BIOMEDICAL AND ENVIRONMENTAL SCIENCES **20**, 56-63 (2007)

# Effect of Pre-treatment of α-Ketoglutarate on Cyanide-induced Toxicity and Alterations in Various Physiological Variables in Rodents

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**Objective** To investigate the effects of pre-treatment of α-ketoglutarate (α-KG) on cyanide-induced lethality and changes in various physiological parameters in rodents. **Methods** The $LD_{50}$ of potassium cyanide (KCN) given orally (po), intraperitoneally (ip), subcutaneously (sc) or intravenously (iv) was determined in male mice, in the presence or absence α-KG given po, ip or iv. α-KG was administered 10, 20 or 40 min prior to KCN at 0.50, 1.0 or 2.0 g/kg by po or ip route, and at 0.10, 0.20 or 0.40 g/kg by iv route. Protection index (PI) was calculated as the ratio of $LD_{50}$ of KCN in the presence of α-KG (protected animals) and $LD_{50}$ of KCN in the absence of α-KG (unprotected animals). In a separate experiment, several physiological variables viz. mean arterial pressure (MAP), heart rate (HR), respiratory rate (RR), neuromuscular transmission (NMT) and rectal temperature (RT) were measured in anesthetized female rats pre-treated (-10 min) with po (2.0 g/kg) or iv (0.125 g/kg) α-KG and then administered sub-lethal (0.75 $LD_{50}$) or lethal (2.0, 4.0 or 8.0 $LD_{50}$) doses of KCN (po). **Results** PI of 4.52, 6.40 and 7.60 at -10 min, 3.20, 5.40 and 6.40 at -20 min, and 1.40, 3.20 and 5.40 at -40 min of po administration with α-KG was observed for 0.50, 1.0 and 2.0 g/kg doses, respectively, against KCN given by po route. When KCN was given ip, a PI of 3.38, 4.79 and 5.70 was observed for 0.50, 1.0 and 2.0 g/kg α-KG given ip (-10 min), respectively. A lower PI of 3.37, 2.83 and 2.38 was observed when KCN given sc was challenged by 2.0 g/kg α-KG given ip at -10, -20 or -40 min, respectively. Similarly, a PI of 3.37, 2.83 and 2.0 was noted when KCN given sc was antagonized by 2.0 g/kg α-KG given po at -10, -20 or -40 min, respectively. No appreciable protection was observed when lower doses of α-KG (ip or po) challenged KCN given by sc route. Pre-treatment of iv or po administration of α-KG did not afford any protection against KCN given po or iv route. Oral treatment of 0.75 $LD_{50}$ KCN caused significant decrease in MAP and HR after 15 min, RR after 30 min and NMT after 60 min. There was no effect on RT. No reduction in MAP, HR, RR and RT was observed when rats received 2.0 or 4.0 $LD_{50}$ KCN after pre-treatment of α-KG (po; 2.0 g/kg). However, no protection was observed on NMT. Protective efficacy of α-KG was not observed on MAP, HR, RR, and NMT decreased by 8.0 $LD_{50}$ KCN. Decrease in MAP and NMT caused by 2.0 $LD_{50}$ KCN (po) was resolved by iv administration of α-KG. **Conclusions** Cyanide antagonism by α-KG is best exhibited when both α-KG and KCN are given by po route. The protective effect of α-KG on cyanide-induced changes in several physiological parameters also indicates a promising role of α-KG as an alternative cyanide antidote.

**Key words**: Cyanide; Toxicity; Physiological variables; Protection; α-Ketoglutarate

## INTRODUCTION

The highly toxic nature of cyanide is known for many years[1]. It is extensively used in industrial and mining operations throughout the world. Cyanide is also a potent suicidal, homicidal and chemical warfare agent[2]. Occupational exposure, ingestion of cyanide-containing foods and inhalation of hydrogen cyanide (HCN) present in fire smoke are known to cause severe toxicity[3-5]. Cyanide is a rapid poison that adversely affects the cellular respiration by inhibiting cytochrome *C* oxidase of mitochondrial respiratory chain[6]. There are numerous cyanide antidotes and no unanimity of opinion on which is the most effective regimen[7]. However, combination of sodium nitrite (SN) and sodium thiosulfate (STS) is the most widely used effective treatment for cyanide poisoning[8]. SN converts hemoglobin to methemoglobin, which reversibly binds to cyanide to produce cyanmethemoglobin, and STS enzymatically enhances cyanide metabolism to thiocyanate[9]. Many therapeutic problems are associated with the use of these antidotes[10-11]. This has prompted extensive research in the last two decades to develop more

