

ALPHA-LIPOIC ACID PROTECTS AGAINST POTASSIUM CYANIDE-INDUCED SEIZURES AND MORTALITY

BY

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ABSTRACT

This study was proposed to investigate the potential protective effect of alpha-lipoic acid (α -LA) against potassium cyanide (KCN) -induced seizures and lethality in mice. The intraperitoneal ED_{50} value of KCN, as measured by induction of clonic and tonic seizures was increased by pretreatment of mice with α -LA (25, 50 and 100 mg/kg) intraperitoneally in a dose-dependent manner. Similarly, the intraperitoneal LD_{50} value of KCN, based on 24 h mortality, was increased by pretreatment with α -LA in a dose-dependent manner. Intraperitoneal injection of the estimated ED_{50} of KCN (4.8 mg/kg) increased, 1h later, nitric oxide (NO) production and brain glutamate and lipid peroxidation levels and reduced intracellular reduced glutathione (GSH) level and glutathione peroxidase (GSH-Px) activity in mice showing convulsions. Also, administration of the estimated LD_{50} of KCN (6 mg/kg) produced, 24 h later, similar marked changes in these parameters in surviving animals. Pretreatment of mice with α -LA inhibited, dose-dependently KCN (ED_{50} and LD_{50}) - induced an increase in NO production and lipid peroxidation level as well as a decrease in intracellular GSH level and GSH-Px activity. It can be concluded that the protective effect of α -LA against KCN - induced seizures and lethality may be due to inhibition of NO overproduction and maintenance of intracellular antioxidant defense mechanisms.

Keywords: Potassium cyanide; Alpha-lipoic acid; Lipid peroxidation; Nitric oxide.

INTRODUCTION

Cyanide is a well established poison known for its rapid lethal action and toxicity. The brain is especially sensitive to cyanide toxicity (Borowitz et al., 1992). In addition to several other clinical signs, acute poisoning with cyanide produces clonic

and tonic seizures in humans and animals (Egekeze and Ochme, 1980). The acute neurotoxicity of cyanide has been attributed to its induction of lethal cytotoxic hypoxia in brain (Ballantyne, 1984) which results in releasing of glutamate into the extracellular spaces (Benvensite et al., 1984).

It has been reported that cyanide-induced neurotoxicity is associated with activation of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors (Sun et al., 1997). Furthermore, Patel et al. (1991) have shown that incubation of brain slices with cyanide resulted in extracellular accumulation of glutamate. Activation of the NMDA receptors by cyanide was found to generate nitric oxide (NO) and reactive oxygen species in cerebellar granule cells (Gunasekar et al., 1996) and in cultured cortical neurons (Shou et al., 2000). It has been suggested that activation of NMDA receptors, NO synthase and free radicals formation may contribute to the development of neurotoxicity induced by cyanide (Yamamoto and Tang, 1998). In addition, cyanide was found to inhibit brain antioxidant defense mechanisms that predispose to oxidative injury (Gunasekar et al., 1996).

Alpha-lipoic acid (α -LA) has been identified as an ideal antioxidant found naturally in our diets, but appears to have increased functional capacity when given as a supplement. This metabolic antioxidant can scavenge a number of free radicals both in hydrophilic and lipophilic environments (Biewenga et al., 1997; Moini et al., 2002). In addition, α -LA was found to be capable of regenerating endogenous antioxidants in the body including vitamins C and E and intracellular reduced glutathione (GSH) (Biewenga et al., 1997).

Therefore, it has been proposed that α -LA is a therapeutic agent in the prevention or treatment of pathological conditions mediated via oxidative stress (Bliska and Wlodek, 2005). Furthermore α -LA was found to inhibit lipopolysaccharide and cytokine mixture-induced NO production in isolated rat Kupffers cells and murine macrophages by preventing the upregulation of iNOS (Kierner et al., 2002; Demarco et al., 2004). In addition, α -LA was found to protect against cellular damage induced by peroxynitrite (Trujillo and Radi, 2002).

