

Protective role of alpha-ketoglutarate against massive doses of cyanide in rats

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Abstract: Cyanide is a highly toxic cellular poison that requires immediate and aggressive treatments. Combination of sodium nitrite (SN) and sodium thiosulfate (STS) is the treatment of choice but oral treatment of alpha-ketoglutarate (A-KG) has also been shown to significantly antagonize cyanide poisoning in laboratory animals. This study reports the efficacy of various treatment regimens as: (i) repeated doses of A-KG after simultaneous treatment of A-KG and STS, (ii) repeated doses of A-KG after pre-treatment of SN, STS and A-KG, (iii) repeated doses of STS after pre-treatment of SN, STS and A-KG, and (iv) repeated doses of A-KG and STS after pre-treatment of SN, STS and A-KG on mortality of female rats exposed to massive doses of potassium cyanide. A maximum of 40-folds protection was observed when A-KG at 1.0 g kg^{-1} after 2 hr and 0.5 g kg^{-1} after 4 hr was repeated following the pre-treatment of SN (0.025 g kg^{-1} ; subcutaneous; -45 min), STS (1.0 g kg^{-1} ; intraperitoneal; -15 min) and A-KG (2.0 g kg^{-1} ; oral; -10 min). Similar protection was also conferred by repeating 0.5 g kg^{-1} each of A-KG and STS 2 hr after pre-treatment of SN, STS and A-KG. Also, 38-folds protection after simultaneous administration of 2.0 g kg^{-1} A-KG and 1.0 g kg^{-1} STS, followed by 2.0 g kg^{-1} A-KG after 2 hr was noteworthy. The results indicate that repeated treatment of A-KG alone after simultaneous treatment of A-KG and STS or repeated treatment of A-KG alone or with STS after pre-treatment of A-KG, SN and STS have immense potential in challenging extremely high doses of cyanide as compared to the antidotes given once. The study has implications in the development of A-KG as an alternate treatment for cyanide poisoning.

Key words: Cyanide, Toxicity, Protection, Alpha-ketoglutarate
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Introduction

The use of cyanide as a potential suicidal, homicidal and chemical warfare (CW) agent has long been recognized (Way, 1984; Borowitz *et al.*, 1992; Baskin and Brewer, 1997; Rotenberg, 2003). Wide industrial application (Peden *et al.*, 1986), dietary intake of cyanogenic food, cigarette smoking (Osuntokun, 1980; Adewusi and Akindahunsi, 1994) and administration of certain drugs (Kalyanaraman *et al.*, 1983; Vesey and Cole, 1985) are other probable sources of cyanide exposure. Hydrogen cyanide (HCN) and carbon monoxide (CO) together are largely responsible for severe toxicity in victims of fire smoke inhalation from residential or industrial fires (Barillo *et al.*, 1994; Alarie, 2002). Firefighters are an occupational group at significant risk of this source of exposure (Silverman *et al.*, 1988).

Cyanide inhibits cytochrome c oxidase, an end chain respiratory enzyme present in mitochondria, leading to hypoxia and death (Borowitz *et al.*, 2001). There are four antidotes used in various countries for the treatment of cyanide poisoning: (i) the cyanide antidote kit containing amyl nitrite, sodium nitrite (SN) and sodium thiosulfate (STS), (ii) hydroxocobalamin (Cyanokit), (iii) dicobalt-edetate (Kelocyanor), and (iv) 4-dimethylaminophenol (DMAP) (Baskin *et al.*, 1992; Marrs *et al.*, 1996). A serious drawback with nitrites is that its administration may be accompanied by serious cardiovascular embarrassment, particularly in children, for whom an adjusted dose is recommended (Berlin, 1970). Secondly, the methemoglobin generation by nitrites is very slow and methemoglobinemia usually impairs oxygen transport. Therefore, it

