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Effect of α-Ketoglutarate on Cyanide-induced Biochemical Alterations in Rat Brain and Liver

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Objective To investigate the biochemical changes in rat brain and liver following acute exposure to a lethal dose of cyanide, and its response to treatment of α-ketoglutarate (α-KG) in the absence or presence of sodium thiosulfate (STS). **Methods** Female rats were administered 2.0 LD_{50} potassium cyanide (KCN; oral) in the absence or presence of pre-treatment (–10 min), simultaneous treatment (0 min) or post-treatment (+2-3 min) of α-KG (2.0 g/kg, oral) and/or STS (1.0 g/kg, intraperitoneal, –15 min, 0 min or + 2-3 min). At the time of onset of signs and symptoms of KCN toxicity (2-4 min) and at the time of death (5-15 min), various parameters particularly akin to oxidative stress viz. cytochrome oxidase (CYTOX), superoxide dismutase (SOD), glutathione peroxidase (GPx), reduced glutathione (GSH) and oxidized glutathione (GSSG) in brain, and CYTOX, sorbitol dehydrogenase (SDH), alkaline phosphatase (ALP), GSH and GSSG in liver homogenate were measured. **Results** At both time intervals brain CYTOX, SOD, GPx, and GSH significantly reduced (percent inhibition compared to control) to 24%, 56%, 77%, and 65%, and 44%, 46%, 78%, and 57%, respectively. At the corresponding time points liver CYTOX and GSH reduced to 74% and 63%, and 44% and 68%, respectively. The levels of GSSG in the brain and liver, and hepatic ALP and SDH were unchanged. Pre-treatment and simultaneous treatment of α-KG alone or with STS conferred significant protection on above variables. Post-treatment was effective in restoring the changes in liver but failed to normalize the changes in the brain. **Conclusions** Oral treatment with α-KG alone or in combination with STS has protective effects on cyanide-induced biochemical alterations in rat brain and liver.

Key words: Gyanide; Neurotoxicity; Hepatotoxicity; Protection; α-ketoglutarate; Sodium thiosulfate

INTRODUCTION

Cyanide is an extremely toxic chemical of both defence and civil interest[1]. Both acute and chronic exposures of cyanide can cause severe poisoning in human and animals[1-6]. Cyanide is a potential suicidal, homicidal and chemical warfare agent[1-3], and can be used for military or terrorism purposes. Occupational exposure, ingestion of cyanide-containing foods and combined inhalation of hydrogen cyanide (HCN) and carbon monoxide (CO) in fire smoke contribute to cyanide toxicity[4,7-8]. The treatment of choice for cyanide poisoning includes combination of methemoglobin inducers like amyl nitrite and/or sodium nitrite (SN) and sulfane sulfur-donor like sodium thiosulfate (STS)[1,9-11]. Nitrites are contraindicated in many instances of cyanide poisoning because of pronounced cardiovascular embarrassment produced by them[12-13]. In a recent research, efforts have been expended to develop antidotes that rapidly complex cyanide without compromising with the cardiovascular system[2].

Cyanide is known to bind to carbonyl moieties to form cyanohydrins[14]. Many keto carboxylic acids like α-ketoglutarate (α-KG) have been shown to significantly antagonize cyanide poisoning in experimental animals[15-19]. It had been shown that parenteral or oral administration of α-KG significantly antagonizes high doses of cyanide in rodents[20-22]. Cyanide is a potent neurotoxin and its effects are mediated through lipid peroxidation. Various antioxidant enzymes are affected by cyanide[23-25]. Although there is no information on acute toxic effects of cyanide on liver, a recent report indicates hepatotoxicity and nephrotoxicity following prolonged oral exposure of cyanide[26]. The present study addressed the biochemical changes in rat brain and liver, particularly the oxidative stress

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predisposing to acute cyanide poisoning, and its attenuation by various treatments with α-KG alone or in combination with STS.

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. Sodium thiosulfate (STS) and other chemicals of analytical grade were from Merck or BDH (India). All the solutions were prepared in 0.9% saline.

