OBSERVATIONS ON THE HAEMATOPOIETIC SYSTEMS IN TROPICAL LEAD POISONING

ANETOR[+] JI, BABALOLA[+] OO, ADENIYI[+], FAA, AKINGBOLA[++] TS

*Departments of Chemical Pathology[+] and Haematology[++],
College of Medicine, University of Ibadan.*

SUMMARY The haemopoietic system was one of the earliest principal targets of lead (Pb) to be recognized and intensely studied but largely from temperate developed countries. This study reports investigations into the haemotobiochemical variations associated with occupational Pb poisoning in a tropical developing country.

One hundred and thirty seven (137) subjects comprising 86 lead workers and 51 appropriately matched controls were studied. The lead workers all males included battery workers, home and autopainters, automechanics, welders, gasoline dispensers and ceramic workers. They were classified according to exposure categories based on the prevailing air lead level (PbA) at the occupational environment.

Blood lead (PbB) was significantly higher in lead workers than in controls (P<0.001). The PbB of controls (occupationally unexposed) was also significantly higher than in communities that have either reduced or eliminated lead from petrol. Erythrocyte protoporphyrin (EPP) and prophobilinogen (PBG) were similar in lead workers and controls. The haem degradative product bilirubin was unlike EPP and PBG higher in controls (P<0.05). Indices of iron (Fe) homeostasis, serum Fe, total iron binding capacity (TIBC), transferrin, and percentage Fe saturation did not differ between lead workers and control (P>0.05) in all cases. There was also no alteration in RNA metabolism as indicated by the absence of basophilic stippling in the erythrocytes of lead workers. Some indices of erythropoietic activity Hb, PCV and MCHC were all significantly decreased in lead workers, compared with controls (P<0.001) in all cases. In contrast, the haem cofactor metals, copper (Cu) and zinc (Zn) levels were significantly elevated in lead workers compared with controls (P<0.01; P<0.001) respectively. There was no variation with exposure category.

These complex observations may suggest the interplay of acute phase and antioxidant responses of Cu in caeruloplasmin and Copper-Zinc super oxide dismutase (Cu-Zn SOD) as well as the inhalation ('supplementation') of Zn fume from the occupational environment. This synergy appears to have significantly restored the activity of the major haem pathway enzyme, d-aminolaevulinate dyhydratase (ALA-D), a Zn dependent enzyme that is exquisitely inhibited by Pb. Thus modulating the deleterious effect of Pb on the haemopoietic system.

These observations imply that the combination of the well known Zn deficiency in many tropical countries and the substantial environmental lead pollution may predispose the general population to a significantly depressed haemopoietic system. This may in turn increase the prevalence of subclinical or overt anaemia of 'uncertain' aetiology in the presence of other haem pathway stressors such as malnutrition.

Key Words: *Antioxidants, Environmental toxin, Haemopoietic system, Iron homeostasis, Lead poisoning.*

Introduction

Lead (Pb) is a non-essential trace element with a toxic potential for all biological systems. Lead is widely used in many occupations and its use is increasing as a result of progressive industrialization and attendant urbanization in most developing countries including Nigeria. It is one of the most important environmental pollutants which seriously affects the health of exposed individuals (Shakman, 1974, Savery and Wills, 1992).

Lead poisoning (plumbism) represents the oldest and one of the most serious worldwide occupational diseases (Legge, 1937). Many physiological systems including those of the renal, nervous, haemopoietic, immune, reproductive and the endocrine are the principal targets of this environmental and occupational toxicant (Damastra, 1977). Of these the haemopoietic system was the earliest to be recognized and most intensely studied. Over a century ago Garrod (1892) first identified porphyrinuria in human lead poisoning. In 1895,

Stovkis demonstrated its occurrence in both clinical and experimental plumbism. It is now well recognized that lead interferes with haemoglobin synthesis at a number of steps (Fig. 1). These interference are responsible for the well-known haematological toxicity of lead.

Most of the previous studies have emanated from the temperate developed countries. The present study is to investigate the haematobiochemical effects of lead poisoning in tropical lead workers.

Materials And Methods

One hundred and thirty-seven (137) subjects comprising 86 lead workers and 51 appropriately matched controls who were not known to be occupationally exposed were studied. The mean age of the lead workers and controls were 36.03 ± 1.0 and 36.66 ± 1.2 years respectively. The lead workers all males included battery workers, auto and home painters, auto-mechanics, welders, gasoline dispensers, and ceramic workers. The number and distribution of these workers have been previously described (Anetor and Adeniyi, 1999). The lead workers were classified according to exposure categories based on the prevailing air lead level (PbA) at the occupational environment (Rudolph et al., 1990). The mean duration of exposure of the Pb workers was $16.57 \pm 23.2.13$ years.

