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ELIMINATION PATTERNS OF ARTERIAL BLOOD CYANIDE ION IN THIOSULFATE-OXYGEN ADMINISTERED RABBIT

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ABSTRACT: *To test the efficacies of thiosulfate in cyanide poisoning with or without oxygen, after the administration of sublethal dose of potassium cyanide, serial arterial blood samples were collected during 60 minutes in 15 rabbits. Cyanide ion concentrations were measured by Conway cell microdiffusion method, and arterial oxygen tensions were also observed. Comparison of elimination constants showed that arterial blood cyanide ion concentration decreased most rapidly in the thiosulfate with oxygen-administered group. The elimination of cyanide ion by the action of thiosulfate in acutely poisoned rabbit accelerated probably due to oxygen and elimination pattern seems to occur by first-order elimination kinetics.*

Keywords: *Arterial cyanide ion, thiosulfate, oxygen, Conway cell microdiffusion*

INTRODUCTION

Sodium or potassium cyanides are widely used chemicals in metallurgy for extraction of gold or silver from their ores, in electroplating, and in organic synthesis of various materials. Hydrocyanic acid is an effective agent for the fumigation of ships, large buildings, flour mills and private dwellings (Clayton and Clayton, 1978; Arena, 1979; Doull *et al.*, 1980). Victims of fires can also be poisoned by hydrogen cyanide and with carbon monoxide which are released from the burinings of nitrogen containing carpet materials, wool or silk (Pitt *et al.*, 1979). In medical fields, Accidental poisonings by nitroprusside (Smith and Kruszyna, 1974; Cottrell *et al.*, 1978) and amygdalin therapy (Humbert *et al.*, 1977; Braico *et al.*, 1979) were also reported to contribute to cyanide poisonings. Cyanide ion has a high affinity to iron in ferric state. It reacts readily with the trivalent iron of cytochrome oxydase (cytochrom a_3) in mitochondria, and inhibits cellular respiration resulting histotoxic hypoxia, in which state cellular utilization of oxygen is blocked. (Gettler and Baine, 1938; Camerino and King, 1966).

After the Pedigo's (1988) first report on therapeutic measures useful for trearment of cyanide poisoning, many authors have studied the control methods and its mechanism to protect against cyanide poisoning, experimentally and/or clinically (Chen *et al.*, 1933; Mushett *et al.*, 1952; Wood and Cooley, 1956; Bain and Knowles, 1967). It is now known whether most effective treatment measures in cyanide poisoning includes nitrite (amyl nitrite or sodium nitrite) and sodium thiosulfate under hyperbaric oxygen (Chen and Rose, 1956; Saunders and Himwich, 1950; Berlin, 1970; Levine, 1959; Cope and Abramowitz, 1960; Cope, 1961; Skene *et al.*, 1966). Although the toxic mechanism of cyanide poisoning is now known to be due to the inhibition of intracellular respiratory enzyme, cytochrome oxidase, the role of oxygen in detoxification of cyanide by thiosulfate is still a controversial one. This experimental study was designed to clarify the role of oxygen in the detoxification of cyanide ion by sodium thiosulfate in arterial blood in rabbit.

MATERIALS AND METHODS

To observe the changing patterns of cyanide ion in arterial blood, 15 rabbits weighing. 1.9-2.3 kg were divided into three groups of 5 rabbits, each according to the cyanide antidotes used (Table 1). The animals were anesthetized with sodium pentobarbital 30 mg/kg, intravenously, and their tracheas were intubated for ventilation. A left external jugular vein was cannulated for the administrations of potassium cyanide and sodium thiosulfate, and whole-body heparinization (1,000 IU) was done through the same catheter. A right external carotid artery was cannulated and a catheter was advanced to 1 cm for sequential blood sampling.