In view of these observations, this work was designated to study the potential protective role of α -LA against cyanide-induced seizures and lethality. The interplay between glutamate, NO, cellular antioxidant defense mechanisms and cyanide-induced seizures and lethality was also investigated.

MATERIAL AND METHODS

Drugs and chemicals :

Potassium cyanide was obtained from Schrarlu Chemie S. A. (Spain). Alpha-lipoic acid was purchased from Fluka Bio-Chemika (Switzerland). Reduced glutathione and Ellman's reagent [(5', 5'-Dithiobis (2-nitrobenzoic acid), DTNB] were obtained from INC Biomedicals Inc (USA). Thiobarbituric acid was obtained from MP Biomedicals, Inc. (France). All other chemicals were of analytical grade.

Animals and treatments :

Male adult Swiss- Webster mice weighing 22-30 g from the Animal House of Assiut University were used in all experiments. Mice were housed in stainless steel cages under a 12h light/dark cycle at 25°C and allowed water and food (laboratory chow) ad libitum. They were divided into groups of 6 animals each. The research was conducted in accordance with the internationally accepted guidelines for laboratory animal use and care. The experiments reported here were approved by our institutional ethics committee.

Fresh solutions of KCN and α -LA were prepared on the day of the experiment in normal saline and sunflower oil, respectively. Different dose levels of KCN (2, 6, 8 and 12 mg/kg) were injected intraperitoneally, each to one group of mice 15 min. after intraperitoneal injection of α -LA (25, 50 or 100 mg/kg) or an equal volume of oil. The animals were observed for 1h after KCN injection for the appearance of generalized seizures. Also, the mortality was determined, 24 h later, in each group of animals. The median effective dose (ED_{50}) and median lethal dose (LD_{50} , based on 24h mortality) for KCN alone or in combination with α -LA were calculated according to the method of Litchfield and Wilcoxon (1949) with 95% confidence limits.

Biochemical measurements :

Groups of 6 animals each were treated with the estimated intraperitoneal ED_{50} of KCN 15 min after intraperitoneal injection of α -LA (25, 50 or 100 mg/kg) or an equal volume of the vehicle. The animals were observed for 1h after cyanide injection for the appearance of generalized seizures in each group. Similar groups of mice were treated with the estimated intraperitoneal LD_{50} of KCN 15 min after injection of α -LA. Survival of animals was monitored for 24h, in each group. Control animals were treated likewise with the pure vehicle. Animals showing convulsions and surviving animals after treatment with estimated ED_{50} and LD_{50} of KCN, respectively were sacrificed by decapitation. Brain and blood tissues were obtained from each animal.

The brain was rinsed in ice-cold saline, blotted carefully, weighed and then homogenized in phosphate buffer (pH 7.4). The homogenate was divided into two equal parts. The first part of homogenate was centrifugated and the supernatant was collected for determination of lipid peroxidation level and GSH-Px activity. Lipid peroxidation was estimated by the measurement of malondialdehyde (MDA) levels in brain tissues. Malondialdehyde is an end product of lipid peroxidation and its level was determined spectrophotometrically by use of thiobarbituric acid reactive substances method previously described by Ohkawa et al. (1979). A stan-

standard reference curve was plotted using 1,1,3,3-tetramethoxypropane. Glutathione peroxidase activity was measured by the method of Paglia and Valentine (1967). The enzymatic reaction in the tube which contained β -nicotinamide adenine dinucleotide phosphate (NADPH), GSH, glutathione reductase and a sample or a standard was initiated by addition of hydrogen peroxide. The change in the absorbance was monitored spectrophotometrically. A standard curve was plotted for each assay.

