The effects of different lead concentrations and different temperatures in ALA-D activity in human blood hemolysate

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Abstract- Heavy metal environmental pollution, especially with lead has become very disturbing, because it causes multiple negative effects in living organisms. It is known that lead is a potent inhibitor of ALA-D activity. The current investigations provide an attempt to find minimal critical level of lead that produces inhibitory effect of ALA-D activity of erythrocyte. In present study it has been also examined the effect of high temperature in activity of ALA-D. Research was performed in human blood hemolysate. For that purpose we selected a healthy human individual who has not been exposed to lead intoxication, does not smoke and does not consume alcohol. The negative correlation was estimated between Pb concentration and ALA-D activity in all concentration (2-100 μg%). Exception from this inhibition trend is the activity of enzyme at 60°C in lead concentration from 7-10 μg%. In concentration of lead above 7 μg% and in temperature of 37°C the activity of enzyme was low while in the same concentration bat in temperature of 60 °C the activity of enzyme was recovered. In high concentrations of Pb, the activity of ALA-D was significantly inhibited at 37°C in comparison to other temperatures (42°C, 50°C and 60°C).

Index Terms- ALA-D, lead, blood, temperature

I. INTRODUCTION

Lead concentration in different environments (air, soil and water) is very variable, and its level in environment during last three centuries is growing up more than 1000 time as a result of human activity (ASTDR, 2005). The most threatened areas concerning of lead contamination are urban areas, industrial zone and areas near the mining and smelter of lead.

In living organisms, lead enters through inhalation (by breathing), or by ingestion (during feeding). The most sensitive target of lead attack is: renal system nervous system, hematological and cardiovascular system, but also other systems in which lead can have influence because of its different way of activity cannot be excluded from attack. Effects are same, whether lead enters through inhalation or by ingestion (ToxFAQs: CABS, 2006). Anemia that results from reduced hemoglobin production and shortened life-spam of erythrocytes is a sensitive indicator of lead exposure and it has been observed only in hard cases of lead intoxication (Ma, 1996). Determination of D-ALA activity in erythrocyte is one of most appropriate ways to evaluate lead exposure, because its activity is very sensitive and specific for different lead concentrations in blood (Sakai, 2000).

Concerning of ALA-D sensitivity on lead, it has been reported in Hernberg et al. 1972. Authors show that ALA-D is very sensitive on lead and they proved that lead inactivate this enzyme without any distinct limit concentration. This enzyme is such sensitive that its activity decrease on half if lead concentration in blood is 15 μ\dl, and in quarter if lead concentration is a 30 μ\dl.

Inhibition of D-ALA activity increases its substrate δ-ALA in plasma and in body liquids, which causes high δ-ALA excretion in urine (Mushak, 2002).

ALA-D is reach with sulfhydryl group therefore thiol are more than needed for maximal activity of enzyme. Because of great affinity between sulfhydryl groups and metal ions especially heavy metals, it can be expected that metals bond for enzyme in normal conditions.

Reports for metal requirements of enzyme from different author sometimes are contradictory. Some authors reported copper as cofactor of D-ALA (Iodice et all., 1958, Komai and Neilnd, 1968, Evans, 1973), while some authors shows that zinc is involved in enzyme acitivity (Ozretic M, dhe Ozretic B. 1980).

Four main enzymes that participate in hem synthesis which are target of different chemical toxicity are: ALA dehydratasae (D-ALA), uroporphyrinogen decarboxilase (UROD), protoporpfyrinogen oxidasa (PPO) and ferrochelatase (FeC). Inhibition of these enzymes results on appearance of deceptive porphyries.

D-ALA is one of the most sensitive enzymes which inhibits hem synthesis caused by toxic substances including also lead.

Inhibition of D-ALA in erythrocytes caused by lead can be restored entirely by heating hemolysate for five minutes at temperature 60°C as is shown in some of publications (Tomokuni).

In the current research the focus was on the influence of different lead concentrations and different temperatures (37°C, 42°C, 50°C and 60°C) of human blood hemolysate in activity of D-ALA.

II. MATERIAL AND METHODS

In the current investigation all experiments were done with blood hemolysate of only one human individual which in this case was selected to be person who is healthy, is not exposed professionally to lead contaminations, does not smoke and does not drink alcohol. The blood lead level was determined in advance.