Animals

Female Wistar rats (150-200 g) bred in the animal facility of Defence Research and Development Establishment (DRDE), were maintained on rice husk in polypropylene cages. Animals had free access to water and rodent pellet food. The study was approved by Establishment's Ethical Committee on Animal Experimentations.

Treatments

Sixty-four rats were divided into eight groups, eight animals each, including (1) control (0.9% saline, oral), (2) KCN (2.0 LD_{50}, oral), (3) KCN +α-KG (2.0 g/ kg, −10 min, oral), (4) KCN + α-KG + STS (1.0 g/ kg, −15 min, intraperitoneal), (5) KCN + α-KG (0 min), (6) KCN + α-KG + STS (0 min), (7) KCN + α-KG (+2-3 min) and KCN + α-KG + STS (+2-3 min). Rats that administered 2.0 LD_{50} KCN (oral) exhibited signs and symptoms of toxicity within 2-4 min. Then 4 animals were sacrificed and the remaining 4 were dissected immediately after death occurred within 5-15 min. Our previous study did not record any mortality of animals receiving 2.0 LD_{50} KCN in the presence of pre-treatment or simultaneous treatment with α-KG or α-KG+STS. However, 33% and 50% mortality were recorded following post-treatment with α-KG and α-KG+STS, respectively (unpublished work). Therefore, animals in group 1 and groups 3-8 were sacrificed corresponding to the time of sampling in KCN-treated groups. Animals were killed by cervical dislocation under light ether anesthesia. The brain and liver were excised quickly to prepare a 3% (w/v) homogenate in chilled phosphate buffer (pH 7.4). Cytochrome oxidase (CYTOX), reduced glutathione (GSH), oxidized glutathione (GSSG) and protein were estimated in both brain and liver homogenates[27-29], and superoxide dismutase (SOD) and glutathione peroxidase (GPx) were determined in brain homogenate alone by the commercial diagnostic kits from Randox Laboratories Ltd., UK. Sorbitol dehydrogenase (SDH) and alkaline phosphatase (ALP) were estimated in liver by the commercial diagnostic kits from Sigma (USA) and Ranbaxy Laboratories Ltd (India), respectively. Above variables were quantitated per milligram protein and the values were expressed as percent change over control.

Statistical Analysis

The values were expressed as $\bar{x} \pm s$ for 4 animals at each time point. Statistical significance was drawn by Student's *t* test ($P<0.05$).

RESULTS

Table 1 shows that at the time of onset of toxic signs and symptoms following oral administration of 2.0 LD_{50} KCN, the levels of brain CYTOX, SOD, GPx, and GSH significantly reduced to 24.3%, 56.4%, 76.8%, and 64.8%, respectively as compared to control. The level of GSSG did not change. Pre-treatment or simultaneous treatment with α-KG alone could maintain the levels of GPx and GSH, while the levels of CYTOX and SOD were still significantly depleted. Adjunction of STS with α-KG could protect all the parameters. Post-treatment with α-KG alone or in combination with STS had no encouraging results. Table 2 indicates the levels of various biochemical indices measured at the time of death following oral administration of 2.0 LD_{50} KCN. Brain CYTOX, SOD, GPx, and GSH significantly reduced to 43.7%, 46.4%, 78.0%, and 56.7%, respectively as compared to control. The level of GSSG did not change. Pre-treatment or simultaneous treatment with α-KG alone could significantly maintain the level of CYTOX. Also, pre-treatment with α-KG alone protected the GPx while pre-treatment or simultaneous treatment with α-KG + STS could protect all the parameters except for SOD (simultaneous treatment). Post-treatment with α-KG+STS could augment the level of CYTOX alone. Table 3 shows that at the time of onset of toxic signs and symptoms following oral administration of 2.0 LD_{50} KCN, the levels of liver CYTOX and GSH significantly reduced to 74.3% and 62.9%, respectively as compared to control. Levels of SDH, ALP and GSSG did not change. Pre-treatment or simultaneous treatment with α-KG or α-KG+STS normalized the levels of CYTOX and GSH in rat liver, but post-treatment with α-KG was effective only in the presence of STS. Table 4 shows that at the