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0895-3988/2007
CN 11-2816/Q

effective and safer cyanide antidotes[12-15]. One striking compound is α-ketoglutarate (α-KG) which exhibits exemplary antagonism of cyanide poisoning in experimental animals[16-19]. Cyanide, a reactive nucleophile, is known to interact with the carbonyl moiety of α-KG to form cyanohydrin[20]. Our recent studies revealed that oral (po) treatment of α-KG alone or in combination with SN and/or STS could confer enormous protection against po cyanide poisoning in rodents[21-23].

Although cyanide is primarily a neurotoxin, its vascular, cardiovascular and cardiorespiratory effects are well documented[24-27]. Cyanide antagonism by nitrites is contraindicated in various instances of cyanide poisoning because of their pronounced cardiovascular effects[10-11]. Therefore, a pejorative cardiovascular embarrassment could be anticipated during treatment of cyanide poisoning by such agents. In view of this we have earlier shown that po treatment of α-KG alone at a dose (2.0 g/kg) offering maximum cyanide antagonism does not cause any biochemical, hematological, histological or physiological alterations of clinical significance in rats[28]. Thereafter, 2.0 g/kg α-KG is considered a safe and effective dose for cyanide antagonism[23]. However, efficacy of α-KG through other routes remains to be exploited. The objective of the present study was (i) to evaluate the time, dose and route specific effects of pre-treatment of α-KG against acute cyanide poisoning in mice through different routes and (ii) to observe the response of po or intravenous (iv) pre-treatment of α-KG to cyanide (po) induced changes in various physiological parameters in anesthetized rats. The results provide a more comprehensive account of cyanide antagonism by α-KG in experimental animals.

## MATERIALS AND METHODS

### *Chemicals*

Potassium cyanide (KCN) and α-ketoglutaric acid disodium salt (α-KG) were purchased from Ferrack, Germany and Sigma-Aldrich, St. Louis, USA, respectively. Other chemicals of analytical grade were from Merck or BDH (India). All the solutions were prepared in 0.9% saline.

### *Animals*

Male Swiss albino mice (25-30 g) and female Wistar rats (200-250 g) bred in the animal facility of Defence Research and Development Establishment (DRDE), Gwalior were maintained on rice husk in polypropylene cages. Animals had free access to water and rodent pellet but were fasted over night prior to experiment. The study was approved by DRDE's Ethical Committee on Animal Experimentations.

### *In vivo Protection Studies*

The $LD_{50}$ of KCN was determined in mice, with or without α-KG. Three to four geometrically constant doses were used, with four animals per dose. Though the mortality of animals was observed for 14 d, death occurred invariably within the first 24 h. $LD_{50}$ and fiducial limits were estimated by the method of Gad and Weil[29]. If the fiducial limits could not be estimated, due to 0 and 100% deaths in the next lower and higher dose, the doses producing 0 and 100% deaths were used as the fiducial limits at 95% confidence intervals. Protection index (PI) was calculated as the ratio of $LD_{50}$ of KCN in the presence of α-KG (protected animals) and $LD_{50}$ of KCN in the absence of α-KG (unprotected animals). Following protection studies were carried.

(i) Routes of KCN and α-KG administration: $LD_{50}$ of KCN in the presence or absence of α-KG was determined in animals who were administered various doses of KCN by oral (po), intraperitoneal (ip), subcutaneous (sc) or intravenous (iv) routes and challenged by α-KG by po, ip or iv routes.

(ii) Dose and time of α-KG administration: α-KG was given 10, 20 or 40 min before KCN administration at a dose of 0.50, 1.0 or 2.0 g/kg. Animals injected α-KG by iv route received 0.10, 0.20 or 0.40 g/kg α-KG.

