AFRICAN JOURNAL OF MEDICINE and medical sciences

VOLUME 41 Second Biomedical Conference Supplement DECEMBER 2012

ISSN 1116-4077

Afr. J Med. med. Sci. (2012) 41, *Suppl.* 91-97

Human erythrocyte membrane Ca^{2+}-ATPase during occupational exposure to lead

JU Imah-Harry[1], MO Akiibinu[2], IO Omotosho[3] and OO Olorunsogo[1]

Laboratories for Biomembrane Research and Biotechnology, Department of Biochemistry[1], College of Medicine, University of Ibadan, Ibadan, Department of Biochemistry[2], Caleb University, Lagos, and Department of Chemical Pathology[1], College of Medicine, University of Ibadan, Nigeria

Abstract

Objective: Erythrocyte membrane Ca^{2+}- ATPase activity was determined in workers occupationally exposed to lead because of the prevalence of elevated blood lead in auto-mobile workers in some urban areas in Nigeria.

Materials and methods: Blood lead levels, biochemical profiles, lipid peroxidation, basal and calmodulin-stimulated Ca^{2+}-ATPase activities were determined in erythrocytes of different categories of workers occupationally exposed to lead. These subjects were mainly battery chargers (BC), spray painters (SP) and auto mechanics (MC).

Results: Estimation of erythrocyte Ca^{2+}-ATPase activity in the absence of calmodulin (basal activity) in test groups indicated that there were significant reductions in the pump function and this correlated very well with the levels of lead in their blood. Specifically, blood lead levels were of the order: BC (5.5 folds) > SP (4 folds) > MC, although there was no significant difference between the blood lead levels in MC ($10.60 \pm 2.55 \mu g Pb^{2+}/dl$) and CT ($8.51 \pm 4.55 \mu g Pb^{2+}/dl$). Similarly, the order of reduction in Ca^{2+}-ATPase activity was BC (69.8%)>SP (52.8%)>MC (32.6%). There was significant difference in the values obtained for MC and CT, ATPase activity being lower in MC compared to CT or healthy individuals. In the presence of calmodulin, basal ATPase activity was increased by at least four fold in erythrocytes from healthy subjects (CT) while the basal activity of the enzymes in membranes of BC, SP and MC was enhanced by about one and half times the activity of the pump in membranes from CT. The levels of total serum protein and albumin increased significantly in BC, SP and MC when compared to healthy subjects (CT). C-reactive proteins (C-RP) levels were higher in BC, SP and MC in comparison to CT. The levels of MDA were high in all lead-exposed workers, BC >SP >MC relative to CT. Although, there were significant decreases in the PCV values of all the groups occupationally exposed to lead compared to values obtained for CT, cholesterol level increased significantly only in BC when compared to the other groups.

Conclusion: These observations are probably due to the integrity of the plasma membrane of these workers and the ability of the heavy metal to compete with Ca^{2+} in the catalytic cycle and Ca^{2+} transport mechanism of the pump protein.

Keywords: *Calmodulin, lead intoxicatin, erythrocyte ghost membranes, Ca^{2+}-ATPase*

Résumé

Objectif: L'activité de la membrane érythrocytaire Ca^{2+}- ATPase a été observée chez des travailleurs dans l'exercice de leur profession en raison de la prévalence du niveau élevée de plomb dans le sang dans la mécanique automobile dans certains milieux urbains au Nigeria.

Matériel et méthodes: Les niveaux sanguins de plomb, les profils biochimiques, la peroxydation lipidique, les activités basales et stimulées par la calmoduline Ca^{2+}-ATPase ont été examinées dans les érythrocytes de différentes catégories de travailleurs professionnellement exposés au plomb. Ces patients étaient principalement des chargeurs de batterie (CB), des peintres au pistolet (PP) et les mécaniciens d'auto (MC).

