Hypophosphatasia in Children: Enzyme Replacement Therapy Using Bone-Targeted, Tissue-Nonspecific Alkaline Phosphatase

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Abstract

Hypophosphatasia (HPP) features low serum alkaline phosphatase (ALP) activity caused by deactivating mutation(s) within the gene that encodes the tissue-nonspecific isoenzyme of ALP (TNSALP). The natural substrates for TNSALP that accumulate extracellularly include inorganic pyrophosphate (PPi), an inhibitor of mineralization, and pyridoxal 5'-phosphate (PLP), the principal form of vitamin B6. Rickets occurs because PPi blocks hydroxyapatite crystal growth within the skeletal matrix. HPP severity spans stillbirth from profound skeletal hypomineralization to osteomalacia late in adult life. There is no established medical treatment. ENB-0040 is a bone-targeted, human recombinant, TNSALP fusion protein that preserved skeletal mineralization and survival in a TNSALP knockout mouse model of severe HPP (J Bone Miner Res 23:777, 2008). Patient trials began in 2008. In a 6-mo, open-label study initially of 6 pts (≤ 3 yrs) with life-threatening HPP, substantial skeletal remineralization, weaning from respiratory support, and improved motor development occurred with ENB-0040 (2 mg/kg IV followed by 1-3 mg/kg SC 3x/wk). Now, we report a phase II, open-label assessment of ENB-0040 in 13 children with HPP, ages 5-12 yr (2 girls, 11 boys), randomized to receive either 2 or 3 mg/kg SC 3x/wk for 6 mo. One pt awaiting scoliosis surgery was withdrawn, and 12 finished Wk 12. The complete (24-week) study will be reported. Transient injection site erythema was common, but there were no drug-related SAEs. Serum PPi and PLP levels, significantly elevated in all patients at baseline, declined in all and were normal in 8 and 10, respectively, at Wk 12. Increases in circulating parathyroid hormone from enhanced skeletal mineralization occurred in most pts, but without symptomatic hypocalcemia from "hungry bones". Skeletal radiographic improvement was present in nearly all pts by Wk 12; in some as early as Wk 6. All pts reported increased strength, endurance, and mobility within several weeks of ENB-0040 treatment. There was a mean increase in walking distance of 84 meters on a 6-minute walk test at Wk 12 (baseline of 325 m was subnormal), and mean strength and agility composite scores increased from the 3rd to the 10th percentile on the BOT-2. This change accompanied the appearance of running, jumping, and hopping skills. Pain troubled 7 pts at baseline, and only 3 at Wk 12. ENB-0040 is a promising enzyme replacement therapy for children with hypophosphatasia.

Introduction

Hypophosphatasia (HPP) is the inborn-error-of-metabolism characterized by low serum alkaline phosphatase (ALP) activity.(16) and caused by autosomal dominant or autosomal recessive inheritance of loss-of-function mutation(s) within the gene that encodes the "tissue-nonspecific" isoenzyme of ALP (TNSALP).(17) TNSALP is ubiquitous and especially rich in bone and liver.

Because TNSALP is normally coupled to cell surfaces,(6) its natural substrates (Fig. 1) accumulate extracellularly in HPP.(17) Among these phosphocompounds, inorganic pyrophosphate (PPi), is a potent inhibitor of hydroxyapatite crystal growth (Fig. 2).(1) Hence, extracellular excesses of PPi in HPP (11,12) cause rickets in children and osteomalacia in adults.(8) Pyridoxal 5'-phosphate (PLP), the major circulating form of vitamin B_6, also accumulates extracellularly in HPP. In severe HPP there may be vitamin B_6 (pyridoxine) responsive epilepsy from insufficient hydrosis of extracellular PLP to provide PL (pyridoxal) for intracellular metabolism (2). There appear to be additional, as yet uncharacterized, natural substrates for TNSALP in HPP.

Expressivity of HPP ranges remarkably, and spans intrauterine death with almost no skeletal mineralization, to poorly characterized dental problems in adults seemingly without bone disease.(16) The severity of HPP correlates with the degree of diminished serum ALP level and TNSALP substrate accumulation.(16,17)

Perinatal HPP manifests in utero. Most affected newborns live only briefly while suffering respiratory compromise. Extreme skeletal hypomineralization causes caput membranaceum and short deformed limbs.(17) Radiographs show severe rachitic changes in the limbs.