All solutions were prepared daily from analytical reagent grade chemicals. Potassium cyanide was injected through the venous catheter at 1 mg/kg dose, a dose which was determined to be a sublethal dose in preliminary experiments. At the same time, sodium thiosulfate (60 mg/kg/hr) was continuously infused intravenously for 30 min with a Sage constant infusion syringe pump. Oxygen, only in S.T. ± O_2 group, was inhaled for 60 min through intubated cannula, through which oxygen content of inhaled air was monitored and FiO_2 maintained at 0.95.

Determinations of arterial blood cyanide ion were made at 1, 3, 5, 8, 10, 15, 30 and

Table 1. Administration protocol of the various experimental conditions

Experimental groups	No. of animals	Administration protocol		
		KCN dose & route	$Na_2S_2O_3$(S.T.) dose & route	O_2 inhalation (F_iO_2=0.95)
CN^- ion elimination in rabbit		*mg/kg, i.v.*	*mg/kg/hr, i.v.*	*1 ATA for 60 min*
Control group	5	1.0	normal saline	ambient air
S.T. group	5	1.0	60	ambient air
S.T. + O_2 group	5	1.0	60	carbogen

60 min sequentially after the KCN injection. The cyanide ion concentrations were measured by microdiffusion method immediately after each sampling (Boxer and Richard, 1951; Sunshine, 1975; Feldstein and Klendshoj, 1954). The procedure was as follows; cyanide may be liberated from the biological fluids by acidification, the evolved hydrocyanic acid liberated from the mixture of blood specimen and 3.6N sulfuric acid in the outer compartment of Conway cell is absorbed in 0.1N NaOH solution in the inner compartment for 12 hours, and the sodium cyanide thus formed can be quantitatively determined by measuring the absorbance of chromophores formed by interaction of the cyanide ion with suitable reagents (barbituric acid, pyridine, 12N HCl). Pye Unicam UV/Visible spectrophotometer, type SP-1750 was used for measuring the absorbance of each solution at 580 nm and every specimen was measured 3 times repeatedly. On the other hand, arterial oxygen tension was determined via carotid arterial catheter just prior to oxygen inhalation, and at 5, 10, 15, 30, and 60 min after KCN injection with Corning Blood Gas Analyzer, model 161.

To compare the sequential changes of arterial blood cyanide ion concentration in each experimental groups, each values was transformed to natural logarithmic of remaining concentration [ln (initial concentration/concentration at time t × 100)] scale and calculated the elimination constants k, which were used for comparing the effectiveness of various therapeutics (Tallarida and Murray, 1981). Statistical comparison