Résultats: L'estimation de l'activité de l'érythrocyte Ca^{2+}-ATPase en l'absence de calmoduline (activité basale) dans les groupes d'essai indique qu'il y avait des réductions significatives de la fonction de pompe et celle-ci est bien en corrélation avec les niveaux de plomb dans le sang. Plus précisément, les niveaux de plomb dans le sang était selon l'ordre suivant: chez les CB (5,5 fois)> PP (4 fois)> MC, bien qu'il n'y avait pas de différence significative entre les niveaux de plomb dans le sang chez les MC ($10,60 \pm 2,55$ìgPb/dl) et CT ($8,51 \pm 4.55$ìgPb/dl). De même, l'ordre de

Correspondence: Prof. O.O. Olorunsogo, Laboratories for Membrane Research and Biotechnology, Department of Biochemstry, College of Medicine, University of Ibadan, Ibadan, Nigeria. E-mail: funsoolorunsogo@yahoo.com or oo.olorunsogo@mail.ui.edu.ng

réduction de l'activité Ca^{2+}-ATPase était CB (69,8%)> PP (52,8%)> MC (32,6%). Il n'y avait pas de différence significative dans les valeurs obtenues pour MC et CT, l'activité ATPase étant inférieure chez les MC par rapport au CT ou aux individus en bonne santé. En présence de la calmoduline, l'activité ATPase de base a été augmentée d'au moins quatre fois dans les érythrocytes de ceux qui sont en bonne santé (CT), tandis que l'activité basale des enzymes dans les membranes des CB, PP et MC a été améliorée d'environ une fois et demie de l'activité de la pompe à membranes des CT. Les niveaux de sérum de protéines totales et de l'albumine ont augmenté de manière significative chez les CB, PP et MC par rapport à ceux en bonne santé (CT). Les protéines C-réactives (C-RP) étaient plus élevées chez les CB, PP et MC par rapport au CT. Les taux de MDA étaient élevés chez l'ensemble des travailleurs exposés au plomb, CB> PP> MT par rapport à CT. Malgré qu'il ya eu des diminutions significatives dans les valeurs de l'hématocrite de tous les groupes professionnellement exposés au plomb par rapport aux valeurs obtenues pour les CT ; le taux de cholestérol a augmenté de façon significative seulement chez les CB par rapport aux autres groupes. *Conclusion*: Ces observations sont probablement dues à l'intégrité de la membrane plasmique de ces travailleurs et de la capacité du métal lourd qui oppose le Ca^{2+} dans le cycle catalytique et le mécanisme du transport de Ca^{2+} de la protéine de la pompe.

Introduction

Lead (Pb^{2+}) is a pervasive and common environmental toxicant which causes serious occupational diseases that have globally become significant and a major public health challenge. Whereas the metal has no known physiological function, its deleterious and toxic potentials constitute a continuum from clinical or overt defect to highly complicated lesions involving biochemical, physiological and behavioral dysfunctions [1]. There is incontrovertible evidence that its toxicity is multifaceted involving a number of biochemical processes including covalent binding to proteins [2], oxidative damage by inducing generation of reactive oxygen species and by reducing cellular antioxidant defense mechanism [3-6] and competition with calcium for stereospecific sites for divalent cations [7]. Its interaction with specific sites to which calcium (Ca^{2+}) binds, appears to be responsible for several biologically important calcium dependent enzymatic and non-enzymatic processes ranging from ion transport and energy metabolism to apoptosis, cell adhesion, biosignalling, protein maturation and genetic regulation [7].

Lead potentially induces oxidative stress and accumulated evidence support the role of oxidative stress in the pathophysiology of lead toxicity [8,9]. Several epidemiological studies have reported that even at low levels, lead has a graded association with several ill health conditions including renal and cognitive impairment [10-12]. Recent studies also report positive association between lead exposure and oxidative stress markers [13,14]. Although both moderate and subclinical effects of occupational lead poisoning are common in many countries of the world, occupational exposure is entirely unregulated in developing countries like Nigeria, India, etc and a little monitoring has been conducted in developed countries [15].