Infantile HPP presents before age 6 months.(18,20) Development seems normal until poor feeding, hypercalcemia, failure-to-thrive, hypotonia, and wide fontanels are noted. Rachitic deformities then appear, and pyridoxine-responsive seizures may occur which predict a fatal course.(2) Radiographic changes include a hypomineralized calvarium that gives the illusion of widely open fontanels, yet there may be functional craniosynostosis. Spontaneous improvement or relentless deterioration is possible.(16,17) Progressive skeletal demineralization and thoracic deformity with fractures herald a lethal outcome.(18,20)

Childhood HPP presents after age 6 months, and nearly always causes premature loss of teeth (< age 5 years).(5) Premature fusion of cranial sutures can lead to bony craniosynostosis.(2) Weakness, delayed walking with a waddling gait, short stature, and dolichocephaly are common.(2) Static myopathy is a poorly understood complication (Fig. 3).

Adult HPP usually presents during middle age, often with poorly-healing, recurrent fractures.(8)

Odonto-HPP refers to dental manifestations of HPP without rickets or osteomalacia.(16,17)

Fig. 1

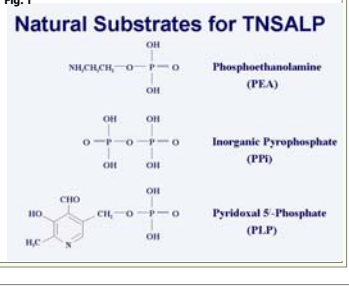


Fig. 2

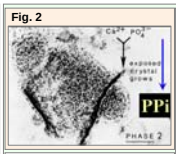


Fig. 3

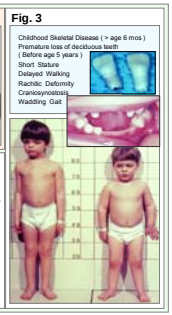
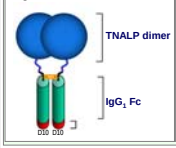


Fig. 4



Treatment

There is no established medical treatment for HPP. Unsuccessful attempts to rescue infantile HPP patients by intravenous infusions of soluble ALP in plasma from patients with Paget bone disease,(18,20) or purified from human placentas, suggested that TNSALP must be within skeletal tissue to mineralize bone and cartilage.(16-18,20)

ENB-0400 is an investigational, recombinant, fusion protein that couples human TNSALP to a deca-aspartate motif for binding to hydroxyapatite and thereby skeletal targeting (Fig. 4).(9) *tnsalp* knockout (null) mice recapitulate infantile HPP.(9) When these mice were given EnzRT using ENB-0040 daily by subcutaneous (SC) injection, the skeletal and dental manifestations and epilepsy of HPP were prevented.(9)

A phase I trial in 6 adults and a Phase II protocol for infants or growing children with life-threatening HPP showed minimal safety concerns, or, on preliminary study, anti-ENB-0040 antibodies. Here, we report a six-month Phase II trial of EnzRT using ENB-0040 for children with HPP.

Methods

ENB-0040 was produced by Laureate Pharma, Inc. (Princeton, NJ) in transfected Chinese Hamster Ovary cells.

This six-month, Phase II, multinational, open-label investigation of the safety, tolerability, pharmacokinetics (PK), and efficacy of ENB-0040 treatment involved 12 children (ages 5—12 years, Tanner Stage ≤ 2) with rickets and gross motor deficits from HPP. Inclusion criteria included low serum ALP activity for age, plasma PLP ≥ 2x normal, serum 25-hydroxyvitamin D ≥ 20 ng/ml, and radiographic rickets. Exclusion criteria included significant co-morbid disease, a treatable rickets, bisphosphonate exposure, hypocalcemia or hypophosphatemia, or experimental treatment for HPP (e.g., bone marrow transplantation). The protocol was approved by the appropriate ethics committees, and informed consent and assent were obtained. The U.S. clinicaltrials.gov registration number is NCT00952484 (http://clinicaltrials.gov/). An extension study is in progress.

