Hypophosphatasia and Alkaline Phosphatase – What Are The Clinical Issues?

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Center for Metabolic Bone Disease and Molecular Research, Shriners Hospital for Children; and
Division of Bone and Mineral Diseases, Department of Internal Medicine, Washington University School of Medicine;
St. Louis, Missouri, U.S.A.

Alkaline Phosphatase: We’ve Been Assaying It For 93 Years, But What Does It Do?
(Role Revealed in Hypophosphatasia)

Disclosure
Honoraria, Travel, and Research Grant Support;
Alexion Pharmaceuticals, Cheshire, CT
asfotase alfa (Strensiq ™)*

* biologic for pediatric-onset hypophosphatasia

Robert Robison, Ph.D (1883-1941)

C. R. Harington
Obituary Notices of Fellows
of the Royal Society,
Vol. 3, No. 10 (Dec., 1941), pp. 929-939

Biochemical Journal 17:p.286-293, 1923
XXXIII. THE POSSIBLE SIGNIFICANCE
OF HEXOSEPHOSPHORE S ESTERS
IN OSSIFICATION.
By ROBERT ROBISON.
From the Biochemical Department of the Lister Institute.

I believe that both mechanisms play a part in normal calcification of the skeleton—the phosphatase producing in the matrix of hypertrophic cartilage and osteoid tissue the necessary degree of supersaturation, and the second mechanism assisting in the deposition of the bone salt from this supersaturated solution in some way not yet understood.

“Bone Phosphatase”
Evidence that ALP Acts in Skeletal Mineralization

• Robison’s discovery that bone/cartilage is rich in ALP Activity
• Initial site of hydroxyapatite crystal formation is within ALP-rich extracellular matrix vesicles
• Serum ALP activity reflects rates of skeletal formation

Evidence Against a Role For ALP in Skeletal Mineralization

• ALP assay not “physiologic” [alkaline pH (e.g., 10.2) with nonphysiologic substrates]
• ALP abundant in tissues that do NOT calcify

Suggested Roles for ALP in Skeletal Mineralization

• Locally increases Pi Concentration (Robison’s Hypothesis)
• Pi Transport
• Phosphotyrosine-specific phosphoprotein phosphatase
• Ca++ Binding protein
• Ca++ ATPase
• Hydrolysis of Inhibitor
Human Alkaline Phosphatases (ALPs)

1. Placental
2. Intestinal
3. Germ Cell

Tissue-specific

4. Ubiquitous (Bone & Liver)

Tissue Nonspecific (TNSALP)

Bone and Liver ALP are Isoforms of TNSALP (differ by post-translational modifications)

John C. Rathbun, M.D. (1915-1972)

Homodimeric TNSALP

Structure of the Human Liver/Bone/Kidney Alkaline Phosphatase Gene*

To cite, please use the reference:

Am J Diseases Child 75 : 822-31, 1949

"HYPOCHONDRASIA"
A New Developmental Anomaly

J. C. Rathbun, M.D.
TORONTO, CANADA
Hypophosphatasia (HPP)

- ~800 Literature Cases
- Affects all races
- Incidence: 1:100,000 Births

Hypophosphatasia

- Heritable Metabolic Bone Disease
  - Defective Skeletal Mineralization
    - Infants & Children → Rickets
    - Adults → Osteomalacia
- Biochemical Hallmark
  - Low Serum ALP Activity
- Inborn Error of Metabolism
  - Autopsy: Global Deficiency of TNSALP Activity
    (Intestinal & Placental ALP Are Normal)

Vol. 85, pp. 7666-7669

Hypophosphatasia

- Circulating calcium, phosphate, and vitamin D levels are not low
- Hypercalcemia (severe disease)
  - Hyperphosphatemia

Hypophosphatasia

- Greatest range of severity of all metabolic bone diseases
- Last rickets/osteomalacia to have a medical treatment
Loss-of-Function Mutations of TNSALP

~ 300 different defects worldwide
(~ 80% missense)

Inheritance

• Severe: Autosomal Recessive

• Mild: Autosomal Recessive
  Autosomal Dominant

Perinatal Hypophosphatasaia

Clinical Forms

- Perinatal
- Infantile
- Childhood
- Adult
- Odonto

Perinatal

• Skeletal Disease (Obvious at Birth)
• Stillbirth
• Deformed Limbs
• Respiratory Compromise
• Periodic Apnea and Bradycardia