Incidentally, the cation has been shown to be localized in the erythrocytes of individuals who are chronically exposed to the toxicant and its concentration is about ninety times higher than in plasma [16] thus making the erythrocyte the vehicle for spreading the cation to different parts of the human body [17]. In this regard, the effect of lead on erythrocyte calcium homeostasis has been investigated. Specifically, it has been shown that the calcium channel present on the erythrocyte membrane possesses a very high permeability to Pb^{2+} with approximately ten times that of Ca^{2+} [18] and thus indicating that lead diminishes calcium influx by competing at the calcium binding site on the calcium channel. Previously, Mas-Olivia [19] demonstrated that lead inhibits the basal activity of erythrocyte membrane calcium ATPase *in vitro* and that the cation has a direct action on calmodulin. Quintanar-Escorza et al [20] recently reported the effect of lead intoxication on the uptake and the influx of calcium in erythrocyte of lead-exposed workers of a recycled automobile battery factory and concluded that enhanced intracellular free calcium concentration was associated with an increase in the influx of calcium and an inhibition of the calcium ATPase activity. These effects were accompanied by oxidative damage and changes on the erythrocyte shape and fragility. There is however, no report in the literature on the influence of lead on the erythrocyte calcium homeostasis in individuals whose exposure to lead is not as chronic or intense as battery or accumulator production workers.

This paper therefore assessed the status of the erythrocyte calcium ATPase in individuals who were not heavily occupationally exposed to lead as a means of gauging the toxicity effect of the heavy metal in professionals who otherwise would be ignored when considering chronic lead intoxication. This has become imperative against the backdrop of the prevalence of elevated blood lead levels in automobile workers in some urban areas of Nigeria [21].

Materials

Subjects

Forty male workers between the ages 25 and 55 years occupationally exposed to lead for an average of 30 years were used for this study. These subjects were battery chargers, automobile mechanics, and motor spray painters, working in various automobile workshops in Ibadan, Nigeria. The control group was made up of clinically healthy volunteers who were not occupationally exposed to lead. As a rule, subjects were not on any medication at the time of the study and had no previous history of serious cardiovascular, renal, hepatic, endocrine, metabolic or gastrointestinal disease. Ethical approval was obtained from the UCH/UI Ethical Committee on human research.

Reagents

ATP (disodium salt vanadium-free), HEPES{4 (2-hydroxyethyl)-1-piperazine-ethanesulphonic acid}, fatty acid-free bovine serum albumin, ethyleneglycerol-bis-{aminoethylether} N,N,N-tetraacetic acid (EGTA), phenylmethylsulphonylflouride, and bovine brain calmodulin were purchased from Sigma chemical Co. (St. Louis, MO). All reagents were of the highest purity grade available. Lead nitrate stock standard solution {1mg/ml} was obtained from British Drug Houses {BDH} Chemical Co, UK.

Methods

Determination of blood lead levels.

Blood was collected by venous puncture in heparinized tubes and kept at 4°C until used. Blood samples were spun at 6000rpm for 10mins in a refrigerated centrifuge. The plasma and white cells were carefully removed by aspiration. Lead concentration was determined in plasma and lysed red cells according the method of Zinterhofer *et al* [22] using a Perkin-Elmer model 2380 Atomic Absorption spectrometer. The packed cell volume (PCV) of blood samples was determined by the method of Pearson *et al* [23].

Preparation of calmodulin-deficient erythrocyte ghost membranes.

Haemoglobin-free and calmodulin-deficient membranes were prepared by the procedure of Niggli *et al* [24] using the principle of hypotonic lysis developed by Dodge *et al* [25] and as previously reported by Olorunsogo *et al* [26]. Whole blood samples collected in acid-citrate dextrose buffer were spun at 5800g for 10mins at 4°C in an MSE angle 13 refrigerated centrifuge. The plasma and buffy layers were removed by aspiration to obtain packed erythrocytes which were washed twice in 5vols of ice-cold 130mM KCl, 20mM Tris-HCl (pH 7.4) by centrifuging the cell suspension at 5800g for 10mins. The washed cells were haemolysed in 5 volumes of ice-cold 1mM EGTA, 10mM Hepes (pH 7.4) and spun at 20,000g for 30mins. This step was repeated at least six times to ensure complete haemolysis and removal of haemoglobin and endogenous calmodulin. The membranes were finally resuspended in ice-cold 130mM KCl, 20mM Hepes (pH 7.4), 500μM $MgCl_2$, 50μM $CaCl_2$ and stored at -80°C following washing for at least eight times in 10mM Hepes (pH 7.4).

Determination of Ca^{2+}- ATPase activity.