cannot be recommended for fire victims concomitantly exposed to HCN and CO (Baud, 2007). Since CO also impairs oxygen carrying capacity of blood, administration of nitrites would further exacerbate the hypoxic condition. SN is also not advocated for individuals with glucose-6-phosphate dehydrogenase deficient red cells because of the possibility of serious hemolytic reactions (Van Heijst and Meredith, 1990). The cobalt compounds and DMAP are also not free from side effects (Van Heijst *et al.*, 1987). This limits the use of these antidotes, particularly outside health care facility (Bowden and Krenzelo, 1997). The onset of action of STS is very slow, and is not recommended alone (Santiago, 2003). Several limitations of cyanide antidotes prompted research on modification of cyanide toxicodynamics by new mechanistic based antidotes (Isom and Borowitz, 1995). Alpha-ketoglutarate (A-KG) was one such compound which was vigorously pursued as cyanide antidote in experimental animals (Moore *et al.*, 1986; Dalvi *et al.*, 1990; Dulaney *et al.*, 1991; Bhattacharya and Vijayaraghavan, 1991). Further studies from this laboratory revealed that oral treatment of A-KG in combination with SN and STS increased the LD_{50} of cyanide by 29 and 26 folds in rats and mice, respectively (Bhattacharya and Vijayaraghavan, 2002; Bhattacharya *et al.*, 2002). One interesting observation during the protection studies was that, usually the unprotected animals would die within a few minutes after administration of cyanide and those protected with A-KG alone or with STS and administered extremely high doses of cyanide would either survive or perish overnight after protracted struggle. This was in contrast to the general observation in experimental cyanide poisoning where animals likely to succumb would not live beyond a

Table - 1: Effect of simultaneous treatment of alpha-ketoglutarate (A-KG) and sodium thiosulfate (STS) followed by repeated doses of A-KG on mortality of female rats exposed to high doses of cyanide

Repeated doses of A-KG (g kg ⁻¹) at different time intervals							Total dose of A-KG (g kg ⁻¹)	Dose of KCN challenged (mg kg ⁻¹)	Percent mortality		Protection index	
0 hr	1 hr	2 hr	3 hr	4 hr	6 hr	8 hr			Single dose	Repeated dose	Single dose	Repeated dose
0.5	-	0.5	-	0.5	0.5	0.5	2.5	100	50	0	7.1	11.3
0.5	-	0.5	-	0.5	0.5	-	2.0	100	50	0	7.1	11.3
0.5	-	0.5	-	0.5	-	-	1.5	100	50	0	7.1	11.3
0.5	-	0.5	-	-	-	-	1.0	100	50	0	7.1	11.3
1.0	1.0	1.0	1.0	-	-	-	4.0	200	75	25	14.2	19.0
1.0	1.0	1.0	-	-	-	-	3.0	200	75	25	14.2	19.0
1.0	1.0	-	-	-	-	-	2.0	200	75	0	14.2	22.6
1.0	-	1.0	-	1.0	-	-	3.0	200	75	0	14.2	22.6
1.0	-	1.0	-	0.5	-	-	2.5	200	75	0	14.2	22.6
1.0	-	1.0	-	-	-	-	2.0	200	75	0	14.2	22.6
1.0	-	0.5	-	-	-	-	1.5	200	75	0	14.2	22.6
2.0	2.0	2.0	-	-	-	-	6.0	200	50	100	14.2	11.3
2.0	2.0	-	-	-	-	-	4.0	200	50	100	14.2	11.3
2.0	-	2.0	-	2.0	-	-	6.0	200	50	25	14.2	19.0
2.0	-	2.0	-	-	-	-	4.0	200	50	25	14.2	19.0
2.0	1.0	1.0	-	-	-	-	4.0	200	50	50	14.2	14.2
2.0	0.5	0.5	0.5	-	-	-	3.5	200	50	50	14.2	14.2
2.0	0.5	0.5	-	-	-	-	3.0	200	50	25	14.2	19.0
2.0	0.5	0.5	-	-	-	-	3.0	200	50	0	14.2	22.6
2.0	-	0.5	-	-	-	-	2.5	200	50	25	14.2	19.0
2.0	-	2.0	-	2.0	-	-	6.0	300	100	75	21.3	26.9
2.0	-	2.0	-	-	-	-	4.0	300	100	25	21.3	38.0
2.0	-	1.0	-	1.0	-	-	4.0	300	100	75	21.3	26.9
2.0	-	1.0	-	0.5	-	-	3.5	300	100	50	21.3	32.0
2.0	-	1.0	-	-	-	-	3.0	300	100	75	21.3	26.9
2.0	-	0.50	-	-	-	-	2.5	300	100	75	21.3	26.9