For determination of GSH and glutamate levels, an equal volume of perchloric acid (1mol/l) was added to the second part of the homogenate and mixed by vortexing. The mixture was allowed to stand for 5 min at room temperature. After centrifugation for 5 min, the supernatant was collected. The GSH contents of the neutralized supernatant were assayed using Ellman's reagent [5, 5'-dithiobis-2-nitrobenzoic acid (DTNB solution)] according to the method of Griffith (1980). A standard reference curve must be prepared for each assay. The glutamate content in the supernatant was measured spectrophotometrically via its enzymatic dehydrogenation with conversion of NAD^+ to NADH according to the method of Lund (1986). A standard reference curve must be prepared for each assay.

Nitric oxide formation was measured in serum samples by assaying nitrite, one of

the stable end products of NO oxidation. Serum nitrite concentration was measured spectrophotometrically using Griess reagent [1% sulfanilamide in 5% phosphoric acid (sulfa - nilamide solution) and 0.1% N-1-naphthylethylenediamine dihydrochloride in bidistilled water (NED solution)] as described by Green et al. (1982). A standard curve must be run simultaneously with each set of samples.

Statistical analysis :

The variability of results was expressed as the mean \pm standard error of mean ($X = \text{S.E.M.}$). The significance of differences between mean values was determined using one-way analysis of variance (ANOVA) and Student's t-test.

RESULTS

The results presented in table (1) indicate that intraperitoneal injection of KCN into mice produced clonic and tonic seizures. The severity of convulsions were dose-dependent. The ED_{50} value of KCN was 4.8 mg/kg. This value was increased (1.1-2 fold) by pretreatment of mice with α -LA intraperitoneally in a dose - dependent manner.

It can be seen in table (2) that the LD_{50} of KCN was 6 mg/kg. Pretreatment of mice with α -LA dose-dependently increased the intraperitoneal LD_{50} of KCN (1-1.9 fold).

Treatment of mice with the estimated intraperitoneal ED₅₀ of KCN produced 1h later in animals showing convulsions, a significant increase in NO production and brain glutamate level. Concurrently this treatment resulted in an increase in lipid peroxidation level and a decrease in intracellular GSH level and GSH-Px activity in mice brain (Table 3). Similar marked biochemical changes were observed in surviving animals after 24h of administration of the estimated intraperitoneal LD₅₀ of KCN into mice (Table 4). Pretreatment of mice with α -LA dose dependently inhibited ED₅₀ and LD₅₀ induced alterations in lipid peroxidation and intracellular GSH levels, GSH-Px activity and NO production. The changes induced by cyanide in brain glutamate level were not affected by pretreatment with α -LA (Tables 3 and 4).

DISCUSSION

Alpha-lipoic acid is unique in its ability to act as an antioxidant in both lipid and aqueous phases of the cell (Moigni et al., 2002). Four distinct antioxidant properties of α -LA and its reduced form have been elucidated: ability to chelate transition metals thus inhibiting the formation of hydroxyl radical, capacity to scavenge reactive oxygen species, capacity to regenerate endogenous antioxidants such as vitamin C, vitamin E and GSH and ability to repair oxidatively dam-

aged protein, such as alpha-1 antiprotease (Biewenga et al., 1997; Lu and Liu, 2002).

It has been reported that cyanide increases lipid peroxidation in brain when produced neurotoxicity (Ardelt et al., 1994). Thus free radicals formation and subsequent lipid peroxidation may contribute to the neurotoxicity induced by cyanide (Yamamoto and Tang, 1996).

Data of the present study demonstrate that cyanide-induced seizures and lethality was associated with increased lipid peroxidation in brain of mice. Pretreatment of mice with α -LA protected against cyanide-induced seizures and mortality and prevented cyanide-induced lipid peroxidation in a dose-dependent manner. It is of interest that administration of toxic doses of cyanide to mice, in our study, resulted in inhibition of enzymatic antioxidants (e.g. GSH-Px) and depletion of non-enzymatic antioxidants (e.g. intracellular GSH) in the brain. These effects were also inhibited by pretreatment of mice with α -LA in a dose - dependent manner.