Ca^{2+}-ATPase activity was determined by following the rate of liberation of inorganic phosphate from á - position of ATP, as previously described by Olorunsogo *et al* [26]. Reaction medium contained in final concentrations: 120mM KCl, 50mM Hepes (pH 7.4), 5 mM $MgCl_2$, 2 mM $CaCl_2$, 5mM EDTA and 50-100μg membrane protein in a total volume of 900μl. The reaction was started by the addition of 2mM ATP. The assay was run in duplicate with or without calmodulin (120nM). At the end of 30 min, 10% sodium dodecylsulphate was used to terminate the reaction. The inorganic phosphate librated was estimated spectrophotometrically. Ca^{2+}-ATPase activity was obtained by subtracting Mg^{2+}-ATPase activity from total activity in the presence of calcium.

Protein estimation

Protein concentration of erythrocyte membrane preparations was determined essentially according to Lowry *et al* [27]. Membrane fractions were precipitated with 10% $^{w}/_{v}$ trichloroacetic acid following treatment with 0.05% $^{w}/_{v}$ deoxycholic acid. Bovine serum albumin was used as standard. Total plasma protein was estimated using the procedure described by Gornal *et al* [28]. Plasma albumin level was determined according to the method of Webster [29]. C-reactive protein was determined according to the procedure of Surekha *et al* [30]. Plasma cholesterol level was determined using the enzymatic CHOD-PAP method (DIALAB KIT), Richmond [31].

Assessment of lipid peroxidation

Lipid peroxidation was determined by measuring the thiobarbituric acid-reactive substances (TBARS) produced during lipid peroxidation. This was done using the procedure of Vashney and Kale [32] and expressed as nanomolar (nM) of malondialdehyde per milliliter of plasma.

Statistics

Results were analyzed using students t-test and ANOVA. The Pearson correlation, test was used for the determination of the degree of significance and to establish a dose-response relationship for the controls and test.

All data were expressed as mean ± standard deviation. Probability ($P<0.05$) was considered significant.

Table 1: Blood lead levels of lead exposed workers.

Study Groups	Pb^{2+}(μg/dl)	Age (yrs)	Exposure (yrs)
BC	51.50±18.84*	46.30±10.31	23.35±8.39
SP	35.80±10.13*	35.90±5.24	17.60±7.89
MC	10.60±2.55	45.20±9.91	18.60±5.94
CT	8.51±4.55	38.20±4.48	0
F_1, P_a Values	43.16, 0.00*	5.26, 0.00*	3.25, 0.08

* $P < 0.05$, 95% confidence interval. Each value is a mean of several determinations (e" 30) ± SD.

Results

Table 1 shows blood levels of lead in battery chargers (BC), spray painters (SP), auto mechanics (MC) and healthy subjects with no history of occupational exposure to lead. Blood lead concentrations were 5.5 times and 4 times higher in BC (51.50 ± 18.84μg Pb^{2+}/dl) and SP (35.80 ± 10.13 μg Pb^{2+}/dl), respectively as compared to the control subjects (CT) (8.51 ± 4.55μg Pb^{2+}/dl). There was no significant difference between the values obtained for MC (10.60 ± 2.55μg Pb^{2+}/dl) and control subjects (CT). The relatively low concentration of blood lead in healthy volunteers (CT) indicates that the level of lead in the environment in Ibadan municipality is safe. In Table 2, both the levels of total serum protein and albumin significantly increased in all groups occupationally exposed to lead (i.e. BC, SP, and MC) when compared to the healthy individuals. In this regard, maximum values were seen in BC where total protein, albumin, cholesterol, and C-reactive protein levels were elevated by 40.3%, 22.3%, 20% and 43.2% respectively, as compared to the values obtained for healthy volunteers (CT). The order of increases in these parameters in workers occupationally exposed to lead was BC > SP > MC although there was no significant difference in the values obtained for cholesterol levels in SP, MC and CT. On the contrary, the PCV values were significantly lower in all workers occupationally exposed to lead by at least 14% than the values obtained for healthy volunteers (CT). The levels of lipid peroxidation products increased with increasing levels of blood lead (Table 2 and Figure 1). The highest value was observed in BC (9.27 ± 0.75 nM/ml) with a corresponding percentage induction of lipid peroxidation of 81.4%, as compared to healthy volunteers CT. The values obtained for SP and MC were also significantly higher than in normal healthy individuals. These results indicate that the

Table 2: Protein profile, packed cell volume (PCV) and lipid peroxidation products in lead exposed workers.