### *Studies on Physiological Variables*

Rats were divided into five groups of four animals each. Various treatments were as follows: (1) 0.9% saline control (po), (2) KCN 0.75 $LD_{50}$ (po), (3) KCN 2.0 $LD_{50}$ (po) + α-KG 2.0 g/kg (-10 min; po); (4) KCN 4.0 $LD_{50}$ (po) + α-KG 2.0 g/kg (-10 min; po); and (5) KCN 8.0 $LD_{50}$ (po) + α-KG 2.0 g/kg (-10 min; po). In another set of experiments, rats were divided into two groups of four animals each as follows: (1) α-KG 0.125 g/kg (iv) and (2) KCN 0.75 $LD_{50}$ (oral) + α-KG 0.125 g/kg (-10 min; iv). The data of control and KCN 0.75 $LD_{50}$ (po) were common for both experiments. All the animals were anesthetized with urethane (1.6 g/ kg; ip) and various physiological parameters were recorded on Grass Polygraph (Model 7-16 P-35) at different time intervals as discussed elsewhere[28]. Briefly, the trachea was cannulated and connected to a pneumotachometer (Fleisch tube) to record the respiratory rate (RR) through a differential pressure transducer (Hugo Sachs Electronics, Germany). The carotid artery was cannulated to record blood

pressure with a low-level DC preamplifier (Model 7 P1) attached to a pressure transducer (P 23 1D, Gould, USA). Mean arterial pressure (MAP) was calculated from the recorded blood pressure. Pulse signals were also fed into a tachograph preamplifier (Type 7 P4) to record the heart rate (HR). Neuromuscular transmission (NMT) studies were carried out to record the twitch responses. The gastracnemius muscle was opened and the sciatic nerve was stimulated with a supramaximal voltage (1-10 v) of 0.2 msec duration at a frequency of 0.2 Hz using a Grass stimulator model S 88. The twitch response of the muscle was recorded using a Force Transducer (model FT0-3). Rectal temperature (RT) was measured using a rectal probe. Animals were allowed to stabilise prior to various treatments.

## RESULTS

### *Time, Dose and Route-Dependent Effects of α-KG on Cyanide-Induced Lethality in Mice*

Table 1 shows that regardless of route of KCN or α-KG administration, or pre-treatment time of α-KG, a conspicuous dose-dependent protective effect of α-KG was observed when given by po or ip route. When KCN given po was challenged by 10 min pre-treatment of α-KG administered po, a PI of 4.52, 6.40 and 7.60 was obtained for 0.50, 1.0 and 2.0 g/kg dose of α-KG, respectively. For 20 and 40 min pre-treatment time, the PI for the corresponding doses was 3.20, 5.40 and 6.40, and 1.40, 3.20 and 5.40, respectively. Similarly, when KCN given ip was

TABLE 1

Time, Dose, and Route-Dependent Effects of α-Ketoglutarate (α-KG) Against Cyanide-Induced Lethality in Male Mice

