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Lead concentration in endothermic vertebrates and ALAD polymorphism

Z. HREHOVÁ
Institute of the High Mountain Biology, University of Žilina, Tatranská Javorina 7, 059 56-SR, Slovak Republic, e-mail: hrehova@uniza.sk

Introduction

Lead – the heavy metal affects human and animals health. Low levels of exposure of lead can iduced cognitive and neurobehavioral changes. High levels can be responsible for encephalopathy, even it can cause death. Previously even considered Pb as one of the causes contributing to the extinction of the Roman Empire. The main reason of destroying health by lead exposure is because of his potential inhibition of δ-aminolevulinic acid dehydratase (ALAD), coproporphyrinogen oxidase, and ferrochelatase, enzymes that catalyze the second, sixth, and final steps, respectively, in the biosynthesis of heme (Warren *et al.* 1998). In this review it will be discussed ALAD gene that encoded δ-aminolevulinic acid dehydratase. ALA dehydratase (E.C. 4.2.1.24) also known as porphobilinogen synthase is an octameric metaloenzyme. This enzyme catalyzes the second step in heme biosynthesis, condensation of two molecules of δ-aminolevulinic acid (ALA) into one molecule of monopyrrole porphobilinogen (PBG), which is precursor of heme. For full activity ALAD recquires zinc ions as a cofactor. Normally, this ions bind enzyme´ s SH group. But in the presenece of lead in the organism, SH group binds the lead because of the higher affinity for this metal and so activity of enzyme is inhibited. This situation can lead to porphyria.

ALAD gene and their polymorphism

The most studies focus on the human ALAD gene and its polymorphism. Only a few research groups dealing with ALAD gene in mice or rats and no one else for the other animals in molecular level. Despite of some known ALAD gene sequences in another vertebrates like *Pan tryglodytes, Bos Taurus, Canis lupus, Equus cabalus*, no research works were made on them. These sequences were obtained from genome projects.

Human ALAD is encoded by single gene located in chromosome 9q34 with two co-dominant alleles, ALAD1 and ALAD2 (Onaja and Claudio 2000). It was first discovered to be polymorphic in 1981 by Petrucci *et al.* (1982). ALAD2 is less widespread than ALAD1. It ranges from 0 - 20 % depending on the population. Generally, Caucasians have the highest frequency of the ALAD2 allele, with approximately 18% of the Caucasian population being ALAD 1-2 heterozygotes and 1 percent being 2-2 homozygotes. In comparison, African and Asian populations have low frequencies of the ALAD2 allele, with few or no ALAD2 homozygotes being found in such populations (Kelada *et al.* 2001). The difference between the allele ALAD1 and ALAD2 polypeptids is a substitution of asparagines for lysine at residue 59, resulting from a single nucleotide change in position 177 of coding region (Wetmur *et al.* 1991a). The expression of these alleles results in three distinctly charged forms of the isoenzymes – ALAD1-1, 1-2 and 2-2 (Onalaja and Claudio 2000). It is result from different charge in lysine and asparagine. Wetmur *et al.* (1991a) developed a new technique for determination of the ALAD genotype based on polymerase chain reaction. They identified 93 ALAD2 heterozygotes and homozygotes with this technique with 100% genotype-phenotype consensus. The biological plausibility for a differential role of the two alleles lies in the fact that the lysine substitution at residue 59 changes the electrical charge of the enzyme (Battistuzzi *et al.* 1981); the more electronegative ALAD2 enzyme may thus have a higher affinity/stability for the lead cation than ALAD1 (Wetmur *et al.* 1991b). Here some studies differs from another. Some of them showed that homozygous or heterozygous individuals for ALAD2 allele exhibited higher blood lead level (BLL) than homozygous ALAD1-1 individuals. These findings led to the suggestion that ALAD2 may be a determinant for increased susceptibility to lead toxicity (Wetmur *et al.* 1991a). On the other hand, some studies have reported either no differences or only differences with low statisticall significance among individuals homozygous for ALAD1 relative to individuals carrying the ALAD2 allele. The third groups of researchers incline to more complex of ALAD polymorphism in the toxicity of lead and support the possibility that ALAD2 may be protective. The cause of this protective role may be keeping lead bound in the blood compartement by ALAD2. Supporting this notion is the finding that ALAD1-1 subjects transfer more lead into bone even when ALAD2 carriers have higher blood lead level (Fleming *et al.* 1998).

Exactly, the high level of free lead is closely linked with the formation of zincprotoporphyrin (ZPP). This is due to the binding of blood lead into the ferrochelatase, an enzyme that catalyzes the terminal step in the heme biosynthesis by converting of protoporphyrin IX into heme. Lead binding to this enzyme inhibits its activity and leads to the binding of zinc ions by protoporphyrin instead of iron. Consequently, higher

ZPP levels can be detected in ALAD1 homozygous. The presence of ZPP has been proposed as an indicator of recent lead intoxication and thus can be used as a biomarker of exposure. However, because of the abundance of hemoglobin, even in serious cases of lead intoxication, increased ZPP is relatively harmless because it may constitute less than 1% of the total hemoglobin produced (Blumberg *et al.* 1984).