Glutathione and glutathione-related enzymes play a key role in protecting the cells against the damaging effects of reactive oxygen species. Intracellular GSH can act as a reductant, reducing hydrogen peroxide and lipid hydroperoxides directly to H₂O, a reaction catalyzed by GSH-Px. Depletion of intracellular GSH, under con-

ditions of continuous intracellular oxidative stress, leads to oxidation and damage of lipids, proteins and DNA by the reactive oxygen species (Nordberg and Arner, 2001). Alpha-lipoic acid was found to prevent GSH depletion by scavenging reactive oxygen species (Lu and Liu, 2002) therefore, it inhibits the oxidative damage of cellular macromolecules.

Also, α -LA can increase GSH levels by increasing cysteine uptake, which is a rate limiting step for GSH biosynthesis (Han et al., 1997). In addition, Shila et al. (2005) concluded that α -LA acting as an alternative sulfhydryl nucleophile to GSH prevents its oxidation to glutathione disulfide in detoxifying reaction against reactive oxygen species and consequently increases the activity of glutathione-related enzymes. Thus, depletion of intracellular GSH and decrease in the activity of GSH-Px during cyanide toxicity, in this study, is indicative of the increased lipid peroxidation level. Peroxidation of membrane lipids has been implicated as a possible mechanism of oxidative stress-induced lethal injury (Jaeschke, 2000). In support for this view, Hatch et al. (1990) found that ethyl maleate, a glutathione depletory, markedly enhanced cyanide lethality in mice. Therefore, the increased intracellular GSH level and GSH-Px activity in brain tissues in response to α -LA treatment in our study, is indicative of increased free radical scavenging and enhanced detoxifi-

cation of hydrogen peroxide and lipid hydroperoxides. Inhibition of lipid peroxidation by α -LA may, at least partially, suppress the injury cascade induced by cyanide in brain.

It is believed that acute neurotoxicity of cyanide is due to its production of cellular hypoxia in brain. During hypoxia, an increase in the excitatory amino acid glutamate in the extracellular space can be observed following a rapid decrease in energy levels (Benveniste et al., 1984). It has been shown that incubation of brain slices with cyanide resulted in extracellular accumulation of glutamate (Patel et al., 1991). Furthermore, cyanide was found to enhance NMDA receptors response in cerebellar granule cells (Gunasekar et al., 1996; Sun et al., 1999). Thus cyanide indirectly activates the NMDA receptor by inducing neuronal release of glutamate and by enhancing receptors-mediated responses by direct interaction with the receptors complex (Sun et al., 1997). In addition, it has been found that the NMDA specific antagonist, MK-801, prevented cyanide-induced neurotoxicity in rat cerebrocortical neurons in culture (May et al., 1995) and neuronal cell death in hippocampal neuronal cell cultures (Sturm et al., 1993) and inhibited cyanide-induced seizures in mice (Yamamoto and Tang, 1996). Therefore, glutamate may play an important role in cyanide neurotoxicity.

In the present study, seizures and acute lethality induced by cyanide in mice were associated with a marked increase in the brain glutamate level. Unfortunately, pretreatment of mice with α -LA did not inhibit cyanide-induced elevation of brain glutamate level.

It has been reported that glutamate activation of NMDA receptors stimulates Ca influx into cells. Calcium then binds to calmodulin and activates constitutive nitric oxide synthase resulting in stimulating NO formation (Bredt and Snyder, 1992). However, it has been found that the activity of inducible nitric oxide synthase (iNOS), a Ca-independent high-output nitric oxide synthase isoform (Ogden and Moor, 1995) is induced as a consequence of glutamate release and NMDA receptors activation in rat brain cortex during restraint stress (Madrigal et al., 2001) and after transient focal cerebral ischemia in rats (Perez-Asensio et al., 2005). Moreover, Iravani et al. (2004) found that unilateral intrastriatal administration of N-methyl-D-aspartic acid to rats produced marked iNOS expression within both astroglial and microglial cells. Furthermore, Prabhakaran et al. (2006) found that iNOS up-regulation and mitochondrial glutathione depletion mediate cyanide-induced necrosis in rat mesencephalic cells.