| Route α-KG | KCN | Time (min) α-KG | Dose of a-KG(g/kg) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 0.50/*0.10 g/kg | | 1.0/*0.20 g/kg | | 2.0/*0.40 g/kg | |
| | | | $LD_{50}$/0.10 g/kg (mg/kg) | Protection Index | $LD_{50}$/0.10 g/kg (mg/kg) | Protection Index | $LD_{50}$/0.10 g/kg (mg/kg) | Protection Index |
| po | po | -10 | 67.3 (44.0-102.8) | 4.52 | 80.0 (49.0-130.6) | 6.4 | 95.2 (62.3-145.4) | 7.6 |
| po | po | -20 | 40.0 (24.5-65.3) | 3.2 | 67.3 (44.1-102.8) | 5.4 | 80.0 (49.0-130.6) | 6.4 |
| po | po | -40 | 16.8 (11.0-25.7) | 1.4 | 40.0 (24.5-65.3) | 3.2 | 67.3 (44.1-102.8) | 5.4 |
| ip | ip | -10 | 20.0 (12.3-32.7) | 3.38 | 40.0 (24.5-65.3) | 4.79 | 33.6 (22.0-51.4) | 5.70 |
| ip | ip | -20 | 11.9 (7.8-18.2) | 2.02 | 16.8 (11.0-25.7) | 2.85 | 28.3 (20.0-40.0) | 4.79 |
| ip | ip | -40 | 11.9 (7.8-18.2) | 2.02 | 14.1 (10.0-20.0) | 2.4 | 16.8 (11.0-25.7) | 2.85 |
| po | ip | -10 | 16.8 (9.2-30.6) | 2.85 | 23.8 (15.6-36.4) | 4.03 | 28.3 (20.0-40.0) | 4.79 |
| po | ip | -20 | 8.4 (5.5-12.9) | 1.43 | 10.0 (6.1-16.3) | 1.69 | 11.9 (7.8-18.2) | 2.02 |
| po | ip | -40 | 8.4 (5.5-12.9) | 1.43 | 10.0 (6.1-16.3) | 1.7 | 10.0 (6.1-16.3) | 1.7 |
| ip | sc | -10 | 16.8 (9.2-30.6) | 2 | 20.0 (12.3-32.7) | 2.38 | 28.3 (20.0-40.0) | 3.37 |
| ip | sc | -20 | 16.8 (9.2-30.6) | 2 | 20.0 (12.3-32.7) | 2.38 | 23.8 (15.6-36.4) | 2.83 |
| ip | sc | -40 | 14.1 (10.0-20.0) | 1.68 | 16.8 (11.0-25.7) | 2 | 20.0 (12.3-32.7) | 2.38 |
| po | sc | -10 | 20.0 (12.3-32.7) | 2.38 | 23.8 (15.6-36.4) | 2.83 | 28.3 (20.0-40.0) | 3.37 |
| po | sc | -20 | 16.8 (11.0-25.7) | 2 | 20.0 (12.3-32.7) | 2.38 | 23.8 (15.6-36.4) | 2.83 |
| po | sc | -40 | 10.0 (6.1-16.3) | 1.19 | 11.9 (7.8-18.2) | 1.42 | 16.8 (11.0-25.7) | 2 |
| Iv* | po | -10 | 16.8 (11.0-25.7) | 1.36 | 14.1 (10-20) | 1.13 | 14.1 (10-20) | 1.13 |
| Iv* | po | -20 | ND | - | ND | - | ND | - |
| iv* | po | -40 | ND | - | ND | - | ND | - |
| Po | iv | -10 | 14.1 (10-20) | 1.13 | 14.1 (10-20) | 1.13 | 14.1 (10-20) | 1.13 |
| Po | iv | -20 | ND | - | ND | - | ND | - |
| po | iv | -40 | ND | - | ND | - | ND | - |

*Note.* α-KG was given intraperitoneally (ip), orally (po) or intravenously (iv) 10, 20, or 40 min prior to KCN administered po, ip, iv or subcutaneously (sc). Protection Index = $LD_{50}$ of KCN in protected animals/ $LD_{50}$ of KCN in unprotected animals. $LD_{50}$ of KCN in male mice by po, ip, iv and sc routes were 12.5 (7.7-20.6), 5.9 (4.0-8.8), 8.4 (5.7-12.5), 2.8 (2.0-4.0) mg/kg, respectively. *α-KG by po or ip route was given at 0.50, 1.0 or 2.0 g/kg dose and for iv route was given at 0.10, 0.20 or 0.40 g/kg. Values in parentheses are fiducial limits at 95% confidence intervals. ND=Not determined.