In single dose, potassium cyanide (KCN; oral) was followed by simultaneous treatment of 2.0 g kg⁻¹ A-KG (oral) and 1.0 g kg⁻¹ STS (intraperitoneal). In repeated dose, KCN was followed by simultaneous treatment (0 hr) of 0.5-2.0 g kg⁻¹ A-KG and STS. Thereafter A-KG was repeated at indicated doses at different time intervals. Protection index is the ratio of LD₅₀ of KCN in female rats in the presence of STS+A-KG and LD₅₀ of KCN alone (14.1 mg kg⁻¹)

few minutes and those recovering within a few hours would not die over night. Now, it was of interest to see if the animals given such high doses of cyanide could be saved by repeated treatments of A-KG alone or with STS and/ or SN. Therefore, anticipating additional protection by repeated administration of the antidotes, the present study was undertaken in female rats with the following objectives: (i) if by repeating the dose of A-KG, more cyanide could be challenged; (ii) reducing the dose of A-KG and repeating it at regular intervals would reduce the load of A-KG; (iii) adjunction of single or repeated treatment of STS would enhance protection, and (iv) adjunction of single dose of SN and STS would further enhance the protection. In cyanide poisoning the therapeutic window is very narrow and if repeated administration of the antidotes could provide additional protection or even notably extend the survival time, it would provide ample opportunity for other therapeutic interventions. This study attempts to resolve the problems associated in the management of cyanide poisoning.

Materials and Methods

Chemicals: Potassium cyanide (KCN), sodium thiosulfate (STS) and sodium nitrite (SN) were purchased from Merck (Germany),

and alpha-ketoglutaric acid disodium salt (A-KG) was from Sigma-Aldrich (St. Louis, MO, USA). All the solutions were prepared fresh in 0.9% saline and administered in a volume < 10 ml kg⁻¹ body weight.

Animals: Female Wistar rats (130-150 g) were obtained from the animal facility of Defence Research and Development Establishment (DRDE), Gwalior. They were maintained on rice husk in polypropylene cages with constant access to water and rodent pellet diet (Ashirwad Brand, Chandigarh, India) *ad libitum*. The animals were acclimatized for 7 days and fasted overnight prior to experiment. The Ethical Committee on Animal Experiments of DRDE approved the protocol for the experiments.

Treatments: The animals were administered KCN, SN, STS and A-KG by oral (po), subcutaneous (sc), intraperitoneal (ip) and po routes, respectively. The dose and treatment time of various agents have been defined below:

Effect of simultaneous treatment of A-KG and STS followed by repeated doses of A-KG: In single dose, KCN (100, 200 or 300 mg kg⁻¹) was followed by simultaneous treatment of 2.0 g kg⁻¹

Table - 2: Effect of pre-treatment of sodium nitrite (SN), sodium thiosulfate (STS) and alpha-ketoglutarate (A-KG) followed by repeated doses of A-KG on mortality of female rats exposed to high doses of cyanide