Sample Collection

About 15ml of venous blood was obtained from the ante cubical fossa using disposable pyrogene free needles and syringes (Becton-Dickinson, Dublin, Ireland). Two ml of blood was dispensed into haematology tubes (EDTA) for haematological studies. 5.3ml was dispensed into heparinizied tubes containing Pb free lithium heparin for lead determination (Vacutainer system, Inc. Rutherford, New Jersey). The remaining blood, about 7.7ml was carefully dispensed into plain vacutainer tubes containing inert polymer substance to aid separation of serum (Vacutainer systems Inc. Rutherford, New Jersey).

The haematological parameters, haematocrit (HCt) or packed cell volume (PCV), haemoglobin (Hb), basophilic stippling by examination of thin blood film were performed on the same day. Protoporphyrin studies were carried out. The whole blood samples for blood lead level (PbB) were frozen at $-20^{\circ}C$ until time for analysis. All serum samples were also frozen at $-20^{\circ}C$ until time for analysis.

Analytical Methods

Blood lead level was determined by atomic absorption spectrophotometry using the method of Hessel (1968). Serum iron (Fe) level was determined by the method described by Persijin et al. (1971). This same method was also used for the determination of unsaturated iron binding capacity (UIBC) from which total iron binding capacity (TIBC) and percent (%) were computed employing sigma iron and total iron binding capacity kit (Sigma Diagnostics, St. Louis, USA). Semiquantitation of erythrocyte protoporphyrin (EPP) was assessed by the procedure of Varley et al. (1980). Transferrin level was computed by the formula described by Young and Wesser (1991). Porpholilinogen level was determined by the inverse Ehrlichs reaction originally described by Hoesch (1947) and modified by With (1970). Red blood cell morphology including examination for basophilic stippling were carried out according to standard practice. Packed cell volume, and mean cell haemoglobin concentration were performed also according to standard laboratory procedures. Haemoglobin concentration was determined by the method evaluated by Khabbaza and Lot (1983) employing potassium ferricyanide using reflectance photometry. Total bilirubin, a degradative product of protoporphyrin was determined by the classical Vander Bergh reaction as modified by Malloy and Evelyn (1937). The haem cofactor metals copper (Cu) and zinc (Zn) were determined by atomic absorption spectrophotometry as for lead using the methods of Osheim (1983) and Smith et al. (1979) respectively.

Results

Tables 1, 2 and 3 show the results obtained from this study. Blood lead (PbB) level was significantly higher in lead workers than in control, 56.3 ± 0.95 and 30.47 ± 1.4 ug/dl respectively (P<0.001) (Table 1). The PbB of controls (occupationally unexposed) was also significantly higher than in communities that have either reduced or eliminated lead from petrol. Serum copper level was significantly higher in lead workers than in controls, 118.0 ± 3.40 and 104.0 ± 3.07 ug/dl respectively (P<0.005) (Table 1). Zinc, a d-amino-laevalinate dehydratabe (ALA-D) active site constituent was also significantly raised in lead workers compared with controls 112.0 ± 6.11 and 85.0 ± 2.65 ug/dl respectively (P<0.001) (Table 1).

Table 1 also shows the levels of serum iron, total iron binding capacity, transferrin and percent iron saturation. Both serum iron and transferrin levels were lower in lead workers than

in controls but these did not reach statistical significance. The values being 77.0 ± 4.93 versus 87.0 ± 6.37 ug/dl for Fe and 183.0 ± 6.60 versus 193.97 ± 9.73ug/dl for transferrin (P>0.05) in both cases. Total iron binding capacity was higher in lead workers than in controls. The values were 302.0 ± 37.2 and 286.0 ± 0.31 ug/dl respectively than in controls. This also however, did not reach statistical significance (P>0.05). Similar to serum Fe and transferrin levels, percent iron saturation was also lower in lead workers than in control 29.74 ± 1.69% versus 31.81 ± 2.39% but this was also not significant (P>0.05).

Table 2 shows the levels of haem precursors and the protoporphyrin degradative product, bilirubin. Erythrocyte protoporphyrin levels were similar in lead workers and controls. Furthermore, qualitative tests for porphobilinogen were negative in both lead workers and controls. Total bilirubin level was surprisingly significantly lower in lead workers than in control (i.e. higher in controls). The level were 0.78 ± 0.03 and 1.0 ± 0.06mg/dl respectively.