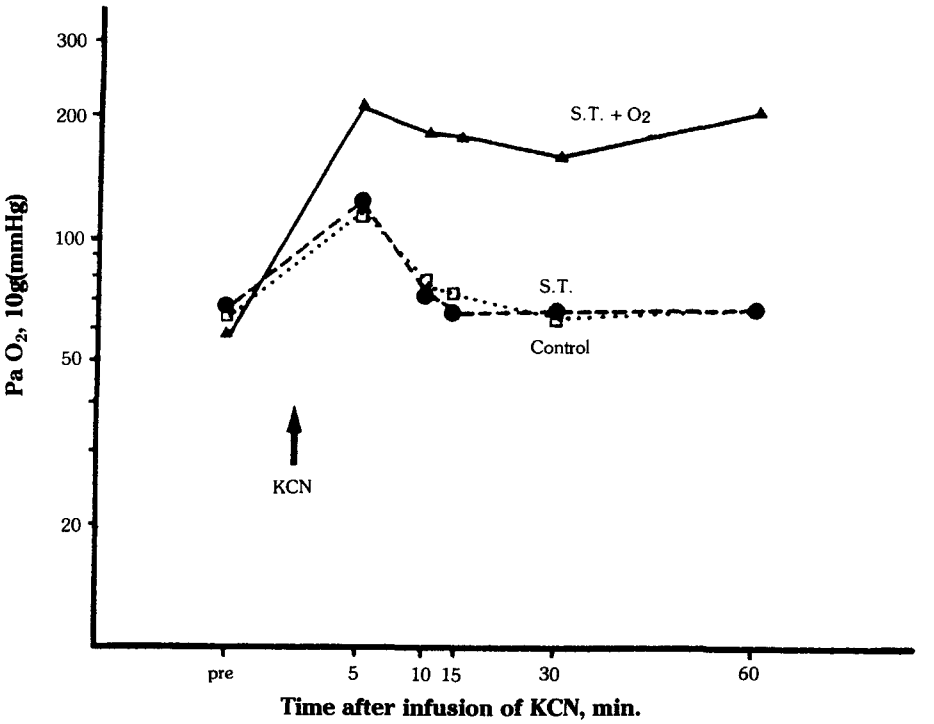

Fig. 1. Sequential changes of arterial blood oxygen tensions determined just prior to oxygen inhalation and at 5, 10, 15, 30 and 60 min after KCN injection in ribit.

was done by the test of the difference between two linear regression coefficients(slopes) with the confidence limits for 95% probability (Petrie, 1978).

RESULTS

Arterial oxygen tension increased significantly from control value of 60-70 mmHg to 100 mmHg at 5 min, thereafter, decreased to the initial level at 60 min in control and S.T. groups, while in the oxygen-administered group, its level increased steadily to 200 mmHg at 5 min and maintained 180-200 mmHg during 60 min.

The mean concentration of arterial blood cyanide ion in control group (Table 2), sampled at 1 min was $3.73 + 0.59$ ug/ml and that in S.T. group was $3.34 + 0.63$ ug/ml and $3.93 + 1.10$ ug/ml in S.T. + O_2 group, which values did not show statistical significance ($F = 0.79$, d.f. $= 3, 16$, $p > 0.05$). Arterial blood cyanide ion concentration decreased rapidly to the level of 1 ug/ml within 10 min in S.T. group and 8 min in S.T. + O_2 treated group. However, a rather slow decreasing pattern of cyanide ion was observed in control group, in which it takes almost 60 min to reach the same level.

To compare the elimination patterns of arterial blood cyanide ion in each treatment conditions, quantitatively, per cent remaining concentrations of cyanide ion within 10 min among groups were used in curvilinear regression analysis. As is shown in Fig. 2, transformation to natural logarithmic scale ($Y = a + b \ln X$) was the best in fitting the equations of each groups, with the statistical significant correlation coefficients of -0.909 to -0.974 ($p < 0.01$).

As a result of the goodness of fit, authors believed that the elimination pattern of cyanide ion in arterial blood should abide by the first-order elimination kinetics, providing the rationale of calculating elimination constants, (k) for the first time. Elimination constants during the first 10 min were -0.068 in control group, -0.135 in S.T. treated group and -0.195 in S.T. with oxygen-administered group.

Table 2. Mean concentration of arterial blood cyanide among experimental groups measured by Conway cell microdiffusion method

(unit: ug/ml)

Experimental groups	No. of animals	Time lapse after KCN infusion (min) 1	3	5	8	10	15	30	60
Control group	5	3.73 ± 0.59	2.98 ± 0.46	2.70 ± 0.57	2.28 ± 0.47	2.01 ± 0.57	1.73 ± 0.45	1.23 ± 0.28	0.95 ± 0.23
S.T. group	5	3.34 ± 0.63	2.27 ± 0.39	1.68 ± 0.49	1.26 ± 0.28	0.95 ± 0.09	0.71 ± 0.15	0.53 ± 0.14	0.52 ± 0.05
S.T. + O_2 group	5	3.93 ± 1.10	2.47 ± 0.90	1.78 ± 0.56	0.97 ± 0.24	0.66 ± 0.15	0.56 ± 0.18	0.47 ± 0.13	0.40 ± 0.09