For blood treatment with lead, two series of test-tubes were prepared with nine tubes for each series. Test-tubes were

wrapped with aluminum folio and heparinizied with 0,02 ml heparin/ml blood.
Blood was treated with lead acetate in different concentrations from 3-10μ% for each subunit and from 20-100μ% in every ten subunits.
Blood lead level was determined with flame atomic absorption spectrometry, method by Milic (Milic, 1985).
The activity of D-ALA was estimated according by standardized method of Berlin and Schaller (1974).
Results of this investigation were also processed statistically and presented as average values with corresponding standard deviations. Statistical software Sigmastat was used for processing results.

III. RESULTS

Results presented in Fig. 1 demonstrate that activity of D-ALA corresponds to linear inhibition starting from 2-10 μg % of lead concentration. It was noticed from Fig. 1 that there was negative correlations between activity of D-ALA and lead concentration in all temperatures. Exception from this inhibition trend is the activity of enzyme at 60°C in lead concentration from 7-10 μg%. In concentration of lead above 7 μg% and in temperature of 37°C the activity of enzyme was low while in the same concentration bat in temperature of 60 °C the activity of enzyme was recovered (Fig. 1).

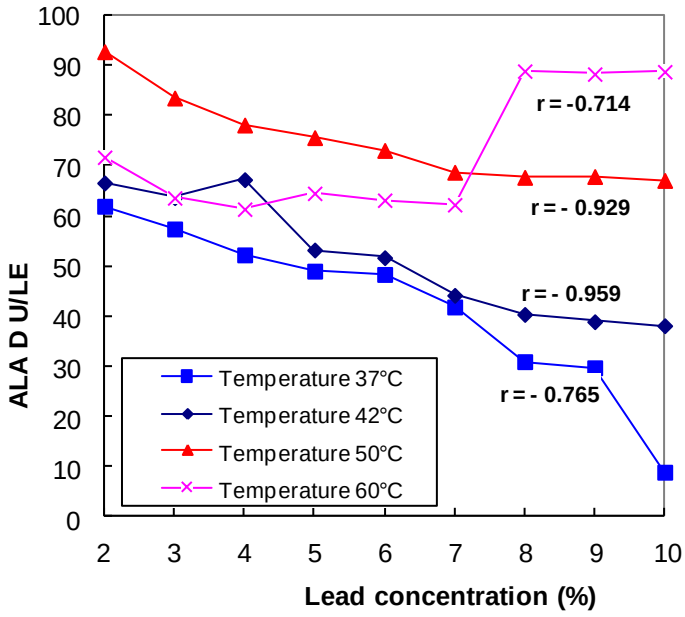

Fig 1: Correlation between ALA-D activity and lead concentration (2-10 μg%) in different temperatures (37°C,42°C, 50°C and 60°C)

Compared with temperature 37°C, in all other temperatures enzyme activity is higher but with negative correlation with lead concentration. From Fig. 1 it is obvious that point of 7μg% at 37°C is critical for enzyme activity, because there appears a significant decline of D-ALA activity.
In other case, in high lead concentration (20-100 μg%) at 37°C, activity of D-ALA shows linear decrease until value of 70μg%, and above this value enzyme activity remain unchanged and very low (Fig. 2).
At temperature of 42°C until the lead concentration of 50μg% the enzyme activity is higher than at temperature of 37°C. At high temperatures such as 50°C and 60°C, enzyme activity is much higher than in temperatures of 37°C and 42°C. Temperature of 60°C has restored activity of enzyme in lead concentration from 20μg% till 50μg%. After that enzyme activity has started to fall down and in lead concentration of 100μg% the enzyme activity is 56 U /LE as is shown in Fig. 2.

