Effect of Chelation with Calcium Disodium EDTA on Haemato-biochemical and Trace Mineral Profile in Blood from Lead Exposed Calves

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ABSTRACT : An experiment was performed using 20 calves of about one-month old to investigate the effect of chelation therapy with calcium disodium ethylenediaminetetraacetate ($CaNa_2EDTA$) alone or along with antioxidant α-tocopherol in lead loaded calves on blood trace minerals, erythrocytic sulfahydryl groups and some haematobiochemical parameters. Fifteen calves were given lead orally at a daily dose of 7.5 mg of 99% pure lead acetate/kg body weight for 28 days. Then the lead was withdrawn on day 28 and the calves were randomly divided into three groups. Each group of five animals was either treated with $CaNa_2EDTA$ alone at the dose rate of 110 mg/kg body weight in two divided doses for 4 days or along with α-tocopherol at the dose rate of 100 mg/kg body weight orally daily for 7 days, keeping the remaining five calves as lead-exposed untreated controls. Blood samples were collected at the end of the lead exposure (day 0) and thereafter on day 2, 4, 7 and 10 from the start of the chelation treatment. The treatment with EDTA alone led to slow but non-significant improvement in blood copper level, but incorporation of antioxidant α-tocopherol in chelation therapy resulted in its significant decline, as recorded on day 7-post treatment. Withdrawal of lead or treatment with $CaNa_2EDTA$ alone or along with α-tocopherol enhanced the erythrocytic thiol contents and the levels of T-SH and P-SH became statistically ($p<0.05$) comparable to those of lead-exposed controls by day 7 and 4, respectively. There was no significant ($p>0.05$) change in serum urea, creatinine, total protein and albumin levels between the treatment groups. It is concluded from the present investigation that treatment with $CaNa_2EDTA$ at the present dose rate is safe to be used for chelation in lead loaded calves. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 8 : 1130-1134)***

Key Words : Chelation, $CaNa_2EDTA$, Lead, Calves, Sulfhydryl Groups, α-Tocopherol

INTRODUCTION

Lead is a common environmental and industrial toxicant having no beneficial biological role. It is one of the commonest causes of poisoning in firm ruminants and young calves are highly susceptible. Calcium disodium ethylenediaminetetraacetate ($CaNa_2EDTA$) has been used as conventional and specific antidote for lead toxicity, and is extensively used for management of lead toxicity in man and animals (Piomelli et al., 1984; Klaassen, 1985; Radostitis et al., 2000). However, experimental study involving administration of $CaNa_2EDTA$ alone in healthy dogs led to sustained urinary loss of zinc, copper and manganese through mobilization and redistribution of these essential elements from storage and soft tissues (Ibim et al., 1992). There are also conflicting reports on tissue specific changes in trace mineral and blood biochemical parameters during or after exposure to lead (Doyle and Younger, 1984). Our earlier report revealed changes in haemato-biochemical parameter and trace mineral profile during exposure to lead (Patra et al., 2001). Also, the biochemical changes during chelation treatment have been reported in lead-exposed rats, and use of EDTA at routine doses and rate of administration was found to be toxic to some animals and unexpected outcome of mortality has also been reported in lead poisoned cows and rabbit (Swartout and Gerken, 1987; Liesegang et al., 1999).

Several vitamins, minerals and amino acids along with chelator have been tried to improve the therapeutic efficacy in management of experimental and natural cases of lead toxicity (Coppock et al., 1991; Olkowski et al., 1991). Treatment with thiamine of lead exposed cattle was more effective than use of $CaNa_2EDTA$ in inducing in remission of clinical signs of poisoning (Coppock et al., 1991). The combined treatment with $CaNa_2EDTA$ and methionine plus zinc was more effective than the chelator alone in decreasing blood and tissue burden of lead and increasing its urinary excretion (Tandon et al., 1994). Recent evidence of oxidative stress and its role, at least in part, in pathotoxicity of lead exposure suggests incorporation of antioxidants to improve therapeutic efficacy of chelator (Ercal et al., 1996; Patra et al., 2000; Chen et al., 2002). α-Tocopherol is a low molecular mass antioxidant that interacts directly with oxidizing radicals, and the status of antioxidant enzymes have been assessed after its supplementation in anoestrus buffaloes (Jones et al., 1995; Kahlon et al., 2003). In the present investigation, $CaNa_2EDTA$ was used alone or along with α Tocopherol, a lipid soluble and chain-breaking antioxidant with the potential to reduce the oxidative stress (Tappel, 1980), in lead-loaded calves to examine its safety with respect to blood trace element profile and haematobiochemical parameters.