Repeated doses of A-KG (g kg ⁻¹) at different time intervals				Total dose of A-KG (g kg ⁻¹)	Dose of KCN challenged (mg kg ⁻¹)	Percent mortality		Protection index	
-10 min	1 hr	2 hr	4 hr			Single dose	Repeated dose	Single dose	Repeated dose
2.0	1.0	1.0	-	4.0	400	50	25	28.4	33.7
2.0	1.0	0.5	-	3.5	400	50	50	28.4	28.4
2.0	0.5	0.5	-	3.0	400	50	25	28.4	33.7
2.0	-	2.0	2.0	6.0	400	50	25	28.4	33.7
2.0	-	2.0	-	4.0	400	50	0	28.4	40.1
2.0	-	2.0	1.0	5.0	400	50	25	28.4	33.7
2.0	-	1.0	1.0	4.0	400	50	25	28.4	33.7
2.0	-	1.0	0.5	3.5	400	50	0	28.4	40.1
2.0	-	1.0	-	3.0	400	50	0	28.4	40.1
2.0	-	0.5	-	2.5	400	50	25	28.4	33.7
2.0	1.0	1.0	-	4.0	800	100	100	28.4	28.4
2.0	-	1.0	1.0	4.0	800	100	50	28.4	40.1
2.0	-	1.0	0.5	3.5	800	100	50	28.4	40.1
2.0	-	2.0	2.0	6.0	800	100	100	28.4	28.4
2.0	-	2.0	1.0	5.0	800	100	100	28.4	28.4
2.0	-	2.0	0.5	4.5	800	100	100	28.4	28.4
2.0	-	0.5	0.5	3.0	800	100	100	28.4	28.4

In single dose, animals were treated with SN (0.025 g kg⁻¹; subcutaneous; -45 min), STS (1.0 g kg⁻¹; intraperitoneal; -15 min) and A-KG (2.0 g kg⁻¹; oral; -10 min) prior to potassium cyanide (KCN; oral) and in repeated dose, animals received the pre-treatment and then administered various doses of A-KG as indicated above at different time intervals. Protection index is the ratio of LD₅₀ of KCN in female rats in the presence of SN+STS+A-KG and LD₅₀ of KCN alone (14.1 mg kg⁻¹)

Table - 3: Effect of pre-treatment of sodium nitrite (SN), sodium thiosulfate (STS) and alpha-ketoglutarate (A-KG) followed by repeated doses of STS on mortality of female rats exposed to high doses of cyanide

Repeated doses of STS (g kg ⁻¹) at different time intervals					Total dose of STS (g kg ⁻¹)	Dose of KCN challenged (mg kg ⁻¹)	Percent mortality		Protection index	
-15 min	1 hr	2 hr	3 hr	4 hr			Single dose	Repeated dose	Single dose	Repeated dose
1.0	1.0	1.0	-	-	3.0	800	100	100	28.4	28.4
1.0	1.0	0.5	-	-	2.5	800	100	100	28.4	28.4
1.0	1.0	0.5	0.5	-	3.0	800	100	100	28.4	28.4
1.0	0.5	-	-	-	1.5	800	100	100	28.4	28.4
1.0	-	1.0	1.0	-	3.0	800	100	100	28.4	28.4
1.0	-	1.0	-	0.5	2.5	800	100	100	28.4	28.4
1.0	-	0.5	-	0.5	2.0	800	100	100	28.4	28.4
1.0	-	0.5	-	-	1.5	800	100	100	28.4	28.4

In single dose, animals were treated with SN (0.025 g kg⁻¹; subcutaneous; -45 min), STS (1.0 g kg⁻¹; intraperitoneal; -15 min) and A-KG (2.0 g kg⁻¹; oral; -10 min) prior to potassium cyanide (KCN; oral) and in repeated dose, animals received the pre-treatment and then administered various doses of STS as indicated above at different time intervals. Protection index is the ratio of LD₅₀ of KCN in female rats in the presence of SN+STS+A-KG and LD₅₀ of KCN alone (14.1 mg kg⁻¹)

A-KG and 1.0 g kg⁻¹ STS once. In repeated dose, KCN was followed by simultaneous treatment (0 hr) of 0.5, 1.0 or 2.0 g kg⁻¹ A-KG and STS once. Thereafter A-KG was repeated at 0.5, 1.0 or 2.0 g kg⁻¹ at 1, 2, 3, 4, 6 or 8 hr after KCN.

