Drugs of the Future 2010, 35(10): 839-843

CCC: 0377-8282/2010
DOI: 10.1358/dof.2010.35.10.1543300

HYPOPHOSPHATASIA: NEW ADVANCES

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CONTENTS

SUMMARY

Hypophosphatasia is a rare, inherited disorder characterized by deficient mineralization of bone and dental tissues. There are six manifestations of the disease based upon their time of onset: perinatal (lethal and benign forms), infantile, childhood, adult and dental. Each form can also be distinguished by characteristic features seen on X-ray and ultrasound. The severity of the disorder varies based on the time of onset and the type of mutations present that cause the disorder, over 200 of which have been identified. These mutations are present in the gene that encodes the bone/liver/kidney form of the enzyme alkaline phosphatase, which plays a role in breaking down pyrophosphates in order to generate inorganic phosphate for bone ossification. Currently, there are no curative treatments for hypophosphatasia and putative approaches have included enzyme replacement therapy, treatment with parathyroid hormone peptides, viral vector-mediated gene therapy, bone marrow transplant and stem cell therapies.

INTRODUCTION

Hypophosphatasia is a rare, inherited disorder characterized by skeletal and dental deformities resulting from defective mineralization. The incidence of hypophosphatasia has been documented worldwide, with prevalence determined at 1 in 100,000 of the population in Canada, although the incidence of the milder forms of the disorder is thought to be much higher (1). Patients with hypophosphatasia display a reduction in tissue-nonspecific alkaline phosphatase (TNSALP) hydrolase activity caused by mutations in the gene encoding the bone, liver and kidney isoform of alkaline phosphatase (*ALPL*). Alkaline phosphatases are present as membrane-anchored proteins on the surface of osteoblasts (bone-forming osteoprogenitor) and chondrocytes (cartilage-forming) (2). The function of TNSALP is not fully understood, but it is believed to be a multifunctional enzyme involved in processes such as bone and dental mineralization, hydrolysis of inorganic pyrophosphates (PPi), collagen and calcium binding, and vitamin B6 metabolism (3-6). To date, 221 mutations and 12 polymorphisms have been associated with the disorder, including missense, deletion, splice, nonsense, insertion/deletion and regulatory mutations (an updated online database of mutations is maintained at http://www.sesep.uvsq.fr) (7).

The disorder varies in severity depending on the type of genetic inheritance, and can be fatal when present in utero and in young children. Individuals carrying mutations in the *ALPL* gene suffer from one of six manifestations of the disorder, which include: perinatal lethal, perinatal benign, infantile, childhood, adult and dental (reviewed in 4, 8, 9). There are currently no therapies available to cure hypophosphatasia, although a number of clinical trials investigating the use of enzyme replacement therapy (10-17), parathyroid hormone treatment (18-20) and bone marrow transplant with or without the addition of stromal stem cells have been examined (21, 22).

FUNCTION OF TISSUE-NONSPECIFIC ALKALINE PHOSPHATASE (TNSALP)

Alkaline phosphatases are a family of four enzymes that catalyze the hydrolysis of phosphomonoesters to release inorganic phosphate (Pi), the rate of which depends on the subunit configuration of the active protein (23). The bone/liver/kidney-specific TNSALP shares 50% homology with the other family members, although the gene encoding it is positioned on a different chromosome (chr: 1p36.1–34) (24) and comprises two or four subunits, each of which contains an active catalytic domain, two Zn^{2+} ions and one Mg^{+} ion (25). TNSALP cleaves the extracellular substrates PPi, pyridoxal-5′-phosphate (PLP, the phosphorylated form of vitamin B6) and phosphoethanolamine (PEA) (3, 5, 26-28). The hydrolysis of PPi promotes osteoblastic mineralization by generating Pi required for the formation of hydroxyapatite crystallization (29, 30). TNSALP hydrolyzes PLP to the apo-vitamer, pyridoxal, which can cross the blood–brain barrier, where it acts as a substrate for the generation of further PLP, which is essential for the synthesis of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (9). Such an inability to produce TNSALP could therefore lead to seizure-like activity due to ineffec-

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tive vitamin B6 metabolism, as has been evidenced in *Alpl* knockout mice (31).

