9th International Meeting on Osteogenesis Imperfecta

June 13-16, 2005
Annapolis, Maryland, USA

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Monday, June 13, 2005	
2:00-7:00 PM	**Registration, Concourse Lobby**
5:00 PM, 5:45 PM and 6:45 PM	Bus Transportation from Sheraton Barcelo to Phillips Restaurant Departures from the Hotel at 5:00 PM, 5:45 PM and 6:45 PM
7:00 PM - 9:00 PM	**Crab Feast at Phillips Seafood Restaurant, Annapolis Waterfront** Joint Social Event of the Scientific and Leadership Conferences
9:00 PM, 9:30 PM and 10:30 PM	Bus Transportation from Phillips Restaurant to Sheraton Barcelo Deparatures from Phillips Restaurant at 9:00 PM, 9:30 PM and 10:30 PM

Tuesday, June 14, 2005		
7:00 AM - 8:00 AM	**Continental Breakfast, Wye Concourse**	
8:00 AM - 12:00 PM	**Registration, Concourse Lobby**	
9:00 AM -1:00 PM	**Poster Set-Up, Severn Room** Posters will remain up for viewing through 4:00 PM on Wednesday June 15. All posters are to be removed by the end of the day on June 15. Posters can be retrieved on June 16 at the Conference Information Desk.	
8:00 AM - 3:00 PM	**Conference Oral Presentations, Wye Room**	
8:00 AM	**Welcome - Marini & Raggio**	
Genetics - Genotype-Phenotype		**Chair - DePaepe**
8:15 AM	Byers	Splice Site Mutations in Type I Collagen Genes and Their Outcomes
8:40 AM	Barnes	Effects of C-Propeptide Mutations in Type I Collagen on Extracellular Matrix Deposition and Fibrillogenesis
8:55 AM	Marini	OI Mutation Consortium: Lethal Regions Align with Binding Sites in Collagen Monomer and Fibril
9:20 AM	Brodsky	Model Peptides: Defining the Alteration in the Folding Pathway of OI Collagens
9:45 AM	Persikov	Investigation of the Relationship Between Triple-Helix Destabilization by Gly to Ser Substitutions and Clinical Severity in Osteogenesis Imperfecta
10:00 AM	Di Lullo	Unraveling the Mysteries of Type I Collagen Structure-Function Relationships: Brittle Bones Break the Silence
10:15 AM	Reigle	Probing the Proteoglycan-Binding Function of the Lethal OI Mutation-Rich Regions in the alpha2(I) Chain of type I Collagen
10:30 AM - 11:00 AM	**Break**	
Biophysical Insights		**Chair - Byers**
11:00 AM	Leikin	Domain Organization of Collagen Triple Helix and Its Role in OI
11:25 AM	Plotkinov	Morphological Comparison of Normal and OI Collagen Fibers Visualized by Second Harmonic Generation Imaging Microscopy
11:40 AM	Canuto	Characterization of Skin Abnormalities Associated with Osteogenesis Imperfecta Via High-Resolution Magnetic Resonance Imaging
12:00 PM - 1:00 PM	**Lunch, Garden Room** (Int. OI Conference Committee meets during lunch)	

Bone and Cell Biology		Chair - Steinmann
1:00 PM	Rauch	Bone and Cell Biology in Osteogenesis Imperfecta:The Bone Tissue Level
1:25 PM	Ashok	Chaperone-Procollagen Interactions Differ with Mutation Location in Osteogenesis Imperfecta
1:40 PM	Osdoby	Bone Cell Interactions in Osteoclast Recruitment, Development, and Survival
2:10 PM	Zhang	Increased Osteoclast Activity in oim Mouse Osteoblast/Osteoclast *In Vitro* Co-Culture System
2:25 PM	Boskey	The Abnormal Mineral in Osteogenesis Imperfecta: How Collagen and Noncollagenous Proteins Affect Mineral Properties
2:50 PM	Forlino	Use of Microarray and Proteomics to Study the Phenotypic Variability in BRTLIV Mice
3:00 PM - 5:00 PM	**Poster Session, Severn Room** (Includes a brief report from the Leadership Conference)	