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Received May 10, 2004; Accepted December 24, 2004

Table 1. Blood lead, copper, cobalt and zinc concentrations during chelation therapy of lead exposed calves

Parameters	Group	Day 0*	2	4	7	10	SEM
Blood lead	A	0.12^{A}	0.09^{A}	0.08^{A}	0.08^{A}	0.11^{A}	0.03
(μg/ml)	B1	$1.11^{b, B}$	$1.04^{b, B}$	$0.84^{a, B}$	$0.93^{ab, C}$	$0.93^{ab, C}$	0.07
	B2	$1.02^{c, B}$	$1.13^{c, B}$	$0.96^{c, C}$	$0.66^{b, B}$	$0.44^{a, B}$	0.10
	B3	$1.04^{c, B}$	$1.05^{c, B}$	$0.74^{b, B}$	$0.63^{ab, B}$	$0.43^{a, B}$	0.08
Copper	A	0.94	0.90	0.92	0.91	0.90	0.09
(μg/ml)	B1	0.87	0.75	0.72	0.72	0.76	0.05
	B2	0.77	0.76	0.87	0.82	0.83	0.06
	B3	0.80	0.71	0.77	0.67	0.69	0.09
Zinc	A	4.04	3.87	4.23	4.29	4.38	0.20
(μg/ml)	B1	4.88	4.35	4.23	4.29	4.38	0.22
	B2	4.65	4.13	4.24	4.19	4.60	0.27
	B3	4.50	4.08	4.01	4.34	4.35	0.19
Cobalt	A	0.46	0.45^{A}	0.47	0.47	0.4	0.03
(ppb)	B1	0.48	0.47^{A}	0.50	0.53	0.51	0.04
	B2	0.56	0.57^{B}	0.57	0.51	0.52	0.03
	B3	0.50	0.59^{B}	0.54	0.56	0.52	0.03

Calves of group B (15) were given lead treatment at the dose of 7.5 mg/kg body weight daily for 28 days and calves of group A was given no lead-treatment. Lead treated calves of Group B1 were given no treatment. Group B2 received $CaNa_2EDTA$ at the dose rate of 110 mg/kg body weight as 10% solution in 2 divided doses daily for 4 days and Group B3 received chelator as group B2 plus α-tocopherol at the dose rate of 100 mg /kg body weight orally for 7 days.

* Day 0 after lead exposure for a period of 28 days.

Values with different superscripts, capital letters column wise and small letters row-wise vary significantly at 0.05.

MATERIAL AND METHODS

Animals and experimental design

Twenty crossbred calves, fed with only whole milk for first one month and subsequently, with whole milk and calf starter and, green fodder *ad lib*, were used for the experiment. At the age of one month the calves were randomly divided into two groups, A and B of 5 and 15 animals each. Calves of group B were given lead treatment with 99% pure lead acetate at the dose rate of 7.5 mg/kg body weight as 10% solution daily through oral route as a drench for 28 days, where as calves of group A was given no treatment to serve as unexposed controls. Then the lead was withdrawn at the end of 28 days and lead-exposed calves were randomly divided into 3 equal groups, B1, B2 and B3 of 5 calves each for therapeutic trial as follows.

- Group B1: No treatment
- Group B2: $CaNa_2EDTA$ at the dose rate of 110 mg/kg body weight as 10% solution in 2 divided doses daily for 4 days
- Group B3: As above plus α-tocopherol at the dose rate of 100 mg/kg body weight orally in the morning hour for 7 days

Blood samples were collected by jugular venepuncture before the start of the chelation treatment (day 0) and, thereafter, on days 2, 4, 7 and 10 for haematobiochemical analysis. Approximately, 3 ml of blood was collected separately into nitric acid washed vials for wet digestion (Kolmer et al., 1951).