antagonized by α-KG administered through the same route, a PI of 3.38, 4.79 and 5.70 was observed for corresponding doses, respectively. Maximum protection was observed when both α-KG and KCN were given po, followed by both administered ip. Also, a PI of 3.37, 2.83 and 2.38 was observed when KCN given sc was challenged by 2.0 g/kg α-KG given ip 10, 20 or 40 min prior to KCN, respectively. Similarly, a PI of 3.37, 2.83 and 2.0 was noted when KCN given sc was antagonized by 2.0 g/kg α-KG given po 10, 20 or 40 min before KCN, respectively. No appreciable protection was observed when lower doses of α-KG (ip or po) challenged KCN given sc. Also, pre-treatment (10 min) of α-KG by iv route did not confer any protection at 0.10, 0.20 or 0.40 g/kg against KCN given po. Conversely α-KG given po did not offer any protection against KCN injected iv. Therefore, further studies with 20 min or 40 min pre-treatment of α-KG (po or iv) were not carried out against KCN given iv or po. The data clearly indicated that the protective efficacy of α-KG was the highest when given 10 min prior to KCN and the effects progressively declined with time when observed up to 40 min, and also 2.0 g/kg α-KG was most effective as compared to its lower doses.

*Response of α-KG to Cyanide-induced Changes in Various Physiological Parameters*

Figure 1 depicts the effect of 0.75 $LD_{50}$ KCN (po) on various physiological variables viz. MAP, HR, RR, NMT and RT in anesthetized rats at different time intervals. The figure also shows the response of various parameters altered by 2.0, 4.0 and 8.0 $LD_{50}$ KCN (po) to pre-treatment of α-KG (-10 min; po). Administration of 0.75 $LD_{50}$ KCN caused a significant decrease in MAP and HR after 15 min of treatment as compared to saline control which persisted for 240 min. The RR and NMT were reduced after 30 and 60 min, respectively which also persisted for a similar time period. The RT remained unchanged following KCN exposure. KCN 2.0 $LD_{50}$ in the presence of α-KG did not reduce the MAP, HR, RR or RT while no protection was observed on NMT which was significantly reduced after 180 min of exposure. Also, α-KG could not prevent 25% and 50% mortality of rats 15 min and 180 min after KCN exposure, respectively. Therefore, at these time points the values were mean of 3 and 2 animals, respectively. The effect of 4.0 $LD_{50}$ KCN in the presence of pre-treatment of α-KG was similar to those produced by 2.0 $LD_{50}$ KCN. The NMT was reduced after 15 min which progressively declined with time. In this treatment group the mortality of animals was similar to that observed in the previous group. Significant decrease in MAP, HR, RR and NMT by 8.0 $LD_{50}$ KCN could not be abolished by pre-treatment of α-KG. Moreover, this dose of KCN caused 75% mortality of animals after 15 min. Therefore, till termination of the experiment the values were recorded only on 1 animal. Figure 2 shows that iv injection of 0.125 g/kg α-KG *per se* did not cause any change in MAP, HR, RR, NMT or RT recorded at various time intervals. Although the initial MAP and