Nosology according to age at Dx

“Skeletal Disease” (~ severity)
Infantile Hypophosphatemia

- Skeletal Disease (Before Age 6 months)
- Wide Fontanels
- Hypotonia
- Hypercalcemia
- Nephrocalcinosis
- Functional Craniosynostosis
- Failure To Thrive
- Flail Chest/Pneumonia
- Seizures

~ 50% succumb
~ 50% improve, but often with sequelae of rickets

Childhood Hypophosphatemia

- Skeletal Disease (After Age 6 months)
- Premature loss of deciduous teeth (Before age 5 years)
- Short Stature
- Delayed Walking
- Rachitic Deformity
- Craniosynostosis
- Static Myopathy
- Waddling Gait
Adult Hypophosphatasia

- Skeletal Disease Typically During Middle Age
- Premature Loss of Adult Teeth
- Osteopenia
- Recurrent Metatarsal Stress Fractures
- Femoral Pseudofractures
- Pseudogout/Chondrocalcinosis/Calcific Periarthritis

Adult Hypophosphatasia with Chondrocalcinosis and Arthropathy

[Image of bone structure with arrows indicating areas of interest]
Odontohypophosphatasia (Childhood or Adult)

- No Radiologic Skeletal Abnormalities
- Premature Loss of Teeth

"One-Tooth" Odonto-HPP

Table 1: Differential Diagnosis of Hypophosphatasia Based on the Temporal Evolution of the Low Serum ALP Values

<table>
<thead>
<tr>
<th>Temporal Evolution of the Low Serum ALP Values</th>
<th>Differential Diagnosis</th>
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<tbody>
<tr>
<td>Periodontal disease</td>
<td>Deficiency of alkali</td>
</tr>
<tr>
<td>Catabolic efficacy</td>
<td>Alkali deficiency</td>
</tr>
<tr>
<td>Osteosclerosis/osteoporosis</td>
<td>Hypophosphatasia</td>
</tr>
<tr>
<td>Pseudohypophosphatasia (PHOS)</td>
<td>Hypophosphatasia</td>
</tr>
</tbody>
</table>

J Bone Miner Res.; 29:1651-60, 2014
Diagnosis

- Hyperphosphatasemia is a marker for bone or hepatobiliary disease
- Remarkable how often hypophosphatasemia is ignored

As mentioned before, low ALP may occur transiently after blood transfusions or cardiopulmonary bypass. Prolonged, severely low levels of ALP occur in hypophosphatasia, a rare inherited disorder of bone metabolism (Whyte, 1996). Decreased ALP can also occur in zinc deficiency, since zinc is a necessary cofactor for ALP activity.
Causes of Hypophosphatasemia

- Hypophosphatasia
- Familial Benign?
- Pernicious or Profound Anemia
- Hypothyroidism
- Vitamin C Deficiency
- Osteogenesis Imperfecta, Type II
- Wilson’s Disease (hemolytic anemia)
- Vitamin D Intoxication
- Inappropriate Reference Range
- Clofibrate Therapy
- Starvation
- Hypoparathyroidism
- ERT in post-menopausal women

- \( \text{Zn}^{2+} \) or \( \text{Mg}^{2+} \) Deficiency
- Cushing’s Syndrome
- Milk-Alkali Syndrome
- Celiac Disease
- Massive Transfusion
- Cleidocranial Dysostosis
- Cardiac Bypass Surgery
- Improperly Collected Blood (e.g., EDTA, oxalate)
- Radioactive Heavy Metals
- Starvation
- Multiple Myeloma
- Achondroplasia

Pathogenesis

Natural Substrates for TNSALP

- Phosphoethanolamine (PEA)
- Pyridoxal 5-Phosphate (PLP)
- Inorganic Pyrophosphate (PPi)

TNSALP (ALPL) mutation analysis

Vitamin B₆
Normal Vitamin B<sub>6</sub> Status in Most Hypophosphatasia Patients

- No symptoms of Vitamin B<sub>6</sub> toxicity or deficiency
- Normal tissue levels of Vitamin B<sub>6</sub> metabolites
- Normal urinary levels of the degradation product, 4-pyridoxic acid
- Normal response to L-tryptophan challenge

PL levels are normal in all but the most severe HPP (low)
Phase 2 Mineralization

Calcium Pyrophosphate Dihydrate

Chondrocalcinosis

Pyrophosphate Arthropathy

TREATMENT?