Simultaneous production of NO and superoxide anion is likely during inflam-

mation and pathological conditions. They react together to form peroxynitrite. The scavenging effect on superoxide anion by NO may be a mechanism by which tissues of host are protected from the deleterious effects of superoxide and superoxide-derived reactive oxygen species (Beckman et al., 1990). peroxynitrite is a potent and versatile oxidant that can attack relatively slowly, a wide range of biological targets. Furthermore, peroxynitrite is toxic by more direct oxidative mechanisms. It modifies tyrosine in proteins to create nitrotyrosine leaving a footprint detectable in vivo. Nitration of structural proteins including neurofilament and actin, can disrupt filament assembly with major pathological consequences (Beckman and Koppenol, 1996).

Data of our study indicate that administration of the toxic doses of cyanide to mice, increased the production of NO. Pretreatment with α -LA dose-dependently inhibited cyanide-induced NO overproduction. Thus, these results are in favor of the possibility that overproduction of NO plays an important role in the pathogenesis of cyanide-induced seizures and mortality. In support for this view, it has been found that NG-nitro-L-arginine, an inhibitor of NOS, inhibited cyanide-induced seizures in mice (Yamamoto, 1995). This effect was abolished by pretreatment with L-arginine, a NO precursor.

Acute cyanide toxicity is attributed to inhibition of cytochrome c oxidase, the terminal enzyme of mitochondrial respiratory chain. Cleeter et al. (1994) found that NO inhibits cytochrome c oxidase. This may be implicated in the cytotoxic effects of NO in the CNS and other tissues. Radi et al. (1994) reported that mitochondria may constitute a key intracellular loci for the toxic effects of peroxynitrite. Similarly, Brown and Borutaite (2007) reported that NO and peroxynitrite inhibit mitochondrial respiration. This stimulates reactive oxygen and nitrogen species production by mitochondria which may contribute to cell death. Furthermore, Leavesley et al. (2008) concluded that the rapid, potent action of cyanide is due in part to mitochondrial generation of NO, which enhances inhibition of cytochrome c oxidase.

It has been reported that α -LA is able to decrease the synthesis of NO by preventing the upregulation of iNOS (Demarco et al., 2004). Another explanation for the reduction of NO level might be due to the direct scavenging effect of NO by the sulphhydryl group of α -LA (Biewenga et al., 1997). Furthermore, α -LA was found to inhibit efficiently the nitration of L-tyrosine by peroxynitrite (Nakagawa et al., 1999) by reacting with the nitrating intermediate of peroxynitrite (Nakagawa et al., 2000). Moreover, it has been shown that α -LA is a very potent protector against peroxynitrite-mediated nitration

of L-tyrosine, direct oxidation of glutathione and cellular damage (Nakagawa et al., 2000; Trujillo and Radi, 2002; Trujillo et al., 2005).

In light of these observations, data of the present study suggest that α -LA by antagonizing the effect of toxic doses of cyanide on NO level may inhibit peroxynitrite anion formation which has potent oxidative and cytotoxic activities. Therefore, α -LA prevented cyanide-induced depletion of intracellular GSH, suppression of GSH-Px activity, elevation of lipid peroxidation level and cellular damage. Thus, decrease of NO level by α -LA resulted in subsequent maintenance of intracellular antioxidant mechanisms and protected against cyanide-induced severe intracellular oxidative stress and damage of brain tissues.

Conclusively, our results demonstrate that α -LA has the ability to protect against seizures and mortality induced by cyanide. The ability of α -LA to provide this protective effect is positively correlated with its ability to suppress cyanide-induced NO overproduction, depletion of intracellular GSH, inhibition of GSH-Px activity and increase of lipid peroxidation level. Thus, the protective effect of α -LA against cyanide-induced seizures and mortality may be due to inhibition of NO overproduction and maintenance of intracellular antioxidant status.