Study Groups	Total protein (g/dL)	Albumin (mg/dl)	Cholesterol (mg/dl)	C-recative proteins (mg/L)	PCV (%)	MDA (nM/ml)
BC	10.51±88*	5.27 ± 0.78*	192.68±26.41*	16.33±10.13*	40.00±3.31*	9.27± 0.75*
SP	10.04±0.57*	5.40±0.45*	170.00±7.00	11.30±5.80*	40.40±3.71*	8.44 ± 1.20*
MC	10.31±0.79*	5.66±0.55*	163.91±14.18	6.00±2.67*	38.80±3.55*	7.33 ± 0.47*
CT	7.49±0.58	4.29±0.69	160.61±8.00	3.07±1.53	47.00±3.57	5.11 ± 0.83
t^a,P^a value	11.41, 0.00	3.81, 0.00	4.52, 0.00	5.01, 0.00	1.06, 0.30	9.03, 0.00
t^b, P^b value	1.53, 0.14	-0.47, 0.65	2.65, 0.01	1.48, 0.16	-5.64, 0.00	3.44, 0.02
t^c P^c value	0.60, 0.55	-1.43, 0.16	3.18, 0.00	3.14, 0.00	-5.84, 0.00	1.97, 0.04

Each value is a mean of several determinations (e" 30) ± SD. *The mean difference is significant at ($P<0.05$) and equal variances assumed. a - BC compared with the controls, b - SP compared with the controls, c - MC compared with the controls

levels of lipid peroxidation products correlate positively with blood lead concentration and this may have adverse effect on the integrity of the red cell membrane. In Table 3, we present the values obtained from estimation of activity of the membrane Ca^{2+}-

Table 3: Ca^{2+} ATPase activity of human erythrocyte ghost membranes of lead exposed workers.

Study groups	ATPase Activity (μmol Pi released/mg prot./ hr) Mg^{2+} - ATPase	Ca^{2+} - ATPase – Calmodulin	Ca^{2+} - ATPase +Calmodulin
BC	0.20 ± 0.06*	0.27±0.02*	0.67 ± 0.08*
SP	0:24 ± 0.09*	0.42±0.03*	1.04 ± 0.12*
MC	0.27 ± 0.06	0.60±0.02*	1.20 ± 0.17*
CT	0.34 ±0.06	0.89±0.03	3.67 ± 0.28
ta, Pa	-	1.58, 0.00	1.30, 0.01
t^b, P^b [a]	–	1.27, 0.02	1.16 , 0.03
t^c, P^c	–	2.81, 0.00	1.47 ± 0.02

*Each value is a mean of several determinations (e"10) ± SD. *The mean difference is significant at (P<0.05) and equal variances assumed. a – BC compared with the controls (a & d), b – SP compared with the controls (b & d), c - MC compared with the controls (c & d).*

basal activity was stimulated four times in erythrocytes from healthy volunteers while the pump action was enhanced by only about one and a half times in BC, SP and MC as compared to the enzyme activity in CT. Surprisingly, the calmodulin-stimulated activity in BC was still lower than the basal activity of healthy volunteers. The results show furthermore that Mg^{2+}-ATPase activity of the erythrocyte ghost membrane of BC and SP was significantly lower than in healthy volunteers (CT).

Discussion

Previous reports have shown clearly that aside from being a cell-target model for cellular lead toxicity in non-excitable cells, erythrocytes are the most important means of transporting lead throughout the human body [17]. It has been reported that lead and calcium use the same transport systems in erythrocytes [17, 18]. More recently, Quintanar-Escorza *et al* [20] reported that the enhanced intracellular free calcium concentration in lead exposed workers was caused by a significant reduction up to 50% in the activity of the Ca^{2+}-Mg^{2+}-ATPase activity in the absence of calmodulin.