CLINICAL PHENOTYPES OF HYPOPHOSPHATASIA

There are currently six known forms of hypophosphatasia (9). Perinatal hypophosphatasia can be detected in utero. In the lethal form, fetal X-ray and ultrasound scan show bone hypomineralization and lack of ossification, specifically in the thoracic region of the spine, and the presence of osteochondral spurs protruding from the forelimbs, patchy ossification of the ribs and shortening of long bones (32, 33). Postnatal lethality occurs due to respiratory complications arising from rickets of thoracic bones and the arrested development of the lungs (8). Infants with this form of hypophosphatasia also show signs of seizures, likely due to malfunction in vitamin B6 metabolism (31). Similar prenatal attributes are seen in the more recently discovered perinatal benign form of hypophosphatasia, although in benign cases postnatal amelioration of skeletal defects occurs and a milder form of the disorder ensues (34).

The onset of infantile hypophosphatasia between birth and 6 months is characterized by rickets and breathing abnormalities due to thoracic deformation, premature fusion of the skull plates (craniosynostosis) leading to increased intracranial pressure, and demineralization in the metaphyses (growing point) of long bones, early loss of deciduous teeth and hypercalcemia (8, 9, 35). This form of the disorder can also be lethal, but those who survive show a general improvement in symptoms, displaying only the persistence of craniosynostosis and short stature in adulthood (9).

In childhood hypophosphatasia there are a range of skeletal deformities, in particular the presence of bony deposits at the end of long bones. Patients are more predisposed to bone fractures, show loss of dentition, rickets and have low bone mineral density compared to other children of similar age (osteopenia), which can lead to childhood osteoporosis, with improvement in adolescence and recurrence in adulthood (35).

The onset of adult hypophosphatasia is seen in middle age and is characterized by early loss of adult teeth, painful pseudofractures of the thighs and stress fractures of the metatarsals. As the patient ages, calcium pyrophosphate dihydrate crystal accumulation in the cartilage of joints is common (36).

The final form, odontohypophosphatasia, is characterized by loss of dentition, severe dental caries, reduced alveolar bone, enlarged pulp chambers and root canals. Patients show biochemical profiles of reduced serum and bone TNSALP activity, but usually do not present the skeletal deformities seen in other forms of hypophosphatasia (37).

In conjunction with radiological findings, the presence of hypophosphatasia can be supported by biochemical analysis of serum and urine. Patients usually present low levels of serum TNSALP activity and elevated levels of serum and urine PPi, as well as elevated levels of urinary PEA and PLP (5, 8).

GENETIC BASIS OF HYPOPHOSPHATASIA

The distinct forms of hypophosphatasia are not inherited in the same manner, which may account for the difference in the severity of the disorder. All forms of hypophosphatasia can be inherited in an autosomal recessive manner, but only adult and dental manifestations may be inherited in an autosomal dominant manner, although there have been reports of the infinite benign form being a dominant inheritance (29, 38-40). Recessive individuals with heterozygous alleles may present biochemical markers associated with hypophosphatasia, such as low serum TNSALP, but may display only mild or absent clinical abnormalities, the severity of which is dependent on the type of *ALPL* mutations present. The likelihood of the sibling of an autosomal recessive patient being a heterozygous carrier themselves is 50%, while 25% will present symptoms (homozygous recessive) and 25% would not present symptoms or be carriers (homozygous dominant). For individuals with autosomal dominant hypophosphatasia, the chance of progeny inheritance of the severe clinical characteristics of the disorder increases to 50% for heterozygous patients (7-9, 38-40).

To date, 221 mutations and 12 polymorphisms associated with hypophosphatasia have been identified. A patient normally possesses one or two mutations of the *ALPL* gene. As with any genetic disorder, these mutations are demographic-specific, with certain mutations being rare in one population and common in another. For example, in the Japanese population over 40% of the mutations in the *ALPL* gene are the T1559del mutation (41), while in Caucasian populations the missense E174K mutation is the most frequent at 7%, increasing to 50% for Caucasians with European ancestry (42). In the USA the most common mutation is the 133A>T mutation, which causes the perinatal benign form of hypophosphatasia (43).

The impact of such mutations on the clinical phenotype of the disorder is difficult to assess, as patients often present more than 1 of the 232 causative mutations and may present in an autosomal recessive or dominant manner. In one case study, siblings with the same two mutations in the *ALPL* gene presented mild perinatal benign hypophosphatasia, although one sibling presented more severe clinical symptoms than the other (44). The reasons for this are unknown and further compound the difficulties in correlating mutation with severity. Despite these anomalies, clear correlations have been associated with some common mutations and mild hypophosphatasia in European populations, namely the missense mutations R119H and E174K (42, 45). Similar correlations have been made for the missense F310L mutation in the Japanese population (41). In addition, experimental site-directed mutagenesis of the TNSALP proteins has provided some evidence of a correlation between specific mutations and enzyme activity. The type of mutation present and how it disrupts the normal function of the enzyme correlate well with the severity of disease. Those mutations causing the production of nonfunctional proteins correlate to the severe infantile forms of hypophosphatasia, whereas mutations causing interference with anchoring of the protein to the cell membrane or a decrease in kinetic activity of the enzyme are generally present in the milder forms (46).