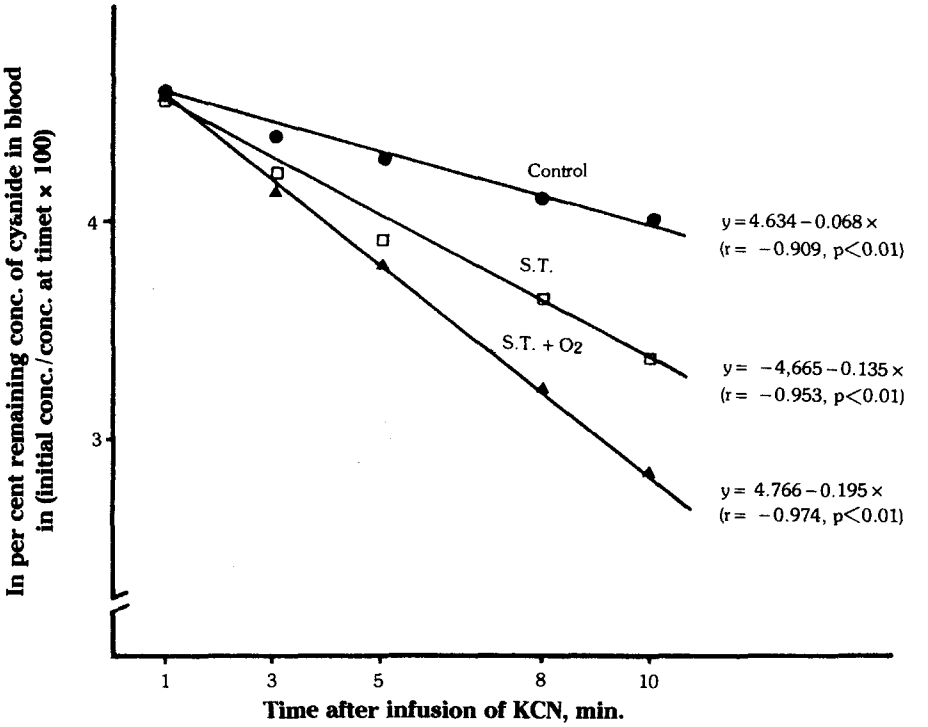

Fig. 2. Linear elimination kinetic patterns of arterial blood cyanide ion during the first 10 min among experimental groups. After transformation to natural logarithmic scale the elimination patterns showed linear relationship, which means the first-order elimination kinetic pattern.

Table 3. Comparison of elimination constants during the first 10 minutes among experimental groups

Experimental group	Elimination constant, $k(min^{-1})$@	Statistical comparison (p value) #		
		Control	S.T.	S.T. + O_2
Control group	−0.068	—	0.01**	0.01**
S.T. group	−0.135		—	0.01**
S.T. + O_2 group	−0.195			—

@Elimination constant k refers to the regression coefficient(b_i) of the regression line calculated from the linear regression method.

$t = \frac{b_1 - b_2}{S_{b1\ -b2}}$, d.f. $= n_1 + n_2 - 4$.

All combinations of the constants were statistically highly significant ($p < 0.01$) by hypothesis testing method between regression coefficients (Table 3). Elimination of cyanide ion from blood when oxygen was given together with thiosulfate was accelerated more significantly than in the cases treated with thiosulfate alone or none.

DISCUSSION

Because free cyanide ion can readily cross the cell and mitochondrial membranes, it rapidly inhibits the electron-transport system. Therefore, the mechanism of cyanide toxicity is due to blocking of the aerobic metabolism, the major pathway for highenergy phosphate production. Theoretically the administration of oxygen should serve no useful purpose in cyanide poisoning, since it is the tissue utilization of oxygen which is impaired, therefore, hemoglobin is fully oxygenated.

But many mortality or toxicological studies have reported that oxygen is of paramount importance in the detoxification of cyanide poisoning even in the case of using oxygen alone. The rational therapeutic approach to cyanide poisoning is to prevent cyanide ion from binding to cytochrome oxidase. This is done by combining the cyanide ion with another iron-containing compound that can compete with cytochrome oxidase in terms of binding affinity. Chen and Rose (1952) proposed firstly nitrite to oxidise hemoglobin to methemoglobin to form a large intravascular pool to bind the cyanide ion preferentially. But nitrite is regarded as being unsatisfactory because it is difficult to achieve adequate level of methemoglobinemia without producing cardiovascular collapse, hypoxia and reverse cyanide poisoning, especially in children. (Chen and Rose, 1952; Berlin, 1970; Graham *et al.*, 1977).