Haematobiochemical parameters

Blood hemoglobin (Hb) level was estimated spectrophotometrically (Van Kampen and Ziglstra, 1961). Samples collected without anticoagulant were used for harvesting serum. Total protein, albumin (Oser, 1965), urea (Netelson, 1961) and creatinine (Tietz, 1987) were estimated in serum samples by colorimetric methods.

Sulfhydryl (-SH) groups : Freshly collected blood samples were centrifuged at 2,000 rpm for 10 min and the supernatant along with buffy coat was discarded. The sedimented erythrocytes were washed with 0.9 percent chilled NaCl solution and the process was repeated three times. Washed erythrocytes were haemolysed with 9-fold volume of distilled water to prepare 10% RBC haemolysate. Total (T-SH), protein bound (P-SH) and non-protein bound (NP-SH) thiol groups in the haemolysate were determined following the method of Sedlak and Lindsay (1968). The molar extinction coefficient of 13, 100 at 412 nm was used to estimate the thiol contents. The protein content of the haemolysate was measured by the method of Lowry et al. (1952) and thiol contents of erythrocytes were expressed in nmol/ mg of protein.

Blood lead and trace mineral concentration

Concentrations of lead, copper, cobalt and zinc in the digested samples were measured using atomic absorption spectrophotometer (Electronic Corporation of India Limited, Hyderabad) at the wavelength of 217, 324.8, 240.7 and 213.9 nm and current of 6, 6, 7 and 7 ma, respectively.

Statistical analysis

Data were analyzed statistically using two-way ANOVA

Table 2. Erythrocytic thiol contents during treatment of lead-exposed calves with chelator alone or along with antioxidant α-tocopherol

Parameters	Group	Day 0*	2	4	7	10	SEM
T-SH	A	35.15^{B}	34.95^{B}	34.96^{B}	34.70	34.88	1.63
(nmol/mg of protein)	B1	31.09^{A}	30.44^{A}	30.91^{A}	32.37	31.50	1.69
	B2	28.52^{A}	28.49^{A}	29.94^{A}	31.31	30.68	0.97
	B3	$30.14^{a, A}$	$30.43^{ab, A}$	$31.40^{ab, A}$	31.89^{ab}	32.93^{b}	0.80
P-SH	A	32.35^{B}	32.11^{B}	32.17	31.89	32.02	1.62
(nmol/mg of protein)	B1	29.00^{A}	28.39^{A}	28.69	30.06	28.94	1.90
	B2	26.13^{A}	26.28^{A}	27.63	28.65	28.15	0.98
	B3	$27.83^{a, A}$	$28.19^{ab, A}$	28.67^{ab}	28.91^{ab}	30.29^{b}	0.82
NP-SH	A	2.81	2.56^{B}	2.73^{B}	2.81^{B}	2.86	0.18
(nmol/mg of protein)	B1	2.09^{ab}	$1.79^{a, A}$	$2.21^{b, A}$	$2.31^{b, B}$	2.55^{b}	0.17
	B2	2.39^{ab}	$2.07^{a, AB}$	$2.31^{a, A}$	$2.65^{b, AB}$	2.54^{ab}	0.11
	B3	2.31^{ab}	$1.84^{a, A}$	$2.73^{b, B}$	$2.97^{c, B}$	2.64^{b}	0.18

Calves of group B (15) were given lead treatment at the dose of 7.5mg/kg body weight daily for 28 days and calves of group A was given no lead-treatment. Lead treated calves of Group B1 were given no treatment. Group B2 received $CaNa_2EDTA$ at the dose rate of 110 mg/kg body weight as 10% solution in 2 divided doses daily for 4 days and Group B3 received chelator as group B2 plus α-tocopherol at the dose rate of 100 mg/kg body weight orally for 7 days.

* Day 0 after lead exposure for a period of 28 days.