Whyte et al., J Peds 101(3):379
Can we increase ALP activity directly in bone?
Infantile Hypophosphatasia: Transplantation Therapy Trial Using Bone Fragments and Cultured Osteoblasts

ALP Targeted To Bone

Asfotase Alfa

CLINICAL CASE SEMINAR
Adult Hypophosphatasia Treated with Teriparatide

PolyAsp (D10)
Mice lacking tissue non-specific alkaline phosphatase die from seizures due to defective metabolism of vitamin B-6

Nature Genetics
Vol. 11:45-51, 1995

Asfotase Alfa for Life-Threatening Hypophosphatasia

Radiographic, respiratory, and functional improvements.

March 8, 2012

Enzyme-Replacement Therapy in Life-Threatening Hypophosphatasia

Enzyme Replacement Therapy for Murine Hypophosphatasia

Abstract

Introduction: Hypophosphatasia (HPP) is a heritable disorder of mineralization that results from embryonic overexpression of alkaline phosphatase (ALP). Consequently, ameliorative therapies for this condition have failed to fully eliminate mineralization defects, insulin-like growth factor (IGF-1), an inhibitor of mineralization, and parathyroid hormone (PTH), a co-factor of vitamin D, besides the intangible treatment of HPP when using vitamin D and other treatments.
Infant Study

Patient 3 (Perinatal HPP)

Baseline

9 Weeks

First Patient

Baseline

Month 21

Patient 2 (Infantile HPP)

Baseline

3/4 months

2 ½ months

Duration Treatment

Age 2 years
2 years of treatment

Summary

- Treatment ≥3 years well tolerated.
- Infants and children with life threatening HPP showed significant improvement in skeletal mineralization, on average at 3 months, and sustained over 3 years
- Nearly all required some respiratory support either at baseline, or early in the study, but only a single patient required respiratory support (supplemental O₂) at last assessment
- Survival was 90%.

Adverse Events

<table>
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<th>Event</th>
<th>Patients, n (%)</th>
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<td>URI</td>
<td>67 (74%)</td>
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All patients reported ≥1 AE during the study

AEs were reported by investigators as primarily mild (73%) or moderate (21%) and unrelated to study drug (82%)

Serious Adverse Events

- Three SAEs were reported by investigators as possibly related to study drug:
  - Craniosynostosis and conductive deafness (same patient)
  - Mild chronic hepatitis in 1 patient, confounded by concomitant montelukast; montelukast was discontinued and liver function tests returned to within normal range by last assessment
- One patient died (septic shock, unrelated to study drug) at 7.5 months of treatment

3 years of treatment

7 years of treatment

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2 years of treatment

3 years of treatment

7 years of treatment

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PHASE II
Asfotase Alfa
FOR
CHILDREN WITH HYPOPHOSPHATASIA

Study design

- Phase II, open-label study; 1 site in USA, 1 site in Canada

- Major inclusion criteria:
  - 5–12 years old
  - Significant HPP-related skeletal disease

- Primary Endpoints:
  - Efficacy, safety and tolerability of asfotase alfa at 6 months
  - Skeletal health assessed radiographically

- Initial dose: 3 mg/kg/week
- Increased to 6 mg/kg/week after 3–9 months via protocol amendment

- Extension Phase (n=12)
  - Endpoints: Radiographic and functional improvements, growth/development, long-term safety
  - Initial dose: 3 mg/kg/week increased to 6 mg/kg/week after 3-9 months via protocol amendment

- Historical Controls (n=16) for radiographs
  - 1 patient withdrew
  - 12 patients continue treatment

Primary Outcome Objective:
Improvement of Radiographic Skeletal Disease

Secondary / Exploratory:
- Physical Performance Assessments
- Transiliac Crest Bone Biopsies
- Biochemical Alterations
- Growth

Primary endpoint:
Radiographic Global Impression of Change (RGI-C)