Wednesday, June 15, 2005		
7:30 AM - 8:30 AM	**Continental Breakfast, Wye Concourse**	
8:30 AM - 6:00 PM	**Conference Oral Presentations, Wye Room**	
9:00 AM - 4:00 PM	**Posters Available for Viewing, Severn Room**	
Insights from Murine Models		**Chair - Prockop**
8:30 AM	Forlino	Maturation or Homozygosity Modulates OI Phenotype In Brtl Mouse
8:55 AM	McBride	Col1a2 G610C Mice: A Knock-In Mouse Model Based on a Large Human OI Kindred With Phenotype Variation
9:20 AM	Kalajzic	Testing Therapeutic Approaches for Osteogenesis Imperfecta Using col1a1 Transgenic Mice
9:45 AM	Aubin	A Deletion in SMPD3 Causes Osteogenesis Imperfecta in the Mouse
10:00 AM	Demetrakopoulos	Effects of Soluble muRANK on Fracture Healing in the oim Mouse Model
10:15 AM	Morello	CRTAP is Required for Prolyl 3-Hydroxylation of Fibrillar Collagens and Loss of its Function Causes Severe Kyphoscoliosis and Osteoporosis in Mice
10:30 AM - 11:00 AM	**Break**	
Orthopedics		**Chair - Raggio**
11:00 AM	Raggio	Orthopaedist + OI: Advances or New Problems
11:25AM	Fassier	Experience with the Fassier-Duval Rod: Effectiveness and Complications
11:50 AM	Pruijis	The Influence Of Bisphosphonates On Orthopedic Management In Osteogenesis Imperfecta
12:15 PM	Marcdargent-Fassier	Radial Head Dislocation in Osteogenesis Imperfecta
12:30 PM	**Group Photo** (Location TBA)	
12:30 PM - 2:30 PM	**Lunch, Garden Room** (Orthopedics Case Conference during lunch)	

Bisphosphonates		**Chair - Bishop**
2:30 PM	Glorieux	Bisphosphonate Therapy in OI: Update on Efficacy and Safety
2:55 PM	Whyte	Bisphosphonate Treatment for Children: Assessing for Toxicity
3:20 PM	Letocha	Controlled Trial of Pamidronate in Children with Type III and IV Osteogenesis Imperfecta Confirms Vertebral Gains but Not Short-term Functional Improvement
3:35 PM	Goldstein	Biomechanical Characterization of the OI Phenotype: Evaluation Of Brtl IV and the Effect of Bisphosphonate Therapy
4:00 PM	Uveges	Alendronate Increases Bone Strength and Bone Volume But Fails to Improve Brittleness or Mineralization in BRTL Mouse Model for Type IV Osteogenesis Imperfecta
4:15 PM	Chevrel	Effects of Oral Alendronate on Bone Mineral Density in Adult Patients with Osteogenesis Imperfecta
4:30 PM	Shapiro	Results of Bisphosphonate Treatment in Adult Osteogenesis Imperfecta
4:45 PM	Malmgren	Effect of Bisphosphonate Therapy on Tooth Eruption in Children and Adolescents with Osteogenesis Imperfecta
5:00 PM	Astrom	Intravenous Pamidronate Treatment to Infants with Severe Form of Osteogenesis Imperfecta
5:15 PM - 6:00 PM	**Panel Discussion**	

Thursday, June 16, 2005		
7:00 AM - 8:00 AM	**Continental Breakfast, Wye Concourse**	
8:00 AM - 12:15 PM	**Conference Oral Presentations, Wye Room**	
Medical Management		**Chair - Shapiro**
8:00 AM	Engelbert	Osteogenesis Imperfecta in Childhood: Habilitation Strategies Regarding Family, Function and Fitness
8:25 AM	Gerber	Technology In The Clinic: Understanding Function In Children With Osteogenesis Imperfecta
8:50 AM	Kuurila-Svahn	Hearing Loss in OI - A Population Study in Finland
9:15 AM	Miyamoto	Cochlear Implantation in Osteogenesis Imperfecta
9:40 AM	Kovero	Skull Base Abnormalities in Osteogenesis Imperfecta - Cephalometric Evaluation of 54 Adult Patients and 108 Adult Controls
9:55 AM	Flor-Cisneros	Pulmonary Function Abnormalities in Children with Osteogenesis Imperfecta Correlate with OI Type and Location of Collagen Mutation
10:05 AM - 10:30 AM	**Break**	
Prospects for Gene and Cell Therapy		**Chair - Marini**
10:30 AM	Chamberlain	Targeted Disruption Of The Type I Collagen Genes In Mesenchymal Stem Cells Restores Properties Of Normal Collagen
10:55 AM	Davidson	RNA Interference for Neurogenetic Disease Therapy
11:20 AM	Wenstrup	Endogenously Expressed Multimeric Self-Cleaving Hammerhead Ribozymes Ablate Mutant Collagen
11:45 AM	Niyibizi	Bone Marrow Derived Osteoprogenitors Transplanted into Neonatal Osteogenesis Imperfecta Mice (oim) Contribute to the Bone Formation *In Vivo*
12:00 PM - 12:15 PM	**Summation**	