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to find out the significance between the means at different periods and of different therapeutic groups (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

In the present investigation, use of $CaNa_2EDTA$ alone or along with antioxidant α-tocopherol in lead treated calves significantly ($p<0.05$) reduced mean blood lead level from the respective day 0 (pretreatment) values (Table 1). The mean blood lead levels on day 10 post treatment in group B2 (0.44±0.13 μg/ml) and B3 (0.43±0.10 μg/ml) were also significantly lower than that of group B1, lead-exposed untreated control (0.93±0.07 μg/ml). In-group B1, blood copper level showed a similar trend of non-significant ($p>0.05$) decline during post lead exposure-observation period of 10 days (Table 1). Treatment with EDTA alone showed a trend in improvement ($p>0.05$) in blood copper level. Incorporation of antioxidant α tocopherol in chelation therapy resulted in non-significant decline its concentration from pre-treatment level by day 7 post treatment, similar to trend recorded for lead-exposed untreated calves. Sharma et al. (2003) emphasized the role of microminerals for production performances in buffaloes. Decreased copper levels in blood, liver and heart following lead ingestion have earlier been reported in bovines (Doyle and Younger, 1984). The present finding in group B3 as compared to that of group B2 might be due to modification in metabolism of copper by addition of antioxidant, α-tocopherol. Mateo and Hoffman (2001) suggested that species and individual variation in sensitivity to lead poisoning among animals might be due to differential resistance to oxidative stress. Ibim et al. (1992) reported increased urinary excretion of copper following treatment with $CaNa_2EDTA$. Zinc supplementation reduced deposition of lead in testes and prevented decrease in δ-Amino levulinic acid dehydrase (ALAD) and superoxide dismutase (SOD) activities. In the present study, there was no significant change in blood zinc and cobalt concentrations from their respective pretreatment (day 0) level following treatment with chelator alone or along with α-tocopherol.

Thiol containing biomolecules are capable of binding toxic metals and thus, neutralize their detrimental effects (Leeming and Donaldson, 1984). Decreased thiol groups in serum have been recorded after oral administration of lead (Vodichenska, 1992). Experimental feeding of lead for 28 days in the present study reduced the erythrocytic T-SH and P-SH level in all the lead exposed groups as compared to respective mean value of lead-unexposed control animals. The major fraction of lead in blood is transported in bound form with erythrocytic membrane. The decreased level of thiol contents in the erythrocytes before the start of treatment might be due to utilization of thiols for chelation of lead. Withdrawal of lead or treatment with $CaNa_2EDTA$ alone or in combination with α-tocopherol led to slow improvement in erythrocytic T-SH and P-SH contents (Table 2). However, the difference between the pretreatment (day 0) and post treatment values (day 10) of T-SH and P-SH did not reach statistical significance ($p>0.05$) in group B2 treated with $CaNa_2EDTA$ alone. Incorporation of antioxidant in chelation therapy in group B3 led to significant (<0.05) increase in erythrocytic T-SH and P-SH concentration by day 10, as compared to respective day 0 values. The decline in free lead after its withdrawal or chelation might be responsible for improvement in thiol contents. However, there is no published literature to compare the above observation.

Lead intoxication has been reported to induce anaemia and alteration in activities of enzymes (Randhawa et al., 1994). Inhibitory effect of lead on heme and globulin

Table 3. Haematobiochemical parameters during treatment of lead exposed calves with chelator alone or along with antioxidant

Parameters	Group	Day 0*	2	4	7	10	SEM
Hb	A	13.94^{B}	13.38^{B}	13.44	13.13	13.36	0.65
(g/dl)	B1	10.63^{A}	10.54^{A}	10.92	10.94	11.02	0.59
	B2	$10.94^{a, A}$	$11.59^{b, A}$	11.73^{b}	13.11^{c}	13.38^{c}	0.39
	B3	$11.68^{ab, A}$	$10.98^{a, A}$	11.46^{ab}	13.01^{b}	12.66^{b}	0.55
Serum urea	A	11.12	11.98	11.28	11.98	13.59	1.47
(mg/dl)	B1	14.88	14.24	13.46	15.10	15.20	1.32
	B2	15.38	16.91	15.13	15.71	14.13	0.88
	B3	15.32	15.79	15.96	15.33	13.49	1.67
Serum creatinine	A	0.96	0.77	0.83	0.78	0.79	0.15
(mg/dl)	B1	0.92	0.83	0.84	0.75	0.88	0.12
	B2	0.90	0.90	0.91	0.77	0.86	0.13
	B3	0.85	0.82	0.80	0.92	0.90	0.12
Total protein	A	7.03	6.70	6.77	6.74	6.95	0.41
(g/dl)	B1	6.60	6.50	6.31	6.07	6.17	0.30
	B2	6.22	6.03	6.19	6.26	6.54	0.24
	B3	6.09	6.19	6.21	6.12	6.13	0.26
Albumin	A	2.41	2.55	2.58	2.66	2.65	0.10
(g/dl)	B1	2.86	2.69	2.75	2.76	2.69	0.19
	B2	2.60	2.61	2.55	2.46	2.49	0.11
	B3	2.42	2.50	2.44	2.51	2.42	0.10