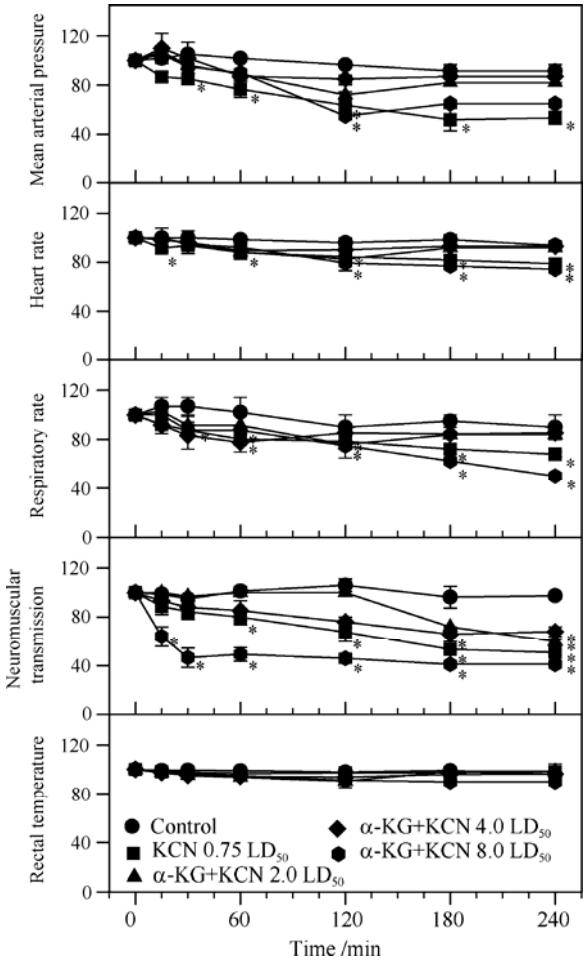


FIG. 1. Effect of oral (po) treatment of α-ketoglutarate (α-KG) on cyanide-induced changes in various physiological parameters in female rats at different time intervals. The control animals received saline (po). The unprotected animals received 0.75.$LD_{50}$ KCN (po) while the animals protected with α-KG (2.0 g/ kg; -10 min; po) were administered 2.0, 4.0 or 8.0 $LD_{50}$ KCN (po). Values are $\bar{x} \pm s$ of 4 animals in all the treatment groups at 0 min. In KCN 2.0 and 4.0 $LD_{50}$ groups, values are mean of 3 and 2 animals after 15 min and 180 min, respectively. In KCN 8.0 $LD_{50}$ group, the values represent only 1 animal after 15 min.

NMT remained decreased in the animals pre-treated with α-KG 2.0 g/kg (iv), significant protection was subsequently observed on all the variables altered by KCN 2.0 $LD_{50}$ (po).

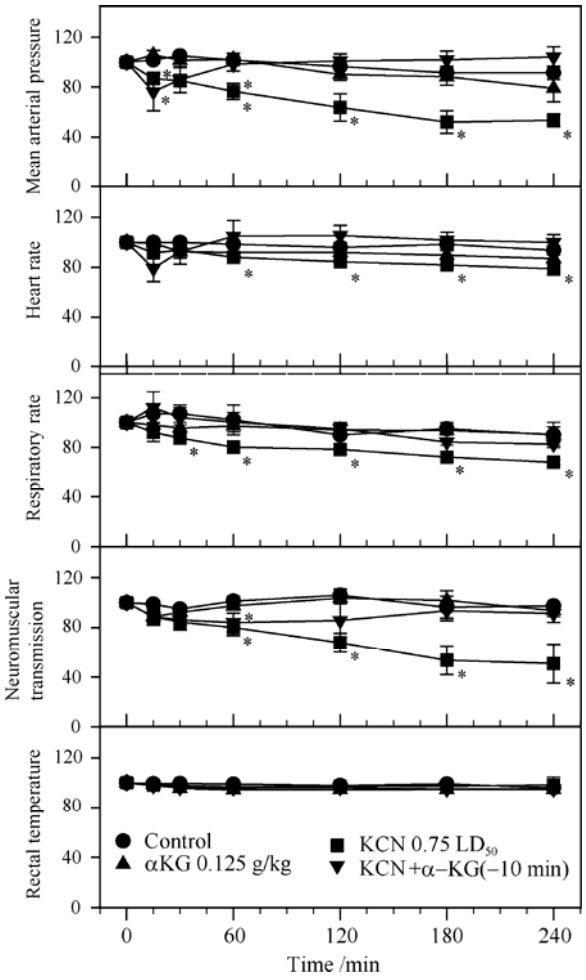


FIG. 2. Effect of intravenous (iv) treatment of α-ketoglutarate (α-KG) on cyanide-induced changes in various physiological parameters in female rats at different time intervals. The control animals received saline (iv) and the treated animals received α-KG (0.125 g/kg; -10 min; iv) in the presence or absence of 0.75.$LD_{50}$ KCN (po).