Effect of pre-treatment of SN, STS and A-KG followed by repeated doses of A-KG: In single dose, animals were treated with SN (0.025 g kg⁻¹; -45 min), STS (1.0 g kg⁻¹; -15 min) and A-KG (2.0 g kg⁻¹; -10 min) once prior to KCN (400 or 800 mg kg⁻¹). In repeated

dose, animals received the pre-treatment and then administered A-KG (0.50, 1.0 or 2.0 g kg⁻¹) at 1, 2 or 4 hr after KCN.

Effect of pre-treatment of SN, STS and A-KG followed by repeated doses of STS: In single dose, animals were treated with SN (0.025 g kg⁻¹; -45 min), STS (1.0 g kg⁻¹; -15 min) and A-KG (2.0 g kg⁻¹; -10 min) prior to KCN (800 mg kg⁻¹). In repeated dose, animals received the pre-treatment and then administered STS (0.50 or 1.0 g kg⁻¹) at 1, 2, 3 or 4 hr after KCN.



Table - 4: Effect of pre-treatment of sodium nitrite (SN), sodium thiosulfate (STS) and alpha-ketoglutarate (A-KG) followed by repeated doses of A-KG and STS on mortality of female rats exposed to high doses of cyanide

Repeated doses of A-KG/STS (g kg ⁻¹) at different time intervals						Total dose of A-KG/STS (g kg ⁻¹)	Dose of KCN challenged (mg kg ⁻¹)	Percent mortality		Protection index	
0 hr	1 hr	2 hr	3 hr	4hr	6 hr			Single dose	Repeated dose	Single dose	Repeated dose
2/1	1/0.5	1/0.5	1/0.5	-	-	5/2.5	800	100	100	28.4	28.4
2/1	1/0.5	1/0.5	-	-	-	4/2	800	100	100	28.4	28.4
2/1	1/0.5	0.5/0.25	-	-	-	3.5/1.75	800	100	100	28.4	28.4
2/1	0.5/0.5	0.5/0.5	0.5/0.5	-	-	3.5/2.5	800	100	100	28.4	28.4
2/1	1/0.25	0.5/0.25	-	-	-	3.5/1.5	800	100	100	28.4	28.4
2/1	-	1/0.5	-	1/0.5	1/0.5	5/2.5	800	100	100	28.4	28.4
2/1	-	1/0.5	-	1/0.5	-	4/2	800	100	100	28.4	28.4
2/1	-	0.5/0.5	-	0.5/0.5	0.5/0.5	3.5/2.5	800	100	100	28.4	28.4
2/1	-	1/0.5	-	0.5/0.25	-	3.5/1.75	800	100	100	28.4	28.4
2/1	-	1/0.25	-	0.5/0.25	-	3.5/1.5	800	100	100	28.4	28.4
2/1	-	0.5/0.5	-	0.5/0.5	-	3/2	800	100	50	28.4	40.1
2/1	-	0.5/0.5	-	-	-	2.5/1.5	800	100	50	28.4	40.1
2/1	-	0.25/0.25	-	0.25/0.25	0.25/0.25	2.75/1.75	800	100	100	28.4	28.4

In single dose, animals were treated with SN (0.025 g kg⁻¹; subcutaneous; -45 min), STS (1.0 g kg⁻¹; intraperitoneal; -15 min) and A-KG (2.0 g kg⁻¹; oral; -10 min) prior to potassium cyanide (KCN; oral) and in repeated dose, animals received various doses of STS and A-KG as indicated above at different time intervals. Protection index is the ratio of LD₅₀ of KCN in female rats in the presence of SN+STS+A-KG and LD₅₀ of KCN alone (14.1 mg kg⁻¹)

Effect of pre-treatment of SN, STS and A-KG followed by repeated doses of A-KG and STS: In single dose, animals were treated with SN (0.025 g kg⁻¹; -45 min), STS (1.0 g kg⁻¹; -15 min) and A-KG (2.0 g kg⁻¹; -10 min) prior to KCN (800 mg kg⁻¹). In repeated dose, animals received A-KG (0.25, 0.50 or 1.0 g kg⁻¹) and STS (0.25 or 0.50 g kg⁻¹) at 1, 2, 3, 4 or 6 hr after KCN.