ALAD polymorphism in mouse

For researches of ALAD polymorphism in laboratory animals was used two strains of mice: DBA/2 and C57BL/6 in order to examine the role of ALAD gene in the lead bioaccumulation. It has been discovered that they differ in their expression of the ALAD gene. Investigator had already shown that hepatic ALAD enzyme activity was higher in DBA/2 mice than in the C57BL/6 strain (Doyle and Schimke 1969, Hutton and Coleman 1969). The cause of the higher enzyme activity in DBA/2 strain is duplication of ALAD gene resulting in two times the doses of ALAD enzyme. Further, hybrid animals (C57BL/6 x DBA/2) were found to have intermediate levels of enzyme activity (Bishop *et al.* 1986). Moreover, another exploration was made to test gender differences in BLL. There were no significantly different in all dosage groups, except in animals exposed to 1,000 ppm of lead acetate. In this group, female animals had higher blood lead levels than males in both strains ($p < 0,05$). To determine if these differences in levels of red blood cells , hematocrit readings were obtained, but this was not significantly different among the groups (Claudio *et al.* 1997). They also reported a higher levels of lead in kidney than in the liver in both strains, but these accumulations were significantly higher in DBA/2 mice.

Comparable situation with ZPP production than in humans has been observed in mice. While DBA/2 strain has double amounts of ALAD gene, and therefore more enzyme in blood available for binding Pb, there is less opportunity for creation ZPP. In C57 mice, situation is reversed. ZPP levels increased with increasing lead exposure. These mouse strains can be used as a model for studying the role of ALAD in lead toxicity. Due to the fact that genetic difference in mice is based on duplication and in humans on polymorphic form, it will be suitable to develope transgenic mice with the ALAD2 gene against already existing ALAD mice gene and these may present a more accurate model of the human system.

Structure of the ALAD gene encoding murine ALAD dehydratase was examined by Bishop *et al.* (1996). Their studies had shown that the gene contains two first exons, 1A and 1B, which are alternatively spliced to exon2, where the coding region begins. Consequently, while ALAD enzyme is identical in all tissues, ALAD mRNA occurs in housekeeping (1A) and erythroid-specific (1B) forms. Each first exon has its own promoter. The promoter driving exon 1A expression is TATA-less and contains many GC boxes. In contrast, the exon 1B promoter bears regulatory sequences similar to those found for β-globin and other erythroid-specific genes. Tissue distribution studies reveal that ALAD mRNA containing exon 1A is ubiquitous, whereas mRNA containing exon 1B is found only in erythroid tissues. This finding, together with our further observation that GATA-1 mRNA levels increase 3-fold during maturation of murine erythroid progenitor cells, may help explain simultaneous 3-fold increases in exon 1B expression. The unexpected result that exon 1A expression also increases 3-fold during CFU-E maturation may be attributable to the action of NF-E2, since there is a potential binding site in a position analogous to the NF-E2 site in the locus control region of the β-globin gene cluster (Bishop *et al.* 1996). The level of exon 1B transcripts varies during different part of life. The most abundant occur is in the fetal life and in adults is scarce. This is due to correlation between exon 1B transcripts and the extent of erythropoiesis.

The comparison between human, mice and rat ALAD gene

The knowledge of ALAD gene structure is necessary because of better understanding of its different expression and so different stage of catalysation in the second step of heme biosynthesis by ALAD enzyme. For these purpose, isolation of ALAD clones was made. A 350 bp fragment of mouse erythroid promoter and the 5‘ half of exon 1B was used as a probe. The comparison of the mouse and human ALAD gene had shown the 70% of nucleotide sequence similarity in 250 bp fragment of this gene. This comparison was used to reveal conserved and thus functionally important regions of ALAD-erythroid-specific promoters (Bishop *et al.* 1996). The most differences from the consesus in all three species was observed in human. A new results in this field could provide a resolution in incorrect hematopoiesis as well as other risks associated with the exposure to lead.

Conclusion

ALAD plays the main role in the heme biosynthesis as already mentioned above, namely in the second step of this pathway. Exchange of Zn with Pb ions in the active site lead to porphyria with the amount of consequences. The principal problem in these deficiencies is the accumulation of porphyrins, the heme precursors, which are toxic to tissue in high concentrations. Various affinity of lead to enzyme is affected by the polymorphism of ALAD gene. It was observed the 20 times higher levels of ALAD than required. On the basis of this observation it can be hypothesized more function of ALAD gene besides the regulation of the second step in the heme biosynthetic pathway. In fact, Guo et al. (Guo *et al.* 1994) reported that ALAD is the 240 kDA proteasome inhibitor CF-2. And the third function of ALAD is binding of lead, as was mentioned above. These multiple ALAD functions may be so critical to erythropoiesis that mutations in the gene are only rarely viable: only four cases of ALAD-deficiences have been reported in the literature (Ishida *et al.* 1992).

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