Thiosulfate can be given because its actual detoxification process involves the conversion of cyanide ion, as it dissociates from the cyanmethemoglobin induced by nitrite, to the less toxic thiocyanate, which is eliminated by the kidneys. This conversion is thought to require an enzyme, rhodanese, which is widely distributed in body tissues. The rate-limiting step in this process is thought to be the supply of sulfur donor such as thiosulfate, which is not stored sufficiently in the body and is also rapidly eliminated by the kidneys (Saunders and Himwich, 1950; Clayton and Clayton, 1978; Arena, 1979).

Cope(1961), in his summary of integrated view of the treatment protocol, suggested that treatment at the intracellular level can be accomplished by maintenance of as high an oxygen pressure as possible, and at the extracellular level consists of a combination with nitrite and thiosulfate. Ivankovich (1980) suggested that, when high plasma concentration of thiosulfate are available, the detoxification mechanism is rapid enough to provide adequate protection. The kinetics of the tissue rhodanese enzyme suggested that three times more thiosulfate than cyanide must be present for successful detoxification and Skene (1966) was also of the opinion that oxygen exerted its therpeutic effect by increasing the rate of detoxification by rhodanese enzyme, but he gave no experimental evidence to support this hypothesis.

At present, several possible mechanisms, by which oxygen may effectively facilitate the action of thiosulfate in cyanide intoxication, can be proposed. Firstly, oxygen may increase the respiratory excretion of hydrogen cyanide or its volatile metabolites by displacement of cyanide from binding sites. Under normal physiological conditions, the

respiratory excretion of cyanide represents only a minor pathway, however, at higher doses, the respiratory excretion may play a major role in cyanide deposition and metabolism. Secondly, oxygen may reactivate cytochrome oxidase or directly displace cyanide ion from binding site on the enzyme. And thirdly, oxygen may alter the biotransformation of cyanide. The predominant cyanide-detoxifying enzyme, rhodanese, does not require oxygen (Green and Westly, 1961), but oxidation of sulfite ion, and end product and inhibitor of the rhodanese reaction [$CN^- + S_2O_3^{-2} \longrightarrow SCN^- + SO_3^{-2}$], can be enhanced in the presence of oxygen, thereby reducing the possibility of end product inhibition.

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REFERENCES

Arena, J.M. (1979); Poisoning. 4th ed., Illinois, American Lecture Series.

Bain, J.T.B. and Knowles, E.L. (1967); Successful treatment of cyanide poisoning. Brit. J. Med. 17: 763.

Berlin, C.M. Jr. (1970); The treatment of cyanide poisoning in children. Pediatrics 46: 793-796.

Boxer, G.E. and Richard, J.C. (1951); Chemical determination of vit-B_{12}. II. The quantitative isolation and colorimetric determinations of millimicrogram quantities of cyanide. *Arch. Biochem. & Biophys.* 30: 372-381. Braico, K.T., Humbert, J.R., Terplan, K.L. and Lehotay, J.M. (1979); Laetrile intoxication, report of a case. *N. Eng. J. Med.* 300(5): 238-240.

Camerino, P.W. and King, T.E. (1966); Studies on cytochrome oxidase, II. A reaction of cyanide with cytochrome oxidase in soluble and particulate forms. *J. Biol. Chem.* 241(4): 970-979.

Chen, K.K. and Rose, C.L. (1952); Nitrite and thiosulfate therapy in cyanide poisoning. *J.A.M.A.* 149(2): 113-119.

Chen, K.K. amd Rose, C.L. (1956); Treatment of acute cyanide poisoning. *J.A.M.A.* 162(17): 1154-1156.

Chen, K.K., Rose, C.L. and Clowes, G.H.A. (1933); Methylene blue, nitrites and sodium thiosulfate against cyanide poisoning. *Proc. Soc. Exper. Biol. & Med.* 31: 250-253.