Protection index: After all the treatments, the protection index (PI) was calculated as the ratio of LD₅₀ of KCN in rats receiving the antidotes and the LD₅₀ of KCN alone, which was 14.1 mg kg⁻¹ (Bhattacharya and Vijayaraghavan, 2002). The LD₅₀ (24 hr) and fiducial limits of KCN were determined in rats using 3 to 4 geometrically constant doses comprising of 4 animals each (Gad and Weil, 1989). The protection observed after single treatment was compared with the repeated treatments.

Results and Discussion

Effect of simultaneous treatment of A-KG and STS followed by repeated doses of A-KG: Table 1 reveals that treatment of 0.50 g kg⁻¹ A-KG at '0' time followed by repetition of the same dose after 2 hr increased the survival of animals by 50% or increased the PI from 7.1 to 11.3, accompanied by a 50% reduction in the dose of A-KG. Similarly, 1.0 g kg⁻¹ A-KG at '0' time followed by the same dose after 1 hr increased the protection by 75% and the PI from 14.2 to 22.6. Total load of A-KG remained at 2.0 g kg⁻¹. Administration of 1.0 g kg⁻¹ A-KG at '0' time followed by 0.50 g kg⁻¹ after 2 hr (total dose 1.5 g kg⁻¹) produced identical protection. Further, administration of 2.0 g kg⁻¹ A-KG at '0' time followed by 2.0 g kg⁻¹ after 2 hr (total 4.0 g) resulted in 75% survival, with PI significantly increasing from 21.3 to 38.0. Also, repetition of 1.0 g kg⁻¹ A-KG after 2 hr and 0.50 g kg⁻¹ after 4 hr (total 3.5 g) resulted in 75% increase in survival, with PI rising from 21.3 to 32.0.

Effect of pre-treatment of SN, STS and A-KG followed by repeated doses of A-KG: The data in Table 2 indicates that repetition of 2.0 g kg⁻¹ A-KG 2 hr after KCN (total dose 4.0 g kg⁻¹) reduced the mortality by 50%, with an increase in PI from 28.4 to 40.1. Repetition of 1.0 g kg⁻¹ A-KG after 2 hr and 0.50 g kg⁻¹ after 4 hr (total dose 3.5 g kg⁻¹) reduced the mortality by 50%, with an increase in PI from 28.4 to 40.1. Similar effects were observed with repetition of 1.0 g kg⁻¹ A-KG after 2 hr alone (total dose 3.0 g kg⁻¹). When 800 mg kg⁻¹ KCN was challenged by repetition of 1.0 g kg⁻¹ A-KG after 2 and 4 hr (total dose 4.0 g kg⁻¹), the PI rose from 28.4 to 40.1 accompanied by 50% increase in survival. When 1.0 g kg⁻¹ and 0.5 g kg⁻¹ A-KG were given after 2 and 4 hr, respectively, an enhanced PI of 40.1 was observed. Further increase in the dose of A-KG did not afford additional protection.

Effect of pre-treatment of SN, STS and A-KG followed by repeated doses of STS: Repeating the doses of STS in challenging 800 mg kg⁻¹ KCN did not yield any additional protection (Table 3).

Effect of pre-treatment of SN, STS and A-KG followed by repeated doses of A-KG and STS: Table 4 shows the effect of repetition of A-KG and STS after pre-treatment of A-KG+SN+STS on survival of rats exposed to 800 mg kg⁻¹ KCN. When treatment of A-KG and STS (0.50 g kg⁻¹ each) was repeated after 2 and 4 hr (total dose: 3.0 g kg⁻¹ A-KG and 2.0 g kg⁻¹ STS), a 50% increase in survival with a corresponding increase in PI from 28.4 to 40.1 was observed. Similarly, repeating the dose of A-KG and STS (0.50 g kg⁻¹ each) after 2 hr alone (total dose: 2.5 g kg⁻¹ A-KG and 1.5 g kg⁻¹ STS) produced identical protection. Other combinations did not enhance the protection.