TABLE 1

Effect of Various Treatments With α-Ketoglutarate (α-KG) Alone or in Combination With Sodium Thiosulfate (STS) on the Levels of Various Biochemical Variables in Brain of Female Rats at Onset of Sign and Symptoms of Toxicity Following Administration of 2.0 LD_{50} KCN ($\bar{x} \pm s$)

Treatments	Percent Change Over Control				
	CYTOX[1]	SOD[2]	GPx[3]	GSH[4]	GSSG[5]
KCN+saline	24.3*±3.3	56.4*±6.8	76.8*±9.7	64.8*±6.9	88.0±7.5
KCN+α-KG(-10 min)	86.8*±8.4	67.8*±9.9	89.9±8.8	79.9±9.9	86.0±9.0
KCN+α-KG(-10 min)+ STS (-15 min)	98.0±5.6	89.8±8.9	91.6±9.7	89.6±9.2	87.9±9.3
KCN+α-KG(0 min)	67.9*±5.6	62.3*±7.9	88.4±10.9	78.4±9.7	101.4±11.2
KCN+α-KG(0 min) + STS (0 min)	86.8±8.4	66.7±9.9	86.8±9.9	82.9±10.0	97.9±9.8
KCN+α-KG(+2-3 min)	68.6*±2.9	46.8*±7.9	67.9*±9.6	70.9*±5.6	86.0±10.4
KCN+α-KG(+2-3 min)+STS (+2-3 min)	59.5*±8.3	60.3*±7.0	88.7±9.8	76.9*±7.9	88.0±9.9

Note. The time of onset of signs and symptoms of toxicity was 2-4 min after KCN administration. [1]Cytochrome oxidase; [2]Superoxide dismutase; [3]Glutathione peroxidase; [4]Reduced glutathione; [5]Oxidized glutathione. KCN and α-KG (2.0 g/kg) were administered orally and STS (1.0 g/kg) was given intraperitoneally. *Significant at $P<0.05$.

TABLE 2

Effect of Various Treatments With α-Ketoglutarate (α-KG) Alone or in Combination With Sodium Thiosulfate (STS) on the Levels of Various Biochemical Variables in Brain of Female Rats at the Time of Death Following Administration of 2.0 LD50 KCN ($\bar{x} \pm s$)

Treatments	Percent Change Over Control				
	CYTOX[1]	SOD[2]	GPx[3]	GSH[4]	GSSG[5]
KCN+saline	43.7*±6.2	46.4*±5.8	78.0*±2.8	56.7*±6.9	87.0±9.9
KCN+α-KG(-10 min)	89.9±7.2	56.7*±6.8	86.2±9.8	69.6*±7.4	108.8±12.3
KCN+α-KG(-10 min) + STS (-15 min)	90.0±11.7	88.6±11.6	88.8±9.9	88.4±5.6	101.4±9.8
KCN+α-KG(0 min)	87.9*±9.3	66.7*±7.9	70.9*±5.6	66.4*±7.0	97.6±8.2
KCN+α-KG(0 min)+STS (0 min)	88.5±9.6	66.7*±8.9	87.9±9.9	87.0±9.3	94.8±9.2
KCN+α-KG(+2-3 min)	56.9*±7.0	49.4*±6.9	56.9*±8.9	59.4*±6.6	90.9±6.8
KCN+α-KG(+2-3 min)+STS(+2-3 min)	87.0*±9.3	58.4*±9.6	60.9±7.0	66.8*±8.2	88.9±9.2

Note. The time of death following KCN administration was 5-15 min. [1]Cytochrome oxidase; [2]Superoxide dismutase; [3]Glutathione peroxidase; [4]Reduced glutathione; [5]Oxidized glutathione. KCN and α-KG (2.0 g/kg) were administered orally and STS (1.0 g/kg) was given intraperitoneally. * Significant at $P<0.05$.

