Arginase and kallikrein activities as biochemical indices of occupational exposure to lead

JADWIGA CHMIELNICKA, ELŻBIETA KOMSTA-SZUMSKA, AND JADWIGA A SZYMAŃSKA

From the Department of Toxicological Chemistry, Institute of Environmental Research and Bioanalysis, Medical Academy of Łódź, 90-145 Łódź, Poland

ABSTRACT In a group of 60 workers occupationally exposed to lead the blood and urine lead concentrations, haematocrit, ALA-D and arginase activities, and urinary 5-aminolaevulinic acid (ALA) and coproporphyrin concentrations, and kallikrein activity were determined. Correlation coefficients of -0.78 and 0.77 for Pb-B/ALA-D and Pb-B/arginase were found respectively for lead concentrations above 40 μg/dl blood, and 0.82, 0.76, 0.74, and -0.64 for Pb-U/ALA, Pb-U/Cp-U, Pb-U/kallikrein, and Pb-U/kallikrein, respectively. It seems that the increase in serum arginase activity may be indicative of liver damage while the decrease in kallikrein activity may indicate kidney damage in workers exposed to lead.

Tissues affected by lead absorption include the haemopoietic system, the central and peripheral nervous systems, kidneys, gastrointestinal tract, skeletal muscles, liver, immune system, reproductive system, and cardiac muscle.[1] Biochemical tests for lead absorption and toxicity have been reviewed by several authors[2-4] while others have discussed the action of lead on the liver[1] and the kidney.[5-7] Biochemical indicators of damage to tissues other than those concerned with haemopoiesis have not been greatly studied.[1 8-10]

Early stages of damage to the internal organs may be signalled by changes in the activity of some enzymes in the urine or serum of workers exposed to lead. Particular attention has been given to urinary kallikrein. This enzyme (EC 3.4.21.8) releases bradykinin, a potent vasodilator polypeptide from an α_2-globulin called kininogen; urinary kallikrein excretion has been found to be very low in lead workers.[11]

Andreasik *et al*[12] studied liver functions in lead workers and found a considerable increase in arginase (EC 3.5.3.1) activity to be the most characteristic symptom, other liver functions showing only small alterations. They concluded that these changes were indicative of damage to hepatocyte mitochondrial functions by lead.

The present study was designed to evaluate the suitability of using the measurement of arginase and

kallikrein activities to estimate exposure to lead by comparison with such classic tests[13 14] as the lead concentration in urine and blood, ALAD activity, or the levels of 5-aminolaevulinic acid (ALA) and coproporphyrins in urine.

Material and methods

Sixty lead workers were studied, from each of whom samples of blood and urine were collected. The activity of 5-aminolaevulinate dehydratase,[15] and the haematocrit[16] were estimated in whole blood and the activity of arginase was determined in serum using the method of Jergovic *et al*.[17] In the urine samples ALA[18] and coproporphyrin[19] concentrations were estimated together with the activity of kallikrein using Bergmeyer's method[20] in which benzyl-L-arginine ethyl ester (BAEE) is used as a substrate.

Blood and urine lead concentrations were determined by the dithizone method described elsewhere.[21 22]

Urinary lead, ALA, and coproporphyrin concentrations and kallikrein activity were estimated in 10 people having no occupational contact with lead compounds (control group).

Results

The ALAD and arginase activities and the blood lead concentrations are shown in the table. The data in this table are separated into two groups on the basis of the blood lead concentration. A twofold

Results of determinations of biochemical indices and lead in blood (mean values and ranges)

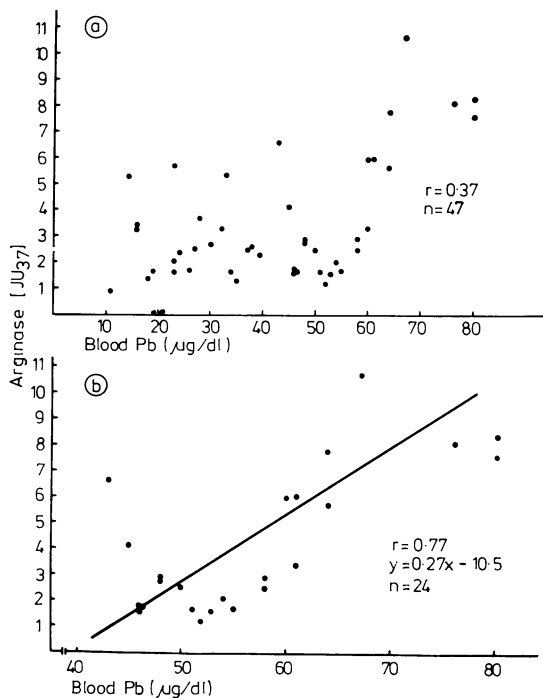| Pb-B μg/100 dl	N*	ALAD activity	ALAD activity ratio	Arginase activity
<40		0·69	0·58	2·45‡
Mean: 27	31	(0·27-1·26)	(0·23-1·10)	(0-5·7)
>40		0·39	0·46	4·25‡
Mean: 58	26	(0·10-0·67)	(0·14-0·88)	(1·2-10·7)

* = Number of samples.