Genetics -
Genotype-Phenotype

Invited Speakers 1 - 3
Posters 1 - 13

S01

SPLICE SITE MUTATIONS IN TYPE I COLLAGEN GENES AND THEIR OUTCOMES

Peter H. BYERS, University of Washington, Seattle, WA

A little more than 90% of individuals with OI have mutations in the two type I collagen genes, *COL1A1* and *COL1A2*. Of these mutations, 20-25% reside at sites or affect splicing and the clinical outcomes extend across the entire spectrum of OI. Splice site mutations can lead to exon skipping, intron inclusion, or the use of cryptic splice sites that can lead to partial exon loss or partial intron inclusion in the resulting mRNA species. The effects of each of these types of outcomes depends on whether the mature mRNA species contain premature termination codons or simply have inserted or deleted in-frame sequences.

Mutations that lead to exons skipping in the *COL1A1* gene are generally lethal if the exon involved is 3' to exon 13. In the *COL1A2* gene, the phenotype is much more variable with exon skips. Splice site mutations in the *COL1A1* gene that lead to premature termination codons because of use of cryptic splice sites generally lead to the mild OI type I phenotype. In the *COL1A2* gene heterozygotes for such mutations may be asymptomatic. In many instances the outcome of splice site mutations is complex so that more than one product results.

From an analysis of portions of both genes, it appears that the outcome of a splice site mutation depends, at least in part, on the order in which the altered intron is removed in the wild-type gene. Mutations in introns that are removed rapidly generally lead to skipping of the adjacent intron while mutations in slowly removed introns usually result in use of cryptic splice sites or intron retention. If these mRNA species do not result in frame-shifts, then the clinical effects are generally moderately severe to severe, but are mild if frame-shifts and mRNA nonsense mediated decay result in loss of the product. At this point a portion of both genes have been mapped but the recent joint accumulation of mutations in the genes with their associated phenotype should permit rapid analysis and may lead to understanding of the sequences in the genes that determine rate of intron removal. (Supported in part by USPHS award AR41223)

Notes

P01

EFFECTS OF C-PROPEPTIDE MUTATIONS IN TYPE I COLLAGEN ON EXTRACELLULAR MATRIX DEPOSITION AND FIBRILLOGENESIS

Aileen M. BARNES[1], Sarah A. MILGROM[1], Emily MORIARTY[1], John P. CASELLA[2], Wayne A. CABRAL[1], James M. PACE[3], and Joan C. MARINI[1] [1]BEMB, NICHD/NIH, Bethesda, MD, [2] CEASR, Univ. of Derby, Derby, UK, [3]Dept. of Pathology, Univ. of Washington, Seattle, WA