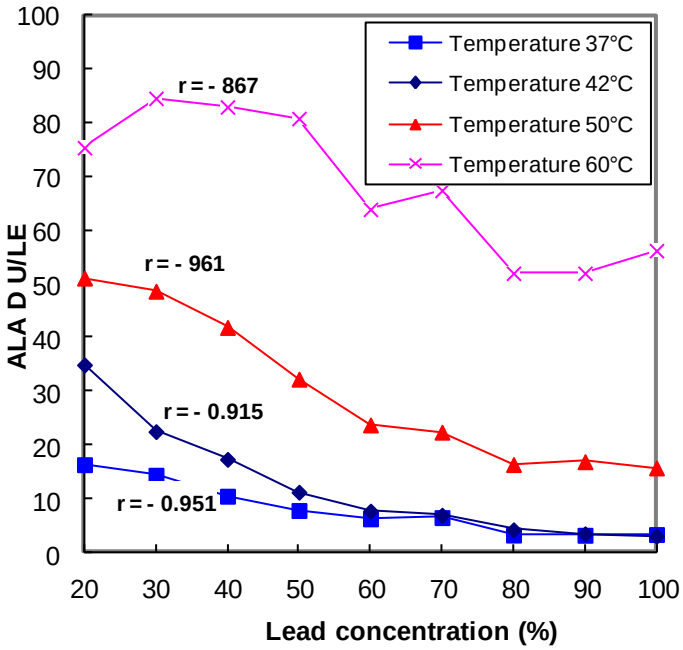

Fig. 2: Correlation between ALA-D activity and lead concentration (20-100 μg%) in different temperatures (37°C,42°C, 50°C and 60°C)

IV. DISCUSSION

The relationships between the amounts of dose and the degree of effect produced by the dose are denoted as dose-effect relationship and are an essential requirement for biological effect monitoring (Sakai, 2000).
Determination of ALAD activity in erythrocytes is one of the most useful methods for evaluation of lead exposure, because the activity is extremely sensitive and specific for blood lead concentration. The activity is inhibited by lead in concentration between 5 and 50μg/100g blood (33-36 Sakai 2000).
The present study has shown negative correlation between lead concentration and D-ALA activity. These results are compatible with data obtained previously in the laboratory of our Institute carried out with different natural animal population and human population near of lead and zinc smelter “Trepca” in Mitrovica (Elezaj et all., 1988, Bakalli et all., 1990, Elezaj et all., 2004).
The research done by Hernberg and Nikanen (1970) with 26 students with blood lead concentration of 15 μg / dl, shows also an exponential negative correlation between ALA-D activity and blood lead concentration.
Campagna et all. (1999) claims that they have identified lowest potential threshold of lead (3.2-4.8 μg / dl), above which ALA-D

might be inhibited, while under these values ALA-D activity might be insensitive.

In our investigation the threshold of lead was 7 μg / dl blood, above witch ALA-D activity was evidently inhibited (37 °C). Unlike to the low lead concentrations, in high concentrations of Pb, the activity of ALA-D was significantly inhibited and above 60μg/dl of blood lead concentration, activity of ALA-D is close to zero (37°C/60 min).

In present study it was also examined the effect of high temperature (42°C/ 60 min., 50°C/60 min. and, 60°C/60 min) in activity of ALA-D. In all temperatures (42°C/ 60 min., 50°C/60 min. and, 60°C/60 min), enzyme activity is higher than in temperature at 37 °C, but with negative correlation with lead concentration. From figure 2 it is evident that in temperature at 60°C, activity of ALA-D is recovered in lead concentration from 20μg/dl until 50μg/dl blood. Achieved results of our investigations about effect of high temperatures in activity of ALA-D are in agreement with the results of other authors, such is Tomokuni, 1975; Sakai et all., 1980; Kajimoto et all., 1983.

Tomokuni (1975) has observed that ALA-D activity of exposed lead workers is more stable in high temperature (60°C) than activity of enzyme in control group, which we have observed also in our investigation. The ALA-D activity is higher and more stable at 60 °C compared to the activity of enzyme at 37 °C.

Based on the research results, there could not be identified a threshold dose of lead that does not have negative effect on organism. In other words there is no safe level of lead below which lead appears to be safe.

In the past 30 years, recommendations for limiting exposure to lead in the community have been largely driven by the need to protect children from the effects of lead on developing brain. (CDC)

Lead exposure may lead to anemia, due to reduced hemoglobin production and shortened life-spam of erythrocytes. Lead has significant effect on hemoglobin synthesis as it inhibits δ-aminolevuilinc acid dehydrogenase (ALAD) thereby decreasing hem synthesis, which leads to an increase in δ-aminolevuilinc acid synthase. The activity of ALAD may be inhibited at PbB concentrations as low as 3 – 34 μg dL-1 with no threshold yet apparent (HPA 2012).

Based on results of current investigation the critical level of lead appears to be at 7μg/dl of blood, from that point the activity of ALAD has decreased significantly.

In Australia NHMRC has recommended an overall goal of blood lead concentration below 10 μg / dl. It was never intended the goal of 10 μg / dl to be interpreted as either a "safe" level of exposure or a "level of concern". CDC, ASTDR, and WHO also have a goal of 10 μg / dl. While some groups have recently lobbied the US Government for a further reduction in this figure, CDC has argued for its retention (CDC, 2005; Brown and Rhods, 2008).

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