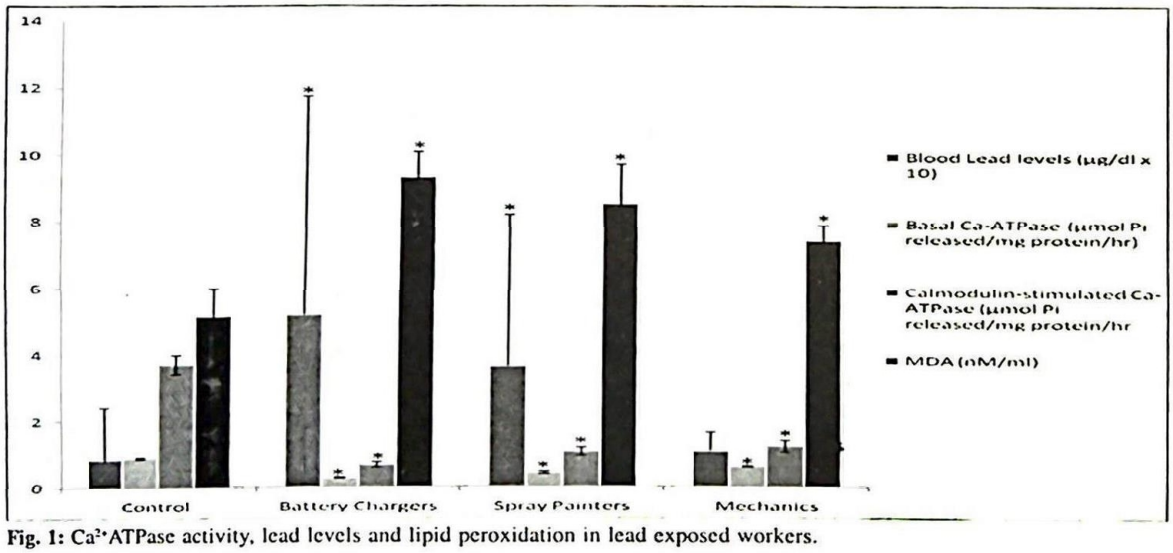

Fig. 1: Ca^{2+}ATPase activity, lead levels and lipid peroxidation in lead exposed workers.

ATPase in all categories of workers exposed to lead and in normal subjects in the absence and presence of calmodulin. Specifically, basal activity of the enzyme was significantly reduced in all subjects occupationally exposed to lead when compared to the pump activity in healthy volunteers (CT). The order of reduction of activity was BC (69.8%) > SP (52.8%) > MC (32.8%). In the presence of calmodulin, the

In this study, our results confirmed that lead exposed workers have significantly higher levels of lead in their blood in comparison with values obtained for healthy individuals. The results show further that there is a positive correlation between the blood lead levels and the basal activity of the erythrocyte Ca^{2+}-ATPase which was least in BC who has the highest concentration of blood lead. The inhibition of the pump

activity in lead exposed workers may be directly due to the interaction of the heavy metal with the sulfhydryl groups of the pump protein as previously suggested by 'in vitro' studies conducted by Mas-Oliva [19] or with the calcium binding sites of the pump.

Although results of previous *in vitro* studies have suggested the possibility of the formation of a lead-calmodulin complex that optimally stimulates the enzyme at lead concentrations up to 10μM, addition of calmodulin to erythrocyte ghost membranes of lead-exposed workers revealed that the basal activity of the pump was stimulable by about one-half times. These observations suggest that although lead may have modified the basal activity of the ATPase, the pump could still be stimulated by calmodulin. However, this effect of calmodulin only makes the enzyme slightly more effective than the pump in healthy volunteers in the absence of calmodulin; thus suggesting that the high intracellular free calcium concentration associated with the erythrocytes of lead-exposed workers is due to the inability of the calmodulin stimulated pump to effectively transport calcium out of the cytosol.

Moreover, the inhibition of the pump action could arise from the presence of lipid peroxidation products caused by lead-induced peroxidation of the erythrocyte membrane. Peroxidation products have been shown to inhibit Ca^{2+}- Mg^{2+}-ATPase activity [33,34]. The finding that the concentration of lipid peroxidation products increased with increase in blood lead levels suggest that lipid peroxidation was lead induced. Also, the observation that the extent of reduction of Ca^{2+}-ATPase activity increased with increasing concentration of lipid peroxidation products indicate that peroxidation of the erythrocyte membrane has a direct effect on the ATPase action as both oxidative damage to plasma membrane and interaction of lipid peroxidation products could adversely affect ATPase activity. This explanation is also applicable to the reduced PCV values seen in lead-exposed workers. The high levels of total serum proteins and C-reactive proteins in these artisans could be due to the secretion of some abnormal proteins or presence of some inflammatory proteins in their blood. This observation may be exploited in respect of early diagnosis of lead intoxication, as values for these parameters increased with increasing blood lead levels.