The study's primary efficacy measurement was the impact of ENB-0040 therapy on the HPP-related rickets assessed radiographically in wrists and knees. Secondary and exploratory objectives included the PK of ENB-0040, patient respiratory status, growth, strength, pain and disability, gross motor functional capabilities,(3) plasma PLP and PPi(4), panorex, iliac crest biopsy changes, and dual-energy x-ray absorptiometry (DXA).

Based on predetermined radiographic characteristics of HPP, three radiologists will "blindly" evaluate the sequential wrist and knee extremity images of the patients and historic controls.

Serum ENB-0040 was quantitated using a colorimetric enzymatic assay in which p-nitrophenol is generated from p-nitrophenyl phosphate at 37°C and pH 9.(22) The method generated levels expressed as U/L which were ~ 25% those reported as IU/L from clinical laboratories for serum total ALP activity. The pharmacodynamics (PD) of ENB-0040 were evaluated by quantifying plasma PLP at Covance Laboratories (Indianapolis, IN), and serum PPi by a research technique.

Safety monitoring included assessments of adverse events (AEs), infusion or injection associated reactions (IARs), and preliminary screening for anti-ENB-0040 antibodies using a solid phase, colorimetric, bridging antidrug antibody assay (Cambridge Biomedical; Cambridge, MA). Safety and efficacy were monitored at 6 week intervals, and *ad hoc* by an independent Data Safety and Monitoring Board (DSMB).

In *tnsalp* null mice, dose-ranging studies had revealed that serum ENB-0040 levels of 650 – 1000 U/L from SC injections preserved skeletal mineralization in most animals.(9) In adult patients with HPP, the Phase I study had shown peak and trough levels of ENB-0040 at this target range (data on file) after one 3 mg/kg body weight IV dose and weekly 2 mg/kg SC doses. For the current pediatric study, patients were randomized to receive either 2 or 3 mg/kg ENB-0040 SC injections thrice weekly (concentration 100 mg/ml). Vitamin D supplementation was not given.

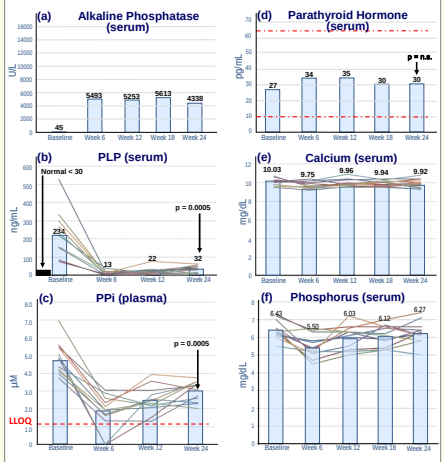
Results

The biochemical responses to EnzRT are summarized in Figs. 5a – f. The radiographic improvement in patient rickets is illustrated in Fig. 6. Following subcutaneous injection of ENB-0040, serum ALP levels increased substantially and were maintained at least at the target range throughout the study. As anticipated, plasma levels of the natural substrates PPi and PLP decreased.

Positive skeletal mineral balance with healing rickets was heralded by increases in serum parathyroid hormone levels, although there was no overt hypocalcemia from "hungry bones". The improvements in patient function and pain are shown in Fig. 7.

Fig. 5

Biochemical Results
(Preliminary Data)



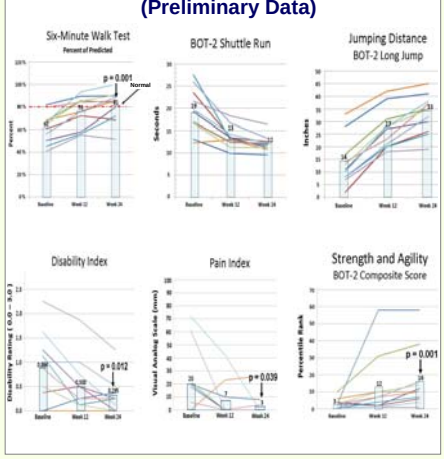
PLP Baseline values for patient 01-08 and 02-01 are not included in this dataset.