Calves of group B (15) were given lead treatment at the dose of 7.5 mg/kg body weight daily for 28 days and calves of group A was given no lead-treatment. Lead treated calves of Group B1 were given no treatment. Group B2 received $CaNa_2EDTA$ at the dose rate of 110 mg/kg body weight as 10% solution in 2 divided doses daily for 4 days and Group B3 received chelator as group B2 plus α- tocopherol at the dose rate of 100 mg/kg body weight orally for 7 days.

* Day 0 after lead exposure for a period of 28 days.

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synthesis has been attributed for reduced haemoglobin (Hb) level in lead exposed animals. In the present investigation, use of chelator significantly improved the haemoglobin by 122% ($p<0.05$) and 108% ($p>0.05$) in group B2 and B3, respectively as compared to day 0 value (Post lead exposure-pretreatment value) (Table 3), which might be due to binding of lead to chelator thereby reducing the effect of lead on synthesis of Hb. Use of $CaNa_2EDTA$ in the present dose rate did not have significant effect on serum urea, creatinine, total protein and albumin level as compared to respective pretreatment (day 0) value (Table 3). The non significant changes in these parameters indicated that use of chelator in lead exposed calves at the present dose rate might not have toxic effects on renal function, particularly on glomerular filtration rate (GFR). It is concluded from the present investigation that use of $CaNa_2EDTA$ at the dose rate of 110 mg/kg body weight given in two divided doses alone or in combination with α-tocopherol did not adversely affect the blood trace elements and haematobiochemical parameters in lead loaded calves, and may be considered safe for management of lead toxicity.

REFERENCES

Chen, L., X. Q. Yang, B. H. Jiao and L. J. Zhao. 2002. Tea catechins protect against lead-induced cytotoxicity, lipid peroxidation and membrane fluidity in HepG2 cells. Toxicol. Sci. 69:149-157.

Coppock, R. W., W. C. Wagner, J. D. Reynolds, R. S. Vogel, S. V. Gelberg and L. Z. Florence. 1991. Evaluation of edetate and thiamine for treatment of experimentally induced environmental lead poisoning in cattle. Am. J. Vet. Res. 52:1860-1865.

Doyle, J. J. and R. L. Younger. 1984. Influence of ingested lead on distribution of lead, iron, zinc, copper and manganese in bovine tissue. Vet. Human Toxicol. 26:201-204.

Ercal, N., P. Treratphan, T. C. Hammond, R. H. Mathews, N. H. Grannemann and D. R. Spitz. 1996. *In vivo* indices of oxidative stress in lead-exposed C57BL/6 mice are reduced by treatment with meso-2, 3-dimercapto succinic acid or N-acetyl cysteine. Free Rad. Biol. Med. 21:157-161.

Ibim, S. E. M., J. Trotman, P. I. Musey and W. E. P. Semafuko. 1992. Depletion of essential elements by calcium disodium EDTA treatment in the dog. Toxicol. 73:229-237.

Jones, D. P., V. E. Kagan, S. D. Aust, D. J. Reed and S. T. Omaye. 1995. Impact of nutrients on cellular lipid peroxidation and antioxidant defense system. Fundam. Appl. Toxicol. 26:1-7.

Kahlon, R. S., Rajiv Singh and R. Singh. 2003. Status of antioxidant enzymes in normal cycling and α-tocopherol supplemented anestrous buffalo heifers (*Bubalus bubalis*). Asian-Aust. J. Anim. Sci. 16:217-221.

Klaassen, C. D. 1985. Heavy metals and heavy metal antagonist. J. Am. Vet. Med. Assoc. 162:781-786.