In conclusion, significant reduction in basal and calmodulin–stimulated calcium pump function during lead intoxication would cause increased intracellular free Ca^{2+} concentration and this would elicit cellular calcium toxicity in addition to the toxic effects of Pb^{2+} in workers occupationally exposed to lead. Indeed, estimation of erythrocyte membrane Ca^{2+}-ATPase activity in the absence and presence of calmodulin may provide insights into the level of cellular damage in lead intoxication.

Acknowledgements

We acknowledge the contribution of Messrs Ayo Famooto (for sample collection), T. Oyewumi (for technical assistance) and Kunle Oladimeji (for typing the manuscript). This work was supported in part by University of Ibadan Senate Research Grant. (SRG/COM/2000/23) to O.O.O

References

1. Calderon-Salinas JV, Hernadez-Luna C, Valdez-Anaya B, Maldonado-Vega M, and Lopez-Miranda A. Evolution of lead toxicity in a population of children. Hum. Exp. Toxicol. 1996; 15: 376-382.
2. Goering PL. Lead protein interactions as basis of lead toxicity. Neurotoxicology. 1993; 14: 45-60.
3. Gurer-Ohrnan H, Sabir HU and Ozegunes H. Correlation between clinical indicators of lead poisoning and oxidative stress parameters in controls and lead-exposed workers. Toxicology. 2004: 147-154.
4. Gurer H and Ercal N. Can antioxidants be beneficial in the treatment of lead poisoning? Free Radic Biol Med. 2000; 29: 927-945.
5. Hsu PC, Liu MY, Hsu CC, Chen LY, and Guo YL. Effects of Vitamin E and/or C on reactive oxygen species related lead toxicity in rat sperm. Toxicology. 1998; 128: 169-179.
6. Osterode W, and Ulberth F. Increased concentration of arachidonic acid in erythrocyte membranes in chronically lead-exposed men. J Toxicol Environ Health. 2000; 59: 87-95.
7. Garza A, Vega R, and Soto E. Cellular mechanisms of lead neurotoxicity. Med. Sci. Monit. 2006; 12: 57-65.
8. Ercal N, Neal R, Treeratphan P, Lutz PM, Hammond TC, Dennery PA and Spitz DR. A role for oxidative stress in suppressing serum immunoglobulin levels in Lead exposed Fisher 344 rats. Arc Environ Contam Toxicol. 2000; 39: 251-256.
9. Farmand F, Ehdaie A, Roberts CK, and Sindhu RK. Lead-induced dysregulation of superioxide dimtase, catalae, guthathion perodide and guanylate cyclase. Environ Res. 2005; 98: 33-9.
10. Ekong EB, Jaar BG, and Weaver VM. Lead-related nephrotoxicity: A review of epidemiologic evidence. Kidney int. 2006; 70: 2074-2084.
11. Lee DH, Lim JS, Song K, Boo Y, and Jacobs JR. Graded association of blood lead and urinary

cadmium concentrations with oxidative stress related markers in the US population: results from the third national health and nutrition examination survey. Environ Health Perspect. 2006; 14: 350 –354.