Table (1) : Protective effect of alpha-lipoic acid (α -LA) against potassium cyanide (KCN) - induced seizures in mice.

Treatment	KCN ED ₅₀ (mg/kg) and its 95% confidence limits	Potency ratio
Control	4.8 (3.9 – 5.8)	—
25 mg/kg α -LA	5.2 (4.1 – 6.5)	1.1
50 mg/kg α -LA	5.8* (4.6 – 7.9)	1.2
100 mg/kg α -LA	9.4** (7.8 – 8.1)	2

Values are means \pm S.E.M. of five observations.

*P < 0.05 vs. control value; **P < 0.01 vs. control value.

Potency ratio was calculated by dividing the ED₅₀ value of KCN with α -LA by the ED₅₀ value without α -LA

Table (2): Protective effect of alpha-lipoic acid (α -LA) against potassium cyanide (KCN) - induced lethality in mice.

Treatment	KCN LD ₅₀ (mg/kg) and its 95% confidence limits	Potency ratio
Control	6 (5.2 – 6.7)	—
25 mg/kg α -LA	6.2 (4.9 – 8.1)	1
50 mg/kg α -LA	7.2* (5.5 – 9.8)	1.2
100 mg/kg α -LA	11.2** (9.4 – 12.8)	1.9

Values are means \pm S.E.M. of five observations.

*P < 0.05 vs. control value; **P < 0.01 vs. control value.

Potency ratio was calculated by dividing the LD₅₀ value of KCN with α -LA by the LD₅₀ value without α -LA

Table (3) : Effect of pretreatment with alpha-lipoic acid (α -LA) on the alterations induced by the estimated intraperitoneal ED₅₀ of potassium cyanide (KCN) in serum nitrite level and brain glutamate, MDA and intracellular GSH levels and GSH-P_x activity in mice showing convulsions.

Treatment	Serum nitrite ($\mu\text{mol/l}$)	Glutamate ($\mu\text{mol/g w.wt}$)	MDA (nmol/g w.wt.)	Intracellular GSH ($\mu\text{mol/g w.wt.}$)	GSH-P _x (U/g w.wt.)
Control	4.20 \pm 0.12	2.96 \pm 0.12	308.49 \pm 20.28	2.82 \pm 0.11	18.86 \pm 1.16
KCN	7.33** \pm 0.32	4.18** \pm 0.21	692.85** \pm 32.35	1.74** \pm 0.08	12.22** \pm 1.06
25 mg/kg α -LA+ KCN	6.86 \pm 0.26	4.20 \pm 0.16	674.34 \pm 41.22	1.82 \pm 0.06	12.86 \pm 1.08
50 mg/kg α -LA+ KCN	5.75** \pm 0.24	4.12 \pm 0.24	527.37** \pm 36.24	2.12** \pm 0.12	15.12** \pm 1.12
100 mg/kg α -LA+ KCN	4.78** \pm 0.18	4.02 \pm 0.18	367.25** \pm 22.40	2.88** \pm 0.14	17.66** \pm 1.06

Blood and brain samples were collected for these biochemical measurements 1h after treatment of mice with 4.8mg/kg KCN or oil.

Values are means \pm S.E.M of five observations **P < 0.01 vs. control value; *P < 0.01 vs. KCN value.

Table (4) : Effect of pretreatment with alpha-lipoic acid (α -LA) on the alterations induced by the estimated intraperitoneal LD₅₀ of potassium cyanide (KCN) in serum nitrite level and brain glutamate, MDA and intracellular GSH levels and GSH-P_x activity of surviving mice.