TABLE 3

Effect of Various Treatments with α-Ketoglutarate (α-KG) Alone or in Combination With Sodium Thiosulphate (STS) on the Levels of Various Biochemical Variables in Liver of Female Rats at Onset of Sign and Symptoms of Toxicity Following Administration of 2.0 LD_{50} KCN ($\bar{x} \pm s$)

Treatments	Percent Change Over Control				
	CYTOX[1]	SOD[2]	GPx[3]	GSH[4]	GSSG[5]
KCN+saline	74.3*±7.9	78.0±9.0	97.9±9.7	62.9*±9.9	89.0±7.4
KCN+α-KG(-10 min)	88.9±9.4	78.0±9.6	86.5±8.9	79.9±9.9	89.0±3.1
KCN+α-KG(-10 min)+STS (-15 min)	92.4±7.6	89.0±10.3	79.9±9.3	88.4±10.2	104.8±10.9
KCN+α-KG(0 min)	78.9*±9.8	89.0±10.2	88.4±10.9	88.4±10.0	89.0±9.2
KCN+α-KG(0 min)+STS (0 min)	89.8±10.4	98.5±10.6	96.8±8.7	87.9±9.6	98.0±10.6
KCN+α-KG(+2-3 min)	77.9*±5.7	86.8±8.4	87.9±9.6	80.9*±9.4	86.0±9.9
KCN+α-KG(+2-3 min)+ STS (+2-3 min)	79.0*±9.8	96.2±3.2	88.7±9.0	86.9*±8.9	88.7±9.9

Note. The time of onset of signs and symptoms of toxicity was 2-4 min after KCN administration. Values are $\bar{x} \pm s$ of four animals (*$P<0.05$). [1]Cytochrome oxidase; [2]Sorbitol dehydrogenase; [3]Alkaline phosphatase; [4]Reduced glutathione; [5]Oxidized glutathione. KCN and α-KG (2.0 g/kg) were administered orally and STS (1.0 g/kg) was given intraperitoneally. *Significant at $P<0.05$.

TABLE 4

Effect of Various Treatments with α-Ketoglutarate (α-KG) Alone or in Combination With Sodium Thiosulphate (STS) on the Levels of Various Biochemical Variables in Liver of Female Rats at the Time of Death Following Administration of 2.0 LD_{50} KCN ($\bar{x} \pm s$)

Treatments	Percent Change Over Control				
	CYTOX[1]	SOD[2]	GPx[3]	GSH[4]	GSSG[5]
KCN+saline	43.7*±6.2	88.4±6.9	89.9±10.8	67.8*±7.9	101.0±9.9
KCN+α-KG(-10 min)	89.9±7.2	86.7±7.9	86.2±9.0	89.0±7.4	89.6±8.9
KCN+α-KG(-10 min)+STS (-15 min)	90.0±9.7	87.7±4.6	79.9±9.9	87.6±9.6	89.8±9.8
KCN+α-KG(0 min)	87.9±9.3	79.9±8.9	89.0±9.0	87.5±8.0	97.6±4.2
KCN+α-KG(0 min)+STS (0 min)	88.5±9.6	86.7±9.0	80.9±9.3	87.3±9.3	90.8±8.2
KCN+α-KG(+2-3 min)	56.9*±7.0	80.0±9.9	86.9±8.9	79.4*±8.9	89.9±9.4
KCN+α-KG(+2-3 min)+ STS (+2-3 min)	87.0*±9.3	79.9±9.9	90.9±9.7	89.6±8.2	88.9±7.3

Note. The time of death following KCN administration was 5-15 min. [1]Cytochrome oxidase; [2]Sorbitol dehydrogenase; [3]Alkaline phosphatase; [4]Reduced glutathione; [5]Oxidized glutathione. KCN and α-KG (2.0 g/kg) were administered orally and STS (1.0 g/kg) was given intraperitoneally. *Significant at $P<0.05$.

time of death, the level of CYTOX further decreased to 43.7% and the level of GSH remained significantly low at 67.8%. The levels of SDH, ALP and GSSG were not affected. Pre-treatment or simultaneous treatment with α-KG and/or STS augmented the levels of CYTOX and GSH but post-treatment with α-KG was effective only in the presence of STS.