Table 3 shows haematocrit, haemoglobin concentration, MCHC, the state of polychromasia and basophilic stippling in lead workers and controls. Haematocrit (PCV), haemoglobin and MCHC levels in lead workers were all significantly decreased in lead workers compared with occupationally unexposed individuals. The values were 41.0 ± 0.33 versus 43.0 ± 0.41%, 13.57 ± 0.09 versus 14.4 ± 0.13 g/dl and 33.0 ± 0.13 versus 34.0 ± 0.18 respectively (P<0.001) in all cases. No polychromasia was evident from erythrocyte morphologic examination. Basophilic stipplings which are granules of RNA caused by derangement in RNA metabolism were also absent in lead workers and controls. The results in this study did not seem to vary with exposure category.

Table 1: Blood lead, serum copper, serum zinc, serum iron, TIBC, transferrin and percent iron saturation in lead workers and controls.

	Lead workers	Controls	t	P
Blood lead ug/dl	56.3±0.95	30.47±1.4	18.91	<0.001
Copper ug/dl	118.0±3.40	104.0±3.07	3.06	<0.005
Zinc ug/dl	112.0±6.11	85.0±2.65	4.06	<0.001
Iron ug/dl	77.0±4.93	87.0±6.37	1.28	<0.05
TIBC ug/dl	302.0±37.12	286.0±0.31	0.42	>0.05
Transferrin (mg/dl)	183.0±6.60	193.97±9.73	0.89	>0.05
Percent (%) iron saturation	29.74±1.69	31.81±2.39	0.71	>0.05

Values are Mean + SEM.

Table 2: Haem-precussors, erythrocyte protoporphyrin (EHP), porpholilinogen (PBG) and haem-degradative products, total bilirubin in lead workers and controls.

	Lead workers	Controls	t	P
Erythrocyte protoporphyrine	±	±	-	-
Porphobilinogen	-ve	-ve	-	-
Total bilirubin mg/dl	0.78±0.03	1.0±0.06	3.35	0.001

Total bilirubin levels are Mean ± SEM ± = t race -ve = absent

Table 3:   Haematocrit (PCV) haemoglobin concentration, mean cell haemoglobin concentration (MCHC), polychromasia and basophilic stipplings in lead workers and controls.

	Lead workers	Controls	t	P
Haematocrit (PCV) (%)	41.0±0.33	43.0±0.41	3.77	<0.001
Haemoglobin (mg/dl)	13.57±0.09	14.4±0.13	4.99	<0.001
Mean cell haemoglobin concentration (MCHC)	33.0±0.13	34.0±0.18	4.53	<0.001
Polychromasia	Absent	Absent	-	-
Basophilic stipplings	Absent	Absent	-	-

Values are mean + SEM where applicable.

Discussion

The significantly elevated PbB level in lead workers indicates lead toxicity and is consistent with most recent studies (Mason et al. 1990; Gennert et al., 1992, Kim et al. 1995). The level of lead found in these lead workers fall into the level currently indicative of severe plumbism. (PbB > 55 ug/dl) 2.66 umol/L) (Harvey, 1994). The blood lead level in the control subjects (occupationally unexposed) gives cause for concern. Recent reports (Landrigan, 1987; Harvey, 1994, Kim et al., 1995) indicate that Pb has no threshold value below which there is no adverse effect. The ideal blood lead level is zero. The level of PbB in controls in this report was at least about three fold current levels in similar populations in developed communities that have either substantially reduced lead in petrol or completely eliminated it (Brody et al. 1994). Pirkle et al. 1994, Kim et al. 1995). These disturbing PbB levels in unexposed subjects (and may be the general population) reflect significant environmental lead contamination largely attributable to the high lead content of Nigeria's petrol (Okoye, 1994, Adeniyi and Anetor, 1999).

The significantly raised Cu and Zn levels appear to be a response to the elevated lead burden. This is partially consistent with the report of Papaioannou et al. (1978) who found significantly elevated copper levels in their Pb (battery) workers but similar Zn levels in Pb workers and controls. Sohler et al (1977) had also earlier reported elevated Cu level in their lead exposed population. The mechanism for the increase in Cu level appears to be dual, first due to increase in caeruloplasmin, an acute phase protein which binds over 90% of serum Cu. Cu is raised in most stress or inflammatory states. Lead has also been described as a stress substance (Sohler et al. 1977). Secondly caeruloplasmin is an antioxidant, copper is also a component of the potent antioxidant Cu-Zn superoxide-dismutase (Cu-Zn SOD). Lead itself in common with some other heavy metals is a stimulator of free radical generation (Costa et al. 1997, Anetor and Adeniyi, 2001). The increase may thus be due to acute phase and antioxidant responses to ameliorate the deleterious effects of lead. Copper caeruloplasmin complex is also a feroxidase required for the appropriate metabolism of iron.