## DISCUSSION

Treatment of cyanide poisoning usually involves iv administration of SN and STS[7-9]. However, they can lead to several therapeutic complications[10-11]. A serious drawback with SN is that its administration may be immediately accompanied by serious cardiovascular embarrassment, particularly in children[10,30]. Treatment of SN is also discouraged in HCN poisoning following exposure to fire smoke[11], because oxygen saturation of the blood is already compromised due to caboxyhemoglobin formation following exposure to carbon monoxide present in the fire smoke, and methemoglobin induction by SN would further diminish the supply of oxygen to tissues[11,31]. Also, methemoglobin values of 30% or more may produce collapse or even fatalities[32]. To circumvent these problems we have proposed α-KG (po) and STS (ip) as a promising treatment regimen for acute and sub-acute cyanide (po) poisoning[21-22,33]. Also, we have recently shown that various treatments of α-KG (2.0 g/kg; po) can significantly antagonize the effects of a sub-lethal dose (0.75 $LD_{50}$) of KCN (po) on various physiological parameters in anesthetized rats[34]. However, the effect of α-KG by other routes and its response to lethal doses of KCN remain to be explored. In the present study, we investigated (i) the effect of dose, time or route of α-KG on acute KCN poisoning in mice through various routes, (ii) the effect of po pre-treatment (-10 min) of α-KG (2.0 g/kg) on several physiological variables altered by lethal doses of KCN (2.0, 4.0, and 8.0 $LD_{50}$; po) and (iii) the effect of iv pre-treatment of α-KG (0.125 g/kg) on different physiological indices altered by a sub-lethal dose of KCN (0.75 $LD_{50}$; po). The doses of α-KG (0.50, 1.0, or 2.0 g/kg) for the studies on PI were based on our previous studies[19,21-22], and the same by iv route was based on our recent unpublished work. In this study, the iv $LD_{50}$ of α-KG in male and female mice was found to be 884 (625-1250) and 526 (345-804) mg/kg, respectively. Therefore, a dose between 100 and 400 mg/kg was selected for the present study on male mice and female rats.

Our previous studies on PI of α-KG alone or with SN and/or STS against acute cyanide poisoning were mostly conducted in male mice[19,22]. At the same time we have shown the protective efficacy of α-KG in female rats as well[21]. The protection studies on various physiological parameters were, however, limited to female rats only[34]. Because of this reason, in the present study, the experiments on PI of α-KG through different routes, were carried out in male mice and conversely, the physiological studies were conducted in female rats as the previous study[34]. This enables us to appreciate the protective efficacy of α-KG in two different species of animals and opposite genders.

The present study revealed a distinct dose and time dependent protective effect of α-KG, regardless of the route of α-KG or KCN administration. The maximum protection was observed when both α-KG and KCN were given po. The most common route of cyanide intoxication is through inhalation or dermal

exposure. However, poisoning through po route is also not uncommon[6]. We have previously shown that po pre-treatment of α-KG confers more protection than α-KG given simultaneously against po KCN[22]. α-KG given po is safe since its $LD_{50}$ in rats is >5.0 g/kg, and a recommended dose of 2.0 g/kg α-KG has enough safety margin[28]. Also, reduction in the dose of α-KG can be considered depending on the severity of cyanide poisoning because a dose as low as 0.50 g/kg produces a PI of 4.5 when given 10 min prior to KCN. Furthermore, this protection can be significantly enhanced by adjunction of STS[21-22]. Also, α-KG offered protection when given 40 min before KCN, albeit the PI progressively declined with the passage of time. The data corroborate our previous findings[22]. Protective efficacy of ip administration of α-KG against KCN given by the same route was also noteworthy. This is in agreement with previous reports[16,19]. Here also, a dose and time-dependent effect of α-KG was observed. When KCN given sc was challenged by 2.0 g/kg α-KG given ip or po, a PI of 2.0 or more was recorded. Marginal protection of 20 min or 40 min pre-treatment of 0.50 or 1.0 g/kg α-KG (po or ip) against KCN given ip, sc or po cannot be over looked because PI <2.0 can also be greatly augmented by supplementation of STS[22]. Significantly enhanced protection by α-KG given po and ip against KCN given by the corresponding routes can be attributed to possible *in situ* interaction of α-KG with $CN^-$ in the stomach and peritoneal cavity, respectively[16,19]. When cyanide is given po, the gastric acid environment favors formation of unionized form of HCN which facilitates absorption[35]. Therefore, besides binding to $CN^-$, the alkaline pH of α-KG is likely to minimize its absorption in the stomach[23]. Additionally, other protective mechanism (s) of α-KG should also be considered[15,36-37]. Cyanide antagonism by α-KG by parenteral route is also documented[16-17,19,38]. It has been shown that distribution of cyanide to the brain stem and heart is significantly reduced by ip treatment of α-KG, indicating a possible interaction of $CN^-$ and α-KG in the vascular system[39]. This binding would minimize the free circulating $CN^-$ and prevent it from distribution to vital tissues. This is validated by the fact that ip injection of α-KG could significantly antagonize HCN poisoning through inhalation route as well[19,38]. However, treatment of α-KG by iv route did not show encouraging results against KCN given po and conversely α-KG given po did not offer any protection against KCN injected iv. As compared to po route, α-KG was found to be more toxic by iv route (our unpublished work).