Treatment	Serum nitrite ($\mu\text{mol/l}$)	Glutamate ($\mu\text{mol/g w.wt.}$)	MDA (nmol/g w.wt.)	Intracellular GSH ($\mu\text{mol/g w.wt.}$)	GSH-P _x (U/g w.wt.)
Control	4.21 \pm 0.15	2.92 \pm 0.14	316.26 \pm 22.56	2.86 \pm 0.14	18.42 \pm 1.10
KCN	17.86** \pm 0.28	5.24** \pm 0.28	834.51** \pm 54.22	1.38** \pm 0.09	11.11** \pm 0.80
25 mg/kg α -LA+ KCN	7.38 \pm 0.24	5.32 \pm 0.25	780.68 \pm 56.34	1.44 \pm 0.08	11.92 \pm 0.86
50 mg/kg α -LA+ KCN	5.92** \pm 0.18	5.18 \pm 0.22	602.34** \pm 42.34	2.21** \pm 0.11	14.86** \pm 1.12
100 mg/kg α -LA+ KCN	4.98** \pm 0.22	5.12 \pm 0.18	402.28** \pm 28.26	2.72** \pm 0.16	17.12** \pm 1.18

Blood and brain samples were collected for these biochemical measurements 24h after treatment of mice with 6 mg/kg KCN or oil.

Values are means \pm S.E.M of five observations **P < 0.01 vs. control value; *P < 0.01 vs. KCN value.

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حمض ألفا ليبويك يحمى من التشنجات والموت المحدث بسيانيد البوتاسيوم

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استهدفت هذه الدراسة بحث التأثير الواقى المحتمل لحمض ألفا ليبويك ضد التشنجات والموت المحدث بسيانيد البوتاسيوم فى الفئران الصغيرة، ولقد وجد أن جرعة سيانيد البوتاسيوم المحقونة فى التجويف البريتونى والتي تحدث التشنجات فى ٥٠٪ من الفئران قد زادت بصورة معتمدة على الجرعة بالإعطاء المسبق لحمض ألفاليبويك فى جرعات متزايدة، وبالمثل وجد أن جرعة سيانيد البوتاسيوم المحقونة فى التجويف البريتونى والتي تحدث الوفاة فى ٥٠٪ من الفئران خلال ٢٤ ساعة قد زادت بصورة مطردة بالحقن المسبق بحمض الفاليبويك، ولقد وجد أن حقن جرعة سيانيد البوتاسيوم التي تحدث التشنجات فى ٥٠٪ من الفئران (٨مجم/كجم) تحدث بعد ساعة من إعطائها زيادة فى إنتاج أكسيد النيتريك ومستوى الجلوتامات والدهون فى المخ وأحدثت نقص فى الجلوتاثيون الخلوى ونقص فى نشاط إنزيم الجلوتاثيون بيروكسيداز فى مخ الفئران التي تحدث فيها التشنجات، أيضاً وجد إن إعطاء جرعة سيانيد البوتاسيوم (٦مجم/كجم) التي تحدث الوفاة فى ٥٠٪ من الفئران تؤدي إلى تغيرات مشابهة فى هذه القياسات فى الفئران التي عاشت، ولقد وجد أن الحقن المسبق لحمض الفاليبويك يشبط اعتماداً على الجرعة الزيادة التي حدثت فى إنتاج أكسيد النيتريك ومستوى الجلوتامات والدهون فى المخ والنقص الحادث فى الجلوتاثيون الخلوى وفى نشاط إنزيم الجلوتاثيون بيروكسيداز فى مخ الفئران الناتج عن إعطاء الجرعة التي تحدث تشنجات فى ٥٠٪ من الفئران والتي تحدث وفاة فى ٥٠٪ من الفئران وقد أستنتج أن التأثير الواقى لحمض الفاليبويك ضد التشنجات والوفيات المحدثه بسيانيد البوتاسيوم قد تكون ناتجة عن تشبيط زيادة إنتاج أكسيد النيتريك وتشبيط مستوى مضادات الأوكسدة الخلوية.