† = Activity ratio = $\dfrac{\Delta \text{ A at pH } 6\cdot 8}{\Delta \text{ A at pH } 6\cdot 0}$ [20].

‡ = Statistically significant differences at p = 0·01.

Fig 1　*Correlation between activity of arginase and lead level in blood: (a) up to 40 μg Pb/100 dl, (b) above 40 μg Pb/100 dl.*

Fig 2　*Correlation between activity of 5-aminolaevulinate dehydratase and lead concentration in blood.*

increase in the blood lead concentration was accompanied by an almost twofold decrease in the ALAD activity while the arginase activity increased considerably. Figure 1 illustrates the dependence of arginase activity on the blood lead concentration. The correlation coefficient was low (r = 0·37) for the whole range of lead concentrations (fig 1(a)) but at higher lead concentrations (above 40 μg/dl) a correlation coefficient of r = 0·77 was found (fig 1(b)). The ALAD activity is plotted against the lead concentration in fig 2; a correlation coefficient of r = 0·78 was obtained for the whole range of lead concentrations (10-80 μg/dl blood).

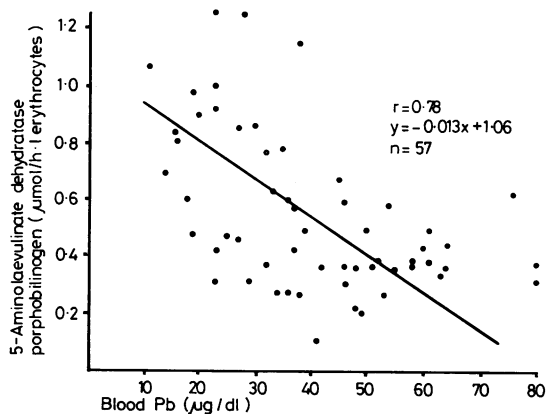In the urine from the control group the mean lead concentration was 52 μg/l, the mean ALA 4·5 mg/l, and the mean coproporphyrin 9·1 μg/dl. In the urine of the lead workers, the mean lead concentration was 110 μg/l, the mean ALA 8·0 mg/l, and the mean coproporphyrin 15 μg/dl.

From the results obtained, correlation coefficients were calculated between the various indices of lead exposure. The dependence of the delta-aminolaevulinic acid level on the lead concentration in urine is shown in fig 3(a) (r = 0·82). High correlation coefficients were also found between the urinary coproporphyrin concentration and delta-aminolaevulinate (r = 0·89; fig 3(b)) and between the CP-U and lead content of urine (r = 0·76; fig 3(c)).

The kallikrein activity was estimated in the control group and in the group of workers of highest occupational lead hazard, the values obtained being 24·8 ± 2·3 U/l and 16·7 ± 4·5 U/l, respectively. The correlation coefficient between kallikrein activity and the lead concentration in urine was −0·74 (fig 4(a)); that between the activity of this enzyme and the concentration of delta-aminolaevulinic· acid was −0·64 (fig 4(b)).
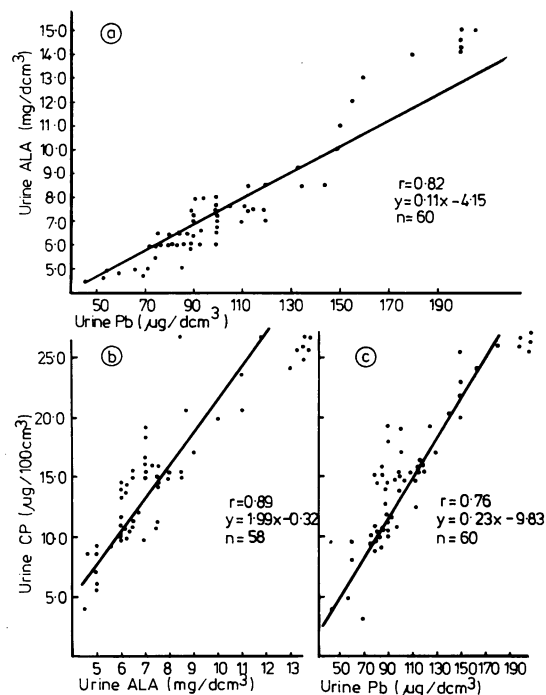
Fig 3   *Correlations between concentrations: (a) delta-aminolaevulinic acid and lead in urine, (b) coproporphyrins and delta-aminolaevulinic acid, (c) coproporphyrins and lead in urine.*
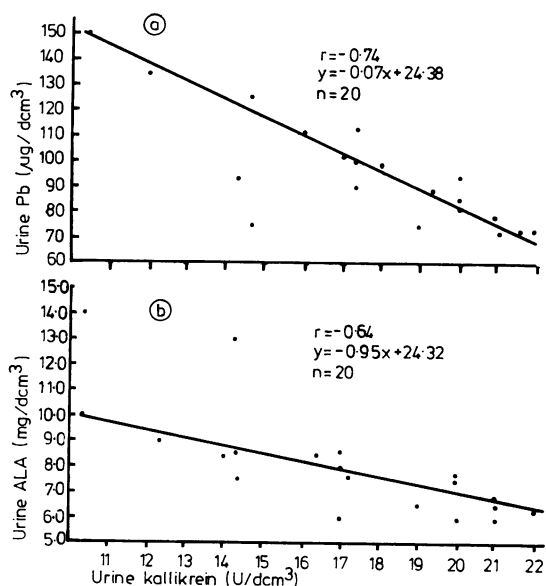