TREATMENT AND MANAGEMENT

There are currently no successful therapies for curing hypophosphatasia and management of symptoms is the present course of action. Nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin are prescribed to help alleviate bone-associated pain in chil-

dren and osteoarthritic inflammation in adults, while teriparatide (recombinant human parathyroid hormone 1-34) has been used successfully to treat metatarsal stress fractures in adults. Supplementation with vitamin B6, calcium and phosphate has been shown to be successful in treating seizure activity associated with infantile and childhood forms of hypophosphatasia. Patients are generally advised to undergo routine dental checkups and exercise dietary caution by avoiding phosphates (47). For patients with the less severe, nonlethal form of dental hypophosphatasia, therapy comes in the form of dental prosthetics rather than any curative measure, and recent case studies illustrate the benefits of such prosthetics on patient well-being (48, 49).

Initial attempts at enzyme replacement therapy using infusions of purified human hepatic alkaline phosphatase as a means to cure infantile hypophosphatasia have so far proven unsuccessful despite improvements in biochemical markers (12). Additional studies utilized infusions of bone alkaline phosphatase-rich plasma from patients with Paget's disease of bone. In these investigations, four patients showed improvements in biomarkers of hypophosphatasia, such as elevated serum enzyme levels, and improvements in hypercalcemia and hypercalciuria, but no improvements in other biomarkers such as urinary PPi. Furthermore, there were no observable improvements in bone mineralization or skeletal defects such as rickets (10, 11).

Investigations into enzyme replacement therapy using alternative approaches have been carried out in murine models of hypophosphatasia. The *Alpl*$^{-/-}$ knockout mouse has a similar phenotype to infantile hypophosphatasia, displaying abnormal bone mineralization, retarded growth, seizures and premature lethality. In one study, lentiviral-mediated delivery of a recombinant virus expressing bone-related TNSALP increased serum alkaline phosphatase activity concomitant with an ablation of epileptiform activity, improvement in bone mineralization and a 30-fold increase in life expectancy (50). In a second study, an engineered bone mineralization-targeting alkaline phosphatase, sALP-FcD10, was injected into *Alpl*$^{-/-}$ mice at birth. These mice adopted a normal phenotype, with the ablation of epilepsy, skeletal and bone deformities, in addition to displaying a reduction in biomarkers of infantile hypophosphatasia such as elevated serum calcium, PPi and pyridoxal (51). In a second *Alpl*$^{-/-}$ murine study, treatment of newborn knockout mice with s.c. injections of ENB-0040 (0.5, 2.0 and 8.2 mg/kg for 43 days), a bone-targeted human recombinant TNSALP fusion protein, induced dose-dependent improvements in skeletal mineralization of the feet, pelvis and ribs. In addition, these mice also showed dose-dependent improvements in life span from 19 days in vehicle-treated animals to 24, 31 and > 44 days in treated animals (52, 53). These studies are a promising example of targeted enzyme replacement therapy, which suggest that earlier attempts at enzyme replacement therapy in hypophosphatasia may have shown poor efficacy due to lack of bone-specific interactions (54).

This form of therapy has now been assessed in treating hypophosphatasia in a multicenter, open-label phase I protocol using ENB-0040. Two patients with infantile hypophosphatasia showed considerable remineralization of the skeleton, improved pulmonary function, growth and motor development within a month after receiving an i.v. injection of ENB-0040 (2 mg/kg) and subsequent s.c. injections of 3 mg/kg three times a week. In the same study, a patient with the perinatal form of hypophosphatasia presented corrected hypercalcemia after 8 weeks of s.c. treatment (13-15). No serious adverse events or immunological responses have been observed in children or adults receiving ENB-0040 (13-16). In adult patients bioavailability was found to be 50% following s.c. administration, with a concomitant increase in ALP serum activity to supraphysiological levels (16).

Finally, an open-label, randomized phase II investigation was conducted in children (5-12 years old) with hypophosphatasia (17). ENB-0040 was administered at 2 or 3 mg/kg s.c. three times for 6 months. There were no serious adverse events related to treatment and improvement in serum alkaline phosphatase activity, bone mineralization, endurance, strength and mobility was seen in all patients within 6 weeks. In seven of eight patients, levels of serum PLP were corrected within 6 weeks. By week 12, 3 patients were assessed for endurance in a 6-minute walk test and displayed distance improvements of 11-29%.