DISCUSSION

Although, combination of SN and STS is the globally accepted treatment of choice for cyanide poisoning, their therapeutic complications in cyanide exposure are well known[1-3,10,12-13]. The beneficial effect of SN is mediated through induction of methemoglobin which combines with cyanide to form cyanmethemoglobin. Methemoglobin values of 30% or more may produce collapse or even fatalities[30-31], though slow detoxification of cyanide is concurrently carried out enzymatically by STS to produce relatively non-toxic thiocyanate[9-11]. Additionally, all these antidotes are administered intravenously. To circumvent these problems we have proposed α-KG and STS as promising antidotes for cyanide poisoning[20-22]. Cyanide is reported to be antagonized by various carbonyl compounds including α-KG[15-19,32]. Our recent studies have shown that oral α-KG in combination with STS protects rodents against acute oral cyanide poisoning[21-22]. In the present study we evaluated the effects of α-KG on various biochemical changes in rat brain and liver following administration of a lethal dose (2.0 LD_{50}) of KCN. The main aim was to characterize the biochemical changes at the initiation of toxicity and just preceding death. The biochemical indices were mostly akin to oxidative stress which is a common phenomenon in cyanide poisoning[23-25].

The present study showed that oral administration of 2.0 LD_{50} KCN significantly inhibited CYTOX activity accompanied with various biochemical alterations predisposing to oxidative damage in rat brain. Inhibition of CYTOX is considered as the main cause of cyanide toxicity[1-3]. Our previous study has also shown a significant decrease in CYTOX activity following KCN administration through parenteral or oral route[21,33]. Other studies have also shown that CYTOX inhibition by cyanide could be significantly prevented by α-KG[21,34]. The central nervous system may be especially vulnerable to hydroperoxide-initiated damage due to high polyunsaturated lipid contents, high rate of oxidative metabolism, and relatively low levels of antioxidant enzymes. Perhaps, for this reason cyanide has been reported to cause lipid peroxidation in mouse brain and inhibit various brain antioxidant enzymes including SOD, catalase, and GPx[23-25]. In the present study also we observed similar effects of cyanide on various antioxidant parameters like SOD, GPx, and GSH. A noteworthy observation in KCN-treated rats is that at the time of death the CYTOX activity in the brain was marginally restored by 20% but this was not sufficient to prevent death. By this time severe oxidative stress already ensued, which was found to be attenuated by α-KG in surviving animals. If we consider the post-treatment, the levels of CYTOX inhibited in α-KG and α-KG+STS groups did not correlate with the survival of the animals, which were 33% and 50% respectively (our unpublished work), indicating that normalization of CYTOX activity alone is not critical to survival. Other parameters predisposing to oxidative stress which could not be corrected by the antidotes, perhaps contribute to

lethality. The protective efficacy of α-KG on the above variables was not pronounced when given alone but with STS, significant ameliorative effects were observed. Post-treatment with α-KG alone or in combination with STS was ineffective. In contrast to brain, the CYTOX activity in liver declined further at the time of death but this was not accompanied with any changes in the levels of liver specific enzymes like SDH or ALP. The significantly reduced levels of GSH remained almost constant at both time points without any reciprocal change in the levels of GSSG. It has been shown that cyanide causes lipid-peroxidation in mouse brain but not in heart or liver[25]. Although liver is not considered as a target organ in cyanide toxicity or associated with acute cyanide intoxication[1], prolonged exposure to cyanide produces hepatotoxicity and nephrotoxicity[26]. Gold potassium cyanide can cause cholestatic hepatitis in human[35]. In the present study, although both hepatic CYTOX and GSH were significantly depleted by KCN, they were not accompanied with any significant change in liver specific enzymes like SDH and ALP, indicating that in acute cyanide exposure the liver toxicity is not critical for death. However, inhibition of temporal CYTOX accompanied with severe oxidative stress could contribute to the toxicity. Protective effects of α-KG on above parameters were also observed in our previous unpublished work. Also, protective effects of α-KG on cyanide-induced convulsions have been reported[36]. Our previous study has shown that α-KG at 2.0 g/ kg does not cause any toxic effects in rats[37]. Therefore, α-KG with STS can be considered as a promising antidote for cyanide poisoning.

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