Fig 4   *Correlations between activity of urinary kallikrein and: (a) lead concentration in urine, (b) concentration of delta-aminolaevulinic acid in urine.*

Discussion

The hazard from lead exposure has usually been estimated on the basis of blood lead concentrations, zinc-protoporphyrin concentration, the activity of 5-aminolaevulinate dehydratase in the blood[3], [13] and the estimation of the concentrations of lead, copro-porphyrin, and ALA in the urine.[23] None of these is linked to damage to the internal organs, however.

The results of this study indicate that enzymatic indices of liver (arginase) and kidney damage (kallikrein) may also be used in estimating occupational exposure to lead. These indices are found to correlate with the concentrations of lead in the blood and urine.

The concentration of arginase is 100-1000-fold higher in the liver than in other organs; serum contains only trace amounts of this enzyme.[24] This specific distribution of arginase can be maintained only if the integrity of the hepatocyte membranes is secured by energy-generating metabolic processes. Lead as a cytotoxic agent may disturb the cell energy metabolism resulting in the disintegration of cellular membranes and the liberation of arginase, an enzyme of relatively low molecular weight, into the serum.

The serum arginase activity was in the range 0 to 11 units at the lead concentrations found. Andreasik *et al*[12] found an upper limit of 49 units in lead workers (although they did not report the corresponding lead concentrations) with a mean of 4 units in a control group. In this study the exposed workers were divided into two groups, with the lead concentration in blood below and above 40 μg Pb/dl. The correlation between arginase activity and blood concentration in the group with the high lead was significant (r = 0·77; fig 1(b)). The difference in arginase activity between these two groups was statistically significant (p = 0·01). A good correlation was also found between the ALAD activity and the lead concentration in blood (r = 0·78; fig 2), confirming earlier findings.[15] [21]

It should be emphasised that, although the correlation coefficients between arginase and lead and ALAD and lead are of the same order of magnitude, the test based on the measurement of arginase activity may be more suitable for clinical purposes since it is indicative of damage to the liver. The biological role of ALAD and the consequences of the decrease in its activity are not clear. One must take into account, however, that an increase in serum arginase activity is also observed in cases of viral hepatitis, cirrhosis, chronic alcoholism, and acute myocardial infarct.[24]

As a result of this study the estimation of urinary kallikrein is also proposed as a new biochemical test of lead toxicity. An inverse correlation was shown

between the kallikrein activity and the lead concentration in urine above 80 μg Pb/l (corresponding to about 40μg Pb/dl blood).

According to the recommendations of a WHO committee, the upper permissible concentration of lead in the urine of people exposed to lead is about 100 μg/l. The decrease in urinary kallikrein activity found with urine lead concentrations of 80 μg Pb/l suggests that this enzyme may be a sensitive index of exposure to lead.

The values of the correlation coefficients: Pb-U/ ALA-U, Pb-U/Cp-U, and ALA-U/Cp-U reported here are similar to those found by other authors.[23][25][26] The correlation coefficients kallikrein-U/Pb-U and kallikrein/ALA-U were similar to those found for ALA-U/Pb-U and Cp-U/Pb-U. Although the biological function of kallikrein is still not completely understood, the decrease in the activity of this enzyme in the urine is probably due to disturbances of the synthesis of this enzyme in the kidneys, induced by the metal.

It is documented in published reports that the symptoms of renal damage in people exposed to lead are not typical and may be overlooked in practice. It seems, however, that changes in kallikrein activity in the urine can be detected earlier than anaemia.

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