12. Menke A, Muntner P, Batuman P, Silbergeld EK, and Guallar E. Blood lead below 0.4 umol/l (100ugl-1) and mortality among US adults. Circulation 2006; 114:1388–1394.
13. Muntener P, He J, Vipputuri S, Coresh J, and Batuman V. Blood Lead and Chronic Kidney disease in the general United States population: results from NHANES III. Kidney Int. 2003; 63: 1044-1050.
14. Nash D, Magder L, Lustberg M, Sherwin RW, Rubin RJ, and Kaufmann RB. Blooc. Lead, blood pressure and hypertension in perimenopausal and post menopausal women. JAMA 2003; 289: 1523-1532.
15. Verrula GR and Noah PK. Clinical manifestations of childhood lead poisoning. Trop Med. Hyg. 1990; 93: 170-177.
16 Cake KM, Bowins RJ, Vaillancourt C, Gordon CL, McNutt RH, Laporte R, Webber CE, and Chettle DR. Partition of circulating lead between serum and red cells is different for internal and external sources of lead. Am. J. Ind. Med. 1996; 29: 440-445.
17. Calderon-Salinas JV, Quintanar-Escorza MA, Hernadez-Luna CE, and Gonzalez-Martinez MT. Effect of lead on the calcium transport in human erythrocyte. Hum.Exp. Toxicol. 1999a; 18: 146-153.
18. Calderon-Salinas JV, Quintanar-Escorza MA, Gonzalez-Martinez MT, and Hernadez-Luna CE. Lead and calcium transport in human erythrocyte. Hum.Exp. Toxicol. 1999b; 18: 327-332.
19. Mas-Oliva J. Effect of lead on the erythrocyte ($Ca^{2+}Mg^{2+}$)-ATPase activity. Calmodulin involvement. Mol Cell. Biochem. 1989; 89: 87-93.
20. Quintanar-Escorza MA, Gonzalez-Martinez MT, Navarro L, Maldonado M, Arevalo B, and Calderon-Salinas JV. Intracellular free calcium concentration and calcium transport in human erythrocytes of lead-exposed workers Toxicol. App. Pharm. 2006; 220: 1-8.
21. Onwukor B, Dosunmu O, Odukoya OO, Arowolo T, and Ademuyiwa O. Biomarkers of lead exposure in petrol station attendants and auto-mechanics in Abeokuta, Nigeria: effect of 2-week ascorbic acid supplementation. Environ Toxicol Pharmacol. 2004; 17: 169-176.
22. Zinterhofer LM, Jotolow PT, and Fappiano A. Atomic absorption determination of lead in blood and urine in the presence of EDTA. J Lab Clin Med. 1971; 78: 664-674.
23. Pearson TC, Guthrine DL, Slater NGP, and Wethealey-Man G. Method of PCV measurement and the effect of IRON deficiency on whole blood viscosity in polycythaemia. British Journal of Haematology. 1982; 52 (1): 166-169.
24. Niggli V, Adunyah ES, Penniston JT, and Carafoli E . ATPase of the erythrocyte membrane. Reconstitution and effect of calmodulin and phospholipids. J. Boil. Chem. 1981; 256: 395-401.
25. Dodge JT, Mitchell C, and Hanahan DJ. The preparation and chemical characteristics of haemoglobin-free ghosts of human erythrocytes. Arc Biochem Biophy. 1963; 100: 119-128.
26. Olorunsogo OO , Okudolo BE, Lawal SOA and Falase AO. Erythrocyte membrane Ca^{2+}-pumping ATPase of hypertensive humans: Reduced stimulation by calmodulin. Biosci Rept (U.K.) 1985; 5: 525-531.
27. Lowry OH, Rosebrough NJ, Farr AL, and Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951 ; 193: 265-275.
28. Gornal AG, Bardwil GS, and David MM. Determination of serum proteins by the mean of the Biuret reactions. Biochemistry. 1949; 177: 751-766.
29. Webster D. An assessment of the suitability of bromocresol green with isolated serum globulin fractions. Clin Chim Acta 1974 ; 53(1): 109- 115.
30. Surekha R , Madhavi G, Srikanth BM, Rao UR, and Jyothy A. Serum ADA and C-reactive protein in rheumatoid arthritis. Int J Human Genetics. 2006 ; 6(3): 195-198.
31. Richmond W. Estimation of serum total cholesterol by enzymatic method. Rev. Clin. Lab. Sci. 1973; 8:198.
32. Vashney R, and Kale RK. Effect of Calmodulin antagonist on radiation induced peroxidation in microsomes. Int J Rad Biol . 1990; 58: 733-743.
33. Okunade WG, and Olorunsogo OO. Effect of reactive oxygen species on the erythrocyte calcium-pump function in protein-energy malnutrition. Bioscience reports 1992; 12: 433-443.
34. McConell EJ, Bittelmeyer AM, and Raess BU. Irreversible inhibition of plasma membrane (Ca^{2+}-Mg^{2+})-ATPase and Ca^{2+} transport by 4-OH-2,3-*trans*-nonenal. Arch Biochem Biophys. 1999; 361: 252-256.