The elevated zinc level is due to simultaneous exposure of lead workers to a source of Zn (Zn fume). This may be analogous to supplementing Pb workers with Zn. The Pb workers in this study include welders who are exposed to alloys of Pb and Zn and other galvanized metals (zinc combined with other metals).

Welding is an occupation that can cause elevated Zn levels (Jacobs et al. 1990). Furthermore, welders, battery workers and auto-painters may occupy the same work environment and are simultaneously exposed to Pb and Zn. This was probably why Papaionnou et al. found similar Zn levls in Pb workers and controls. They investigated only battery workers. Zinc is a component of the active site of ALA-D (Finelli 1977, Abdulla, 1979) and reactivates ALA-D, the major haem pathway enzyme. Zinc is also a component of the potent antioxidant, Cu-Zn SOD. The synergistic effects of the raised Cu and Zn, both cofactor metals in the haem pathway ameliorated the deleterious effect of Pb on this pathway. The observation by Border et al (1976) that where industrial exposure to Zn and Pb are appreciable the Zn might activate ALA-D significantly to mask the toxic effect of Pb, appears consistent with the findings in this study in that simultaneous exposure to Pb and Zn may lead to raised Zn level which may protect the subject against the adverse metabolic effects of

lead. Other earlier investigators had also made similar observations (Haeger-Aronsen, 1971, Thawley, 1979).

The implication of these findings is that the haem pathway in lead exposed individuals was protected. The similar levels of the haem precusor EPP and absence of both porphobilinogen and basophilic stipplings suggest that ALA-D activity was not significantly inhibited despite the markedly elevated BLL in lead workers. Normally, this pathway would have been depressed and there will be attendant adaptive responses such as raised EPP and PBG as well as polychromasia, reflective of increased marrow activity and some degree of basophilic stipplings suggesting altered RNA metabolism.

The indices of iron homeostasis indicate that the second major haem pathway enzyme was also not significantly inhibited, were this the case serum Fe level would be higher in lead workers followed by decreased ALA-D activity. Though the exact mechanism is uncertain. The synergistic effect of Cu and Zn may be protective. It is possible that Zn and Cu sufficiently competed with Pb so that the amount of Pb available was inadequate to inhibit ferrochelatase. This observation is consistent with observed complexity of the relationship between iron metabolism and Pb absorption (Quaterman, 1986). Klander and Petering (1975) have also found copper and zinc to be protective or modify the haem pathway in experimental models when present in adequate amounts.

The haemotological indices in this study suggest haematological toxicity. This finding is consistent with those of Gibson et al (1968), Sohler et al (1977), Papaionnou et al. (1978) and Antonmicz et al. (1991). The mechanism of the observed haemopoietic effect is not definite. Most previous investigators have attributed the anaemia of Pb poisoning solely to decreased haem synthesis due to inhibition of ALA-D and ferrochelatase. These enzymes though not determined are unlikely to be inhibited owing to the unaltered haem pathway precursors. This observation confirms the suggestions of Waldron (1963) and Granic et al (1978) that the anaemia of plumbism is only partly due to impaired haem synthesis.

Increased haemolysis is also unlikely, as bilirubin was significantly lower in Pb workers than in controls. This decreased bilirubin level probably reflects its antioxidant role (Stocker et al. 1987) as it may have been consumed excessively in mopping up free radicals generated by increased lead burden.

A possible explanation is that the renal tubules are also among the principal target sites of lead. Thus lead may have an inhibiting effect on erythropoietin. This is considered plausible because 1, 25-dihydrochoxycholecal ciferol finally activated in these tubules is impaired in plumbism (Goyer and Rhyne, 1973, Goyer 1993).

These complex observations may suggest the interplay of acute phase and antioxidant responses in caeruloplasmin and Cu-Zn SOD as well as the inhalation ('supplementation') of Zn fume from the occupational environment. This synergy appears to have significantly restored the activity of the major haem pathway enzymes ALA-D and ferrochelatase. A-aminolaevulinate dehydratase is a zinc dependent enzyme that is exquisitely inhibited by Pb. The elevated Cu and Zn levels most probably have modulated the deleterious effects of Pb on the haemopoietic system.

One important implication of this observation is that the combination of the well known Zn deficiency in many tropical developing countries (Atinmo, 1982; Gibson, 1994; Underwood and Smitasiri, 1999) and the substantial environmental lead pollution (Adeniyi and Anetor, 1999) may predispose the general population in these countries to a significantly depressed haemopoietic system. This may in turn increase the prevalence of subclinical or overt anaemia of 'uncertain' aetiology especially in the presence of other haem pathway stressors such as malnutrition.

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