- Change in rickets severity assessed by a 7-point scale
- Paired radiographs
- Mean score of 3 independent radiologists blinded to time points after first radiograph

RGI-C scores at each visit

- P < 0.01 for all time points compared with 0

Metaphyseal Flare

Baseline
Week 24
### Physical Assessments

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<tr>
<th>Six-Minute Walk Test</th>
<th>BOT-2 Strength and Agility Composite</th>
</tr>
</thead>
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<tr>
<td>Hand-Held Dynamometry</td>
<td></td>
</tr>
<tr>
<td>• Grip</td>
<td>Running Speed and Agility</td>
</tr>
<tr>
<td>• Knee flexion</td>
<td>• 50 ft shuttle run</td>
</tr>
<tr>
<td>• Knee extension</td>
<td>• side step over beam</td>
</tr>
<tr>
<td>• Hip flexion</td>
<td>• one-legged stationary hop</td>
</tr>
<tr>
<td>• Hip extension</td>
<td>• one-legged side hop</td>
</tr>
<tr>
<td>• Hip abduction</td>
<td>• two-legged side hop</td>
</tr>
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#### Strength
- standing long jump
- push-ups
- curl-ups
- wall sit
- V-up

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#### Bruininks-Oseretsky Test of Motor Proficiency, 2nd Edition (BOT-2)

**Running Speed and Agility Test**

- Stepping over a balance beam
- Shuttle run
- Two-legged side hop
- One-legged side hop

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**Baseline**

Walked 350 meters

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**Patient 4 at Baseline**

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**Week 24**

Walked 401 meters

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**6 minute walk test**

![Image of 6 minute walk test](image-url)

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Patient 4 after 1 ½ years of Rx

Baseline

14 inches

Week 24

37 inches
**Safety**

- Transient, dose-dependent injection site reactions in all patients which are less prominent and less frequent with lower doses on the extension study
- No ENB-0040-Related SAEs
- No evidence of ectopic calcification on retinal examination or renal ultrasound
- Low Titer Anti-ENB0040 Antibodies

**Conclusions**

- The most common AEs were mild-to-moderate ISRs
- During treatment with asfotase alfa, improvements were observed as early as 6 months in children for:
  - Strength
  - Running Speed and Agility
  - Disability
  - Transfer and Basic Mobility
  - Sports and Physical Functioning
  - Pain
- Improvements were sustained through 5 years of treatment
**Asfotase alfa**

- 03 July 2015 – Japan (HPP)
- 14 August 2015 – Canada (Pediatric-Onset HPP)
- 28 August 2015 – Europe (Pediatric-Onset HPP)
- 23 October 2015 – United States (Pediatric-Onset HPP)

*STRENSIQ™*

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**Clinical Vignette**

Atypical Femoral Fracture, Bisphosphonates, and Adult Hypophosphatasia

Michael K. Wasilewski

J Bone Miner Res; 24:1132-4, 2009

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**Unique coexpression in osteoblasts of broadly expressed genes accounts for the spatial restriction of ECM mineralization to bone**

Trung-Duc Hoang, E. Paul Torok-Storb, and Fred H. Sugimura


---

**Adult Hypophosphatasia**

Management of Femoral Fractures and Pseudofractures in Adult Hypophosphatasia

Donald C. Melton, Adam J. Wroblewski, Giacomo Del Palmo, Sarah N. Wroblewski, Cristiano Brondino, Joshua R. Watkins, and John F. Langley


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**Atypical Subtrochanteric and Diaphyseal Femoral Fractures: Report of a Task Force of the American Society for Bone and Mineral Research**

Elizabeth A. Black, Carol A. Burtis, Rex Brown, Susan F. Calder, Robert A. Adami, Thomas J. Ahern, Richard A. Binkley, John W. Callahan, Thomas G. Coates, Stephen J. Cofield, Pippa M. Cowin, Dale D. Driscoll, Timothy M. Eads, Mark W. Engh, Steven C. Franklin, J. Donald Gartland, George H. Glucksman, D. Paul Harris, Peter J. Hutton, John C. Koval, John C. Klabunde, John L. Kiesewetter, Michael E. Koval, Vedant V. Kulkarni, Donald C. Lennarz, James M. Lewis, Donald C. Melton, and Joseph N. Minns
