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Article

Neuromuscular features of hypophosphatasia

Manifestations neuromusculaires de l'hypophosphatasie

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Summary
The pathophysiology of the neuromuscular manifestations of hypophosphatasia (HPP) remains unknown. Pyridoxine-sensitive seizures characterize severe forms of infantile HPP. Young children and infants affected with severe forms of HPP, but also adults often present with myopathy characterized by hypotonia or muscle weakness. Chronic pain, of unclear mechanism is also often present. Tissue-non-specific alkaline phosphatase (*Alkaline Phosphatase-Liver/Bone/Kidney* [*ALPL*]) is expressed in brain neuronal cell and in muscle cells during development and adulthood. The knockout of the *ALPL* impacts neuronal functions in animal models. This may occur through metabolic anomalies involving gamma-aminobutyric acid (GABA) and other neurotransmitters via the metabolism of pyridoxal phosphate (vitamin B6) and phosphoethanolamine. In this context, a greater understanding of the neuromuscular pathophysiology of HPP is critical to assess the potential impact of new therapies.

Résumé
La physiopathologie des manifestations neuromusculaires de l'hypophosphatasie (HPP) est relativement méconnue. Des convulsions sensibles à la pyridoxine accompagnent les formes sévères du nourrisson. Une forme de myopathie avec hypotonie ou faiblesse musculaire est souvent présente, en particulier chez les jeunes enfants et dans les formes sévères, mais se rencontre aussi dans les formes de l'adulte. Des douleurs chroniques dont le mécanisme est peu clair sont aussi présentes. Il existe une expression de la phosphatase alcaline non spécifique de tissu (*Alkaline Phosphatase-Liver /Bone/Kidney* [ALPL]) dans le cerveau, au niveau des cellules neuronales et dans les muscles, lors du développement et chez l'adulte. L'invalidation du gène a des conséquences sur certaines fonctions neuronales dans les modèles expérimentaux. Des anomalies métaboliques impliquant l'acide gamma-aminobutyrique (GABA) et d'autres neuromédiateurs *via* le métabolisme du phosphate de pyridoxal (vitamine B6), mais aussi la phosphoéthanolamine peuvent être en cause. Une meilleure connaissance de la physiopathologie neuromusculaire de l'HPP permettra de mieux apprécier l'impact potentiel des nouvelles thérapeutiques.

1. Introduction

The association between HPP and the neuromuscular clinical signs remains rather mysterious [1,2]. The pathophysiology is probably complex. Elucidating the pathophysiology is particularly important in that the neuromuscular symptoms account for a significant fraction of HPP morbidity [3].

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2. Clinical manifestations

HPP is due to loss of function mutations of the *ALPL* gene coding for ubiquitous tissue nonspecific alkaline phosphatase resulting in low blood alkaline phosphatase (ALP) levels. The neuromuscular symptoms are highly patent in the severe forms of the disease including muscle weakness, leg myalgia and waddling gait [4,5]. In adults, symptoms are sometimes similar to those of fibromyalgia ('low phosphatase unwellness'), including non-systematized chronic painful syndromes [6]. These manifestations are frequently poorly related to the osteoarticular anatomical and clinical findings and unlikely to respond to conventional analgesic and anti-inflammatory therapies.

3. Pathophysiology

Several murine models simulate the severe perinatal or more moderate adult forms of human HPP. However, the neuromuscular involvement has not been clearly documented [7]. ALPL is not only expressed in bone cells but also in endothelial, neuronal, heart muscle and skeletal muscle cells. ALPL activity in vessel walls emerges during prenatal life. Muscles present with endothelial phosphatase activity as of 26 weeks of gestation. The mode of expression in the brain as of 28 weeks of gestation is similar to that in adults [8]. The exact functions of ALPL in the endothelium have yet to be elucidated. However, with regard to the brain, old and more recent studies suggest that the enzyme is involved in blood – brain barrier processes [9]. ALPL is strongly expressed in the developing nervous system in mice and primates [10]. Moreover, brain imaging of children with HPP has evidenced various abnormalities such as cerebral atrophy, ventricular dilatation, low density of the white matter, polycystic encephalopathy and cortical laminar necrosis [11,12]. In mice in which the murine gene for ALPL (Akp2-KO) has been knocked out, thinner spinal nerves, hypomyelinization, axons of smaller caliber and delayed cerebral cortex synapse maturation have been evidenced [13,14]. Clearly, ALPL dysfunctions may affect the development of the nervous system by disturbing several metabolic pathways.

Ultrastructural studies in rodents have shown the presence of ALP activity in the myocardium at sarcoplasmic reticulum level, mainly at Z line level [15]. The low level of ALP activity in muscle fibers, compared to other tissues, may explain the absence of enzymatic activity reported in man. High levels of activity have been reported in animal pathological models or in the context of dermatomyositis – polymyositis. These findings underlie the hypothesis that ALP activation may be associated with denervation or the regeneration of non-innervated muscle fibers.