Fig. 6

Radiographic Results

Fig. 7

Functional Testing
(Preliminary Data)



Safety

Injection Site Reactions

SC injections of ENB-0040 were well tolerated. Injection site reactions, consisting of transient skin discoloration were common. Retinal findings, renal ultrasound studies, and skin examination showed no evidence of ectopic mineralization. Preliminary studies revealed low titer anti-ENB-0040 antibodies in some patients.

Discussion

Childhood HPP has wide-ranging expressivity, but typically features rachitic changes on radiographs and a static myopathy that causes fine and gross motor weakness and delayed motor milestones.

There is no established medical therapy for HPP.(16,17) Earlier attempts to rescue infantile HPP by repeated IV infusions of soluble bone ALP(20) or soluble placental ALP were unsuccessful. It seemed necessary to increase ALP activity directly within skeletal tissue. In addition, avoiding ALP excesses elsewhere, that could cause ectopic calcification if too much PPi was hydrolyzed, appeared optimal. Low circulating PPi levels are pathogenic in generalized arterial calcification of infancy, a lethal genetic disorder featuring defective extracellular production of PPi.

Here, we found a bone-targeted, enzyme replacement therapy, ENB-0040, to be safe and well tolerated and effective in children with HPP. The twelve patients with infantile or childhood HPP completed six months of ENB-0040 treatment. Their skeletal remineralization occurred gradually without symptomatic hypocalcemia from "hungry bones," and was heralded by reduction in urine calcium levels and increases in serum PTH concentrations. Serum 25-hydroxyvitamin D levels were adequate at baseline. Positive mineral balance throughout the skeleton, obvious radiographically after several months of treatment (Fig. 6), paralleled improvement in nearly all complications of childhood HPP. The clinical investigators observed radiographic skeletal improvement by the end of the six-month study in all patients. Responder analysis of radiographic change in treated patients versus historic controls by blinded raters is pending. Most patients experienced significant gains in strength and gross motor skills.

Although the pathogenesis is not understood,(16,17) HPP often causes weakness, seemingly from a static myopathy. When etidronate (a synthetic analog of PPi and first-generation bisphosphonate) was tested for Paget bone disease, excessive doses led to osteomalacia, and muscle weakness that together were reminiscent of HPP (Herbert Fleisch, M.D., personal communication). Possibly, our patients' improved agility and motor function from ENB-0040 therapy partly reflected healing of rickets, but also hydrolysis of PPi or some as yet unrecognized myopathic TNSALP substrate.

Although ENB-0040 treatment markedly elevated serum ALP levels in our patients, we found no evidence that it caused ectopic mineralization. Perhaps this reflected reduction, but not over-correction, of extracellular PPi. Mineral targeted ENB-0040 did not cause nephrocalcinosis or retinal changes. We were also concerned that dermal injections of ENB-0040 could precipitate calcium and Pi locally. Mineralization of extracellular matrix can occur when sufficient ALP activity is expressed in the presence of fibrillar collagen.(22) However, the SC injections of ENB-0040 were well tolerated. We also worried about bony craniosynostosis, but history, physical and fundoscopic examinations at six months and head circumference measurements did not reveal concerns. Our patients receiving ENB-0040 continue to improve during the extension study, now with as much as a total of eleven months of treatment.

Summary

In 12 children with HPP and rickets after 6 months of ENB-0040 therapy:

- ENB-0040 was safe and well-tolerated.
- There were no drug-related SAEs.
- Transient, dose-dependent injection site reactions occurred.
- There was no symptomatic hypocalcemia from "hungry bones".

Efficacy:

- Improved radiographic appearance, motor function, strength, & agility.
- Reduced biomarkers of disease and bone pain.

Conclusions

Recombinant, human TNSALP coupled to a deca-aspartate motif for bone targeting (ENB-0040) was well-tolerated when given by SC injections for six months to twelve children with HPP. Treatment was associated with healing of skeletal disease as well as improved motor function.

ENB-0040 is an effective enzyme replacement therapy for children with hypophosphatasia.

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