Other approaches to treatment have included the use of recombinant parathyroid hormone (PTH) in the form of teriparatide to stimulate osteoblast production of TNSALP in adult hypophosphatasia. In the first successful clinical evaluation of teriparatide, a middle-aged woman was treated with daily s.c. injections. Within 6 weeks of onset of treatment bone-related femoral pain had significantly improved. Furthermore, within 2-4 months both spreading metatarsal stress and proximal femur fractures showed significant repair and healing, respectively. Hypophosphatasia biomarkers also showed significant improvement in bone mineralization (55).

Additional investigations have assessed the use of longer forms of PTH (PTH[1-84], Preotact®). Two female siblings (aged 56 and 64 years), both with adult hypophosphatasia, were treated daily with Preotact® for 7 and 18 months, respectively. Both patients showed elevated serum TNSALP, improvements in bone-related pain and mobility, and healing of fractures over the course of treatment (19). Similarly, following a 2-year course of teriparatide therapy, an elderly female who had suffered fractures since the age of 10 showed marked improvement in hypophosphatasia symptoms (21).

In stark contrast to the above studies is the case of an adult with hypophosphatasia in whom teriparatide treatment did not produce sustainable improvement. In this study, a middle-aged woman showed improvement similar to that noted in the previous studies, but improvement in biomarkers, mobility and bone-related pain was not sustained when assessed 8 months after cessation of treatment (20). Such discrepancies in treatment response likely arise from the genetic heterogeneity of hypophosphatasia. Carriers of certain mutations may respond more favorably to one form of treatment compared to those with a different set of mutations.

Another promising approach to treatment in infantile hypophosphatasia has been the use of cell therapy, including bone marrow transplant (BMT) and the implantation of bone fragments, cultured osteoblasts or nonimmunogenic mesenchymal stromal cells (MSCs). Stem cell therapy using MSCs has been particularly attractive in the search for the origin of the elusive osteoprogenitor, the osteoblast. MSCs can be differentiated into osteoblast cells in vitro, although whether this can occur in vivo remains to be seen (56). In

2003, the first report of successful BMT in an 8-month-old female with infantile hypophosphatasia was published. Three months post-transplant rickets healed and bone mineralization was significantly improved, although by 6 months skeletal defects began to recur. Almost 2 years after the initial transplant the infant received an additional transplant that had been enriched for stromal cells, a small proportion of which were MSCs. This combination of stromal cells and BMT significantly improved and prolonged corrections in skeletal defects, which the authors state were still apparent more than 6 years later, despite the persistence of biochemical markers associated with hypophosphatasia (22).

A trial in a similar patient using i.v. injection of mixed stromal cells and crushed bone fragments concomitant with i.p. injection of bone fragments demonstrated similar results, with skeletal improvements sustained for several years (23). A recently reported study assessed the potential for using a patient's own MSCs to restore function. Katsube et al. introduced a viral vector expressing TNSALP under the control of its own promoter into MSCs derived from a patient with hypophosphatasia. In the absence of the vector, the cells had low levels of TNSALP and were unable to produce bone mineralization when supplied with osteogenic culture conditions; however, cells transduced with the vector elicited a sevenfold increase in alkaline phosphatase and demonstrated the potential for bone mineralization. When used in vivo in a mouse model, cells transduced with the vector were able to form bone with a frequency of 50% compared to those treated with nontransduced MSCs (57).

FUTURE OUTLOOK

Some promising clinical improvements have been noted using BMT enriched with stromal cells and bone fragments in younger patients, and this warrants further investigation in additional clinical trials. The recent positive advances utilizing target-directed enzyme replacement therapy and MSC manipulation also look promising in murine models of hypophosphatasia and in early clinical trials, and future translation to the clinic is an ongoing goal in hypophosphatasia research. Continued investigation into genotype–phenotype correlations, in addition to continued identification of polymorphic variants, could also prove useful in clinical diagnosis, genetic counseling and tailored therapy. Investigations into the specific functions of TNSALP in general could also prove fruitful in identifying additional therapeutic targets for generating novel treatments. Indeed, it has been suggested that one such approach could include inhibition of other proteins that may act to antagonize the function of TNSALP, as has been suggested for proteins involved in the metabolism and trafficking of PPi, the accumulation of which inhibits mineralization (8).

DISCLOSURES

The author states no conflicts of interest.

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