). The decrease in RR however, correlates with the studies on mouse where 50 percent decrease in RR was observed at inhalation of 63 ppm of HCN for 30 minutes (10). These changes are perhaps dose dependent as high concentration of cyanide in the atmosphere, with rapid absorption through the respiratory tract causes immediate swamping of the endogenous detoxification mechanism (2). This also explains the lack of such episode in cyanide given parenterally. The increase in transthoracic pressure could be due to central involvement as the increased TV suggests that the system tried to compensate the decrease in RR by elevating the pressure to increase the depth of respiration (hyperpnoea) (6). Decrease in compliance could be possibly due to direct action of HCN on the pulmonary cells as cyanide is known to be cytotoxic thereby, impairing the recoiling capacity of lungs.

Fig. 2 illustrates the effect of cyanide on lung phospholipids and its fractions when determined after 30 minutes of exposure. No change was observed in TPL or any of the fractions viz. PC, PE or Sphingo at 0.5 LD_{50} dose of KCN. However, in both 1.0 LD_{50} and inhalation group, decrease in TPL, PC and PE was observed which was pronounced in 1.0 LD_{50} group. Deficiency of surfactant can be lethal causing atelactesis, where the recoiling capacity of the lung is impaired resulting in total collapse. In the present study, significant decrease in pulmonary phospholipids (i.e., surfactant) following inhalation of HCN was anticipated to cause rapid collapse of the lung with increased

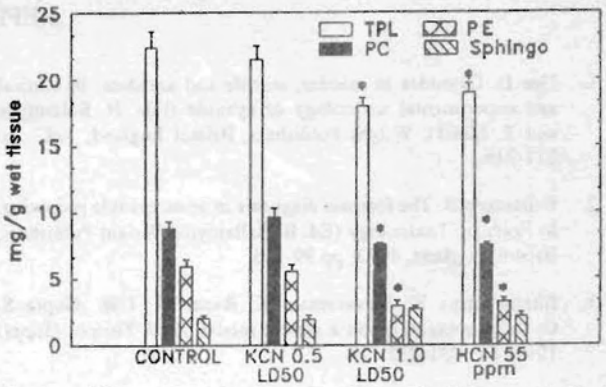

Fig. 2 : Effect of cyanide lung phospholipid level in anaesthetized rats. Values are mean ± SE, n = 6, *P < 0.05. TPL=Total phospholipids; PC = Phosphatidylcholine; PE = Phophatidylethanolamine and Sphingo = Sphingomyelin.

compliance. However, a paradoxical observation on compliance noticed, can be attributed to direct local effect of HCN on the pulmonary cells, thereby, impairing the recoiling property. No change was noticed in the compliance following subcutaneous route (1 LD_{50}), which further supports our contention. Chlorpromazine (a major antipsychotic drug) was found to be beneficial in preventing the cyanide induced impaired phospholipid synthesis in cultured hepatocytes and also in antagonising cyanide toxicity (11). Besides, in experimental animals, it is shown to stimulate phospholipid synthesis, thereby, indicating a possible inhibition of phospholipids by cyanide. Decreased level of pulmonary phospholipid in our present study correlates well with the above findings. In brief, the study suggests that the inhalation of cyanide is more injurious compared to parenteral route.

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