Kolmer, J. A., E. H. Spaudlding and H. W. Robinson. 1951. Approved laboratory techniques. Appletion Century Crafts. New York, 1090-1091.

Leeming, T. K. and W. E. Donaldson. 1984. Effect of dietary methionine and lysine on toxicity of ingested lead acetate in

chick. J. Nutr. 114:2155-2159.

Liesegang, A., J. L. Riond and M. Wanner. 1999. Unexpected outcome after two consecutive infusion of disodium EDTA in dairy cows. J. Vet. Med. 46:149-154.

Lowry, O. H., N. J. Rosenbrough, A. L. Farr and R. J. Randall. 1952. Protein measurement with Folin Phenol reagent. J. Biol. Chem. 193:265-275.

Mateo, R. and D. J. Hoffman. 2001. Difference in oxidative stress between young Canada geese and mallards exposed to lead contaminated sediments. J. Toxicol. Environ. Heal. 64:531-545.

Netelson, S. 1961. Microtechniques for Clinical Chemistry. Ed. Thomas C. C., Springfield, Illionois, p. 434.

Olkowski, A. A., S. R. Gooneratne and B. A. Christensen. 1991. The effect of thiamine and EDTA on biliary and urinary lead excretion in sheep. Toxicol. Letters 59:153-159.

Oser, B. L. 1965. Hawk's Physiological Chemistry. 14th Edition, New Delhi, Mc Graw Hill.

Patra, R. C., D. Swarup and S. K. Dwivedi. 2000. Antioxidant defense and lipid peroxide level in liver and kidneys of lead exposed rats. Asian-Aust. J. Anim. Sci 13:1433-1439.

Patra, R. C., D. Swarup, S. K. Dwivedi and A. Sahoo. 2001. Trace minerals in blood of young calves during exposure to lead. Indian J. Anim. Sci. 71:507-510.

Piomelli, S., J. F. Rosen, J. J. Chisolm and J. W. Graef. 1984. Management of childhood lead poisoning. J. Pediatr. 105:523-528.

Radostits, O. M., C. C. Gay, D. C. Blood and K. W. Hinchcliff. 2000. Veterinary Medicine 9th edn. W.B. Saunders Company Ltd., London, pp. 1477-1566.

Randhawa, S. S., G. Singh, R. S. Brar, D. C. Nauriyal, Gurdeep and F. Trenti. 1994. Comparative evaluation of therapeutic trial in experimentally induced lead toxicosis in crossbred calves. Proceedings 18th World Buiatrics Congress, Bologna, Italy 2:1377-1380.

Sedlak, J. and R. H. Lindsay. 1968. Estimation of total, protein bound and non protein bound sulfahydryl groups in tissues with Ellman's reagent. Anal. Biochem. 25:192-205.

Sharma, M. C., S. Raju, C. Joshi, H. Kaur and V. P. Varshney. 2003. Studies on serum micro-mineral, hormone and vitamin profile and its effct on production and therapeutic management of buffaloes in Haryana State of India. Asian-Aust. J. Anim. Sci. 16:541-548.

Snedecor, G. W. and W. G. Cochran. 1967. Statistical Methods 6th Edition, Oxford and IBH. New Delhi. pp. 258-268.

Swartout, M. S. and D. F. Gerken. 1987. Lead induced toxicosis in two domestic rabbits. J. Am. Vet. Med. Assoc. 191:717-719.

Tandon, S. K., S. Singh and S. J. S. Flora. 1994. Influence of methionine and zinc supplementation during chelation of lead in rats. J. Trace Elem. Electrolytes Health Disease 8:75-77.

Tappel, A. L. 1980. Vit E and selenium production from *in vivo* lipid peroxidation. Annals New York Acad. Sci. 355:18-31.

Tietz, N. W. 1987. Fundamental of Clinical Chemistry. W.B. Saunders Company, Philadelphia. p. 683.

Van Kampen, E. J. and W. G. Ziglstra. 1961. Calorimetric determination of hemoglobin. Clin. Chem. Acta. 6:5388.

Vodichenska, T. S. 1992. The effect of chronic combined exposure to nickel and lead on the enzymatic indices in body uptake with the drinking water. Problknig 17:48-56.