ALPL is an ectoenzyme linked to the cell membrane by glycosylphosphatidylinositol (GPI) anchoring. Cases of hypotonia have been reported in children with low serum ALP levels due to mutations of the Phosphatidylinositol Glycan Anchor Biosynthesis Class T (*PIGT*) gene [16]. The gene is involved in the biosynthesis of a subunit of transaminase GPI, which anchors the GPI complex to precursor proteins such as ALPL. A functional deficiency of transaminase GPI would explain the reduced release of ALPL and thus the low circulating enzyme levels, while GPI abnormalities are more characteristic of hyperphosphatasia [17,18].

4. ALPL metabolites

The functions of ALPL in tissues other than bone have not been fully elucidated; we know that ALPL plays an essential role in mineralization via an inorganic phosphate / inorganic pyrophosphate (Pi/PPi) ratio leading to calcification. Two substrates other than PPi accumulate in the blood and urine of patients with HPP: pyridoxal-5'-phosphate (PLP) and phosphoethanolamine.

5. PLP and vitamin B6

PLP is a substrate of ALPL. ALPL dephosphorylates PLP yielding pyridoxal [PL] (form of vitamin B6) which can then enter cells. Intracellular PL is then converted back to PLP, which is an essential cofactor in numerous enzymatic reactions, particularly those involved in amino acid metabolism. Approximately eighty percent of the PLP is located in skeletal muscle where PLP is a cofactor of glycogen phosphorylase, which is responsible for the first step in the transformation of glycogen into glucose. A reduction in the intracellular quantity of PLP together with ALPL deficiency may thus contribute to muscle deficiency.

In the nervous system, the reduced availability of intracellular PL is likely responsible for the pyridoxine sensitive seizures in neonates affected with severe HPP. GAD65 and GAD67, the enzymes that convert glutamate into gamma-aminobutyric acid (GABA), are PLP-dependent. Interestingly, Akp2-KO mice show a decrease in the quantity of GABA in their brain extracts [19]. GAD67 is more strongly saturated by PLP than GAD65. The latter, located in the synaptic endings, regulates inhibitory transmission. Knockout of ALPL generates epilepsy in mice [20]. Administration of PLP to Akp2-KO mice or neonates with severe HPP only temporarily limits the epileptic seizures and does not improve survival [19,21].

The excitation/inhibition disequilibrium created by GABA reduction (40% in the Akp2-KO mouse) is perhaps not the only factor responsible for seizures in severe HPP. PLP is the cofactor of many enzymes responsible for the synthesis of neuromediators such as serotonin, dopamine, histamine, and taurine whose level is likely impaired in babies with hypophosphatasia [11,21,22].

6. Prurinergic metabolites

ALPL is an ectonucleotidase that hydrolyses ATP, adenosine diphosphate (ADP) and adenosine monophosphate (AMP) to yield adenosine in various tissues (nervous tissue, bone, liver, epithelium of the nasal cavities and bronchi). In the nervous system ALPL thus plays a role in the regulation of synaptic transmission by prurinergic signaling [6,23]. Activation of subunits of protein G associated with adenosine receptors induces, via phosphoinositides, membrane changes that have repercussions on ion channels and Transient Receptor Potential Vanilloid 1 (TRPV1) channels. This mechanism results in changes in neuronal excitability. ALPL is strongly expressed in spine and contributes to regulating membrane excitability in nociceptive neurons [6]. ALPL is also able to transform pro-inflammatory ATP and increase anti-inflammatory adenosine levels.

7. Phosphoethanolamine

Phosphoethanolamine is a phospholipid component and precursor. Its urinary concentration may become very elevated in severe forms of HPP. It has been found in the cerebrospinal fluid of a young patient [22]. It is a natural substrate of ALPL. However, it may also be metabolized by other pathways including a PLP-dependent phosphoethanolamine phospholigase. A relationship between phosphoethanolamine and epilepsy has been reported.

8. Conclusion

Several mechanisms may contribute to explaining the myopathic presentation: modification of phosphorus metabolites, consequences of PLP deficiency and energetic and metabolic consequences, neuronal function consequences of GABA concentration. The metabolic abnormalities, also involving intra-cerebral mediators, may have consequences with regard to the chronic pain syndromes in HPP. In an analysis of the essential importance of biochemistry for progress in physiology and medicine, Arthur Kornberg, who won the Nobel prize for physiology and medicine in 1959 for his discovery of the mechanisms of nucleic acid synthesis, recommended that enzymology, should particularly address investigation of the brain and behavior, fields that remain largely unexplored [24]. The concept holds true. Enhanced understanding of pathophysiology and greater experience of the effects of therapies on the chronic manifestations of HPP would certainly enable improved management of patients' neuromuscular and pain manifestation.

Statement of interests

C. Fonta has no conflicts of interest to report. J.-P. Salles has received fees from Alexion Pharmaceuticals for occasional interventions and expert reviews, and has been or is principal investigator or investigator for trials sponsored by the company.

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