Scrutiny of the results reveal that a maximum of 40-folds protection was observed when A-KG at 1.0 g kg⁻¹ after 2 hr and 0.5 g kg⁻¹ after 4 hr was repeated following the pre-treatment of SN, STS and A-KG. This protection was almost 12-folds more than that observed for pre-treatment of A-KG, SN and STS given only once (Bhattacharya and Vijayaraghavan, 2002). Also, the total load of A-KG was only 3.5 g kg⁻¹. This indicates that at an additional load of 1.5 g kg⁻¹ A-KG, a 12-folds increase in protection was possible. If absolute value is to be considered, a 50% increase in survival of rats exposed to 800 mg kg⁻¹ KCN is note worthy. Also, similar protection was conferred by repeating 0.5 g kg each of A-KG and STS 2 hr after pre-treatment of SN, STS and A-KG. This regimen is better than the previous one because there was an additional load of only 0.5 g kg⁻¹ each of A-KG and STS. If SN is to be omitted from the regimen then 38-folds protection after simultaneous administration of 2.0 g kg⁻¹ A-KG and 1.0 g kg⁻¹ STS, followed by 2.0 g kg⁻¹ A-KG after 2 hr was also appreciable. Here, 75% mortality could be reduced while challenging 300 mg kg⁻¹ KCN. In our previous studies, where A-KG (2.0 g kg⁻¹) was given once as simultaneous treatment with STS (1.0 g kg⁻¹), a PI of 14.2 was observed (Bhattacharya and Vijayaraghavan, 2002). Therefore, a 16-fold increase in protection could be obtained with barely an additional load of 2.0 g kg⁻¹ A-KG.

Cyanide can induce a life-threatening poisoning from which full recovery is possible provided the right antidote is instituted immediately and aggressively. There are cases where people having consumed up to 3.0 g of cyanide were saved with immediate and vigorous therapy (van Heijst *et al.*, 1987). With artificial ventilation and cardio-pulmonary resuscitation, the management of cyanide poisoning becomes easier in human. However, in smaller experimental animals, the treatment purely depends on the pharmacological interventions, resulting in larger morbidity and mortality. The onset of cyanide toxicity is so fast that an antidote given therapeutically practically yields marginal or no protection in animals (Niknahad and O'Brien, 1996). Therefore, in most of the studies, antidotes are given prior to cyanide (Moore *et al.*, 1986). In the present study, although A-KG was given repeatedly with various other combinations including the pre-treatment of SN and STS, it does not imply that these compounds would not be effective when given therapeutically in human. When A-KG was repeated after a pre-treatment of SN, STS and A-KG, 100% animals tolerated 800 mg kg⁻¹ KCN which for a 70 kg man works out to >5.0 g, a dose much higher than the reported dose tolerated by human given nitrite-thiosulfate therapy (van Heijst *et al.*, 1987). Although, A-KG is considered as a monotherapy for moderate cases of cyanide poisoning, its one-time or repeated administration with SN and/ or STS may be useful for patients who are critically ill. Repeated administration of antidotes or their combinations are clinically practiced in hospitalized patients who do not respond satisfactorily to the treatment instituted immediately after cyanide poisoning (van Heijst *et al.*, 1987; van Heijst and Meredith, 1990). Even hydroxocobalamine alone or with another antidote was repeated in

the event of incomplete or transient response in patients exposed to HCN (Borron *et al.*, 2007).

In the likelihood of A-KG being developed as cyanide antidote, the present study would have immense clinical relevance, particularly as prophylaxis for fire fighters, occupational exposures or rescue operations in contaminated areas and as therapy for suicidal, homicidal or accidental cases, casualties of smoke inhalation or as an antidote for CW agent like blood gases (Bhattacharya, 2004). A-KG alone or with STS and/ or SN could be an effective out-of-hospital therapy for cyanide poisoning. Since A-KG is found to be safe at the recommended therapeutic doses (Bhattacharya *et al.*, 2001), its utility as cyanide antidote is anticipated to be far reaching.

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