Clayton, G.D. and Clayton, F.E.(1978); Patty's industrial hygiene and toxicology. 3rd ed., A Wiley-Interscience Publication.

Cope, C. (1961); The importance of oxygen in the treatment of cyanide poisoning. *J.A.M.A.* 175(12): 1061-1064.

Cope, C. and Abramowitz, S. (1960); Respiratory responses to intravenous sodium cyanide, a function of the oxygen-cyanide relationship. *Am. Rev. Res. Dis. 81:* 321-328.

Cottrell, J.E., Casthely, P., Brodie, J.D., Patel, K., Klein, A. and Turndorf, H. (1978): Prevention of nitroprusside-induced cyanide toxicity with hydrocobalamin. *N. Eng. J. Med. 298(5):* 809-811.

Doull, J., Klaassen, C.D. and Amdur, M.D. (1980); Casarett and Doull's toxicology, the basic science of poisons. 2nd ed, N.Y., Macmillan Publishing Co., Inc.

Feldstein, M. and Klendshoj, N.C. (1954); The determination of cyanide in biologic fluids by microdiffusion analysis. *J. Lab. & Clin. Med. 44(1):* 166-170.

Gettler, A.O. and Baine, J.O. (1938); The toxicology of cyanide. *Am.J.M.Sc. 195:* 182-198.

Graham, D.L., Laman, D., Theodore, J and Robin, E.D. (1977); Acute cyanide poisoning complicated by lactic acidosis and pulmonary edema. *Arch. Intern. Med. 137:* 1051-1055.

Green, J.R. and Westley, J. (1961); Mechanism of rhodanese action: Polarographic studies. *J. Biol. Chem. 236(11):* 3047-3050.

Humbert, J.R., Tress, J.H. and Braico, K.T. (1977); Fatal cyanide poisoning, accidental ingestion of amygdalin. *J.A.M.A. 238(6):* 482.

Ivankovich, A.D. (1980); Cyanide antidotes and methods of their administration in dogs. *Anesthesiology 52:* 210-216.

Levine, S. (1959): Oxygen in the therapy of cyanide poisoning. *J.A.M.A. 170*(13): 1585.

Mushett, C.W., Keller, K.L., Boxer, G.E. and Richards, J.C. (1952); Antidotal efficacy of vitamin B_{12}a(hydroxocobalamin) in experimental cyanide poisoning. *Proc. Soc. Exper. Biol. & Med. 31:* 234-237.

Petrie, A. (1978); Lecture notes on medical statistics. Oxford, London, Edinburgh, Melbourne, Blackwell Scientific Publications.

Pitt, B.R., Radford, E.P., Gurtner, G.H. and Traystman, R.J. (1979); Interaction of carbon monoxide and cyanide on cerebral circulation and metabolism. *Arch. Environ. Health 34(5):* 354-359.

Saunders, J.P. and Himwich, W.A. (1950): Properties of the transsulfurase responsible for conversion of cyanide to thiocyanate. *Am. J. Physiol. 163:* 404-409.

Skene, W.G., Norman, J.N. and Smith, G. (1966); Effect of hyperbaric oxygen in cyanide poisoning. Proceedings of the third international conference on hyperbaric medicine, edited by Brown, I.W.Jr. and Cox, B.G. Washington, *Ntl. Acad. Sci. & Ntl. Res. Council.*

Smith, R.P. and Kruszyna, H. (1974); Nitroprusside produces cyanide poisoning via a reaction with hemoglobin. *J. Pharm. Exper. Ther. 191(3):* 557-563.

Sunshine, I. (1975); Methodology for analytical toxicology. Cleverland, CRC Press.

Tallarida, R.J. and Murray, R.B. (1981); Manual of pharmacologic calculation with computer programs. *N.Y., Heidelberg, Berlin, Springer-Verlag.*

Wood, J.C. and Cooley, S.L. (1956); Detoxification of cyanide by cystine. *J. Biol. Chem. 218:* 449-457.