We have earlier reported that various cardio-respiratory changes produced by sub-lethal dose of cyanide could be protected by pre-treatment or post-treatment of α-KG[34]. The present investigation aimed to characterize various physiological changes induced by sub-lethal and lethal doses of KCN in the presence of α-KG given po or iv. Cyanide-induced hypoxia and metabolic acidosis are usually accompanied by several physiological changes[24-25]. Respiratory stimulation may be observed in the early phase of poisoning. The tachypnea and hypernea observed have been ascribed to the inhibition of cytochrome oxidase in chemoreceptors, resulting in an accumulation of acid metabolites which stimulate the chemoreceptors[40]. Cyanide is also reported to produce cardiorespiratory and cardiovascular changes in experimental animals[26-27] and directly act on respiratory center causing respiratory depression[26,40]. Hypothermia and bradycardia are some of the common cardiovascular effects of cyanide in experimental animals[26-27]. Brain stem hypoxia caused by vertebral artery injection of cyanide in artificially ventilated cats depress phrenic and stimulate sympathetic nerve activity with a simultaneous increase in arterial blood pressure[26]. Severe bradycardia, decrease in arterial blood pressure and respiratory paralysis following intravenous injection of sodium cyanide in dogs have also been reported[41]. In the present study we observed a significant reduction in MAP, HR, RR and NMT following administration of 0.75 $LD_{50}$ KCN. Our aim was to further enhance the dose for KCN in the presence of α-KG and for this reason the data of protected animals could not be compared with the corresponding doses for unprotected animals. Animals receiving 2.0, 4.0, or 8.0 $LD_{50}$ KCN would have died instantly if not protected with α-KG. Therefore all the comparisons of 0.75 $LD_{50}$ KCN were made with 2.0, 4.0, or 8.0 $LD_{50}$ KCN protected with α-KG. We have earlier shown that rats receiving oral pre-treatment or simultaneous treatment of α-KG alone could tolerate up to 7.0 $LD_{50}$ KCN given po[21]. However, in the present study, animal mortality was observed at different time intervals in all the protected groups receiving 2.0, 4.0, or 8.0 $LD_{50}$ KCN. This was because the animals were anesthetized and also under surgical trauma. Nevertheless, all the surviving animals showing significant changes in several physiological parameters, particularly on cardio-respiratory variables responded to pre-treatment of α-KG. Although iv treatment of α-KG resolved the physiological changes caused by 0.75 $LD_{50}$ KCN, it cannot be considered in view of the fact that α-KG is very toxic by this route and also this is not a route of choice in field conditions.

In conclusion, the present study provides a comprehensive account on the protective efficacy of α-KG against acute cyanide poisoning in rodents. α-KG affords a dose, time and route-dependent protection against cyanide and is most effective when both α-KG and cyanide are given po. Response of α-KG to various physiological variables altered by cyanide is very encouraging and supplements the promising role of α-KG as an alternative treatment for cyanide poisoning. Our previous observation has shown that α-KG (po) is very safe and does not cause any cardiorespiratory changes *per se*, thus providing an additional advantage[28].

## ACKNOWLEDGEMENT

The authors thank Er. K. SEKHAR, Director and Dr. R. VIJAYARAGHAVAN, Head of Pharmacology and Toxicology Division, DRDE, Gwalior for their keen interest and support in this study.

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(*Received January* 20, 2006 *Accepted July* 7, 2006)