Mutations in the C-propeptide of Type I collagen have been found in a small number of patients with Osteogenesis Imperfecta (OI). These mutations are of special interest because they are located in a region that is cleaved from the procollagen heterotrimer before collagen fibril assembly and are not expected to be present in collagen fibrils in tissues. Thus, their pathophysiological mechanism will differ from helical mutations. We identified 5 novel C-propeptide mutations by RT-PCR and sequencing of COL1A1 cDNA. Four involved substitutions at conserved residues: W1097C, D1233N (Type III OI), T1120I and P1266H (Type IV OI). The 5th proband (Type II OI) had a 6 nt deletion at the E51/I51 junction causing an in-frame insertion of 42 amino acids of I51 in cDNA. We compared proband fibroblasts with a lethal C-propeptide mutation (D1263Y; Pace, J Med Gen 2002), 3 mutations in the helical region adjacent to the C-propeptide (G898S, G967C, G997S) and control cells. All mutant collagens, including helical mutations, showed backstreaking of α1(I) steady-state collagen, suggesting a delay in helix folding. In normal control and G898S, pro-α1(I) chains incorporated into helix in ~20 minutes, as did most pro-α1(I) G967C chains, although a sub-population had longer incorporation time. The helical mutation closest to the C-propeptide, G997S, and all of the C-propeptide mutations had delayed chain incorporation. Processing assays suggested that there was defective C-propeptide cleavage from secreted collagen containing C-propeptide or helical mutations. For many probands, pro-α1 collagen persists and pC-α1 increases, suggesting that processing is blocked at pC-α1. Overmodified mutant collagens incorporated into fibroblast matrix in culture and formed mature cross-links. Skin and bone fibrils from two probands (T1120I, P1266H) were examined by electron microscopy (EM). Proband dermal fibril diameters were ~12% larger than control. SEM revealed disorganized bone fibrils with variable diameters. *In vitro* processing of mutant procollagens with C-proteinase ± procollagen C-proteinase enhancer (PCPE) is in progess. These investigations provide insight into the pathophysiological mechanism by which mutations not expected to be incorporated into ECM structure alter matrix organization and weaken connective tissue.

Notes

S02

CONSORTIUM FOR OSTEOGENESIS IMPERFECTA MUTATIONS: LETHAL REGIONS IN THE HELICAL PORTION OF TYPE I COLLAGEN CHAINS ALIGN WITH COLLAGEN BINDING SITES FOR INTEGRIN AND PROTEOGLYCAN

Joan C MARINI, Antonella FORLINO, Wayne A CABRAL, Aileen M BARNES, James D SAN ANTONIO, Sarah MILGROM, James C HYLAND, Jarmo KORKKO, Darwin J PROCKOP, Anne DEPAEPE, Paul COUCKE, Francis H GLORIEUX, Peter J ROUGHLEY, Alan M LUND, Kaija KUURILA, Daniel H COHN, Deborah KRAKOW, Monica MOTTES, James TROENDLE, Raymond DALGLEISH, Peter H BYERS. NICHD, NIH, Bethesda, MD; Thomas Jefferson Univ, Phila, PA; Tulane University, New Orleans, LA; Univ Ghent, Ghent, Belgium; Shriners Hospital, Montreal, Canada; Rigshospitalet, Copenhagen,Denmark; Vaasa Hosp, Vassa, Finland; Cedars-Sinai, Los Angeles, CA, Univ Verona, Italy; Univ Leicester, UK; Univ of Washington, Seattle, WA

The database of type I collagen mutations has 832 independent mutations in α1(I) and α2(I), including 682 glycine substitutions and 150 exon skipping defects. Distinct genotype-phenotype relationships emerge from the analysis for each chain, supporting different roles for the chains in matrix integrity. Lethality is not directly determined by disruption of collagen stability in either chain, although this may contribute to outcome.

In α1(I), substitutions from first position codon changes predominate; recurrences occur at CpG sequences. One-third of α1(I) Gly substitutions cause lethal OI, especially residues with a charged or branched side chain. The mutations do not follow a gradient or regional model. Substitutions in the first 200 residues are non-lethal and have variable outcomes thereafter. Two regions (691-823 and 910-964) contain only lethal mutations and align with Major Ligand Binding Regions, suggesting a role in collagen binding to integrins, MMPs, fibronectin and COMP.

In the α2(I) chain, substitution occurrence does not support random mutations but the majority of substitutions do not result from first position codon changes. Unlike α1(I), recurrences at the same site are generally concordant for outcome, even from different substituting residues. Only 20% of substitutions cause lethal OI. The lethal mutations are located in 8 regularly spaced clusters along the chain, supporting a regional model. The lethal regions align with proteoglycan binding sites along the fibril, suggesting a role in matrix interactions at the fibril level.

Notes

S03

MODEL PEPTIDES: DEFINING THE ALTERATION IN THE FOLDING PATHWAY OF OI COLLAGENS

Michael BRYAN[1], Tim HYDE[2], Yingjie LI[2], Jean BAUM[2], and Barbara BRODSKY[1]
[1]Robert Wood Johnson Medical School; [2] Rutgers University, Piscataway, NJ 08854

It is known that the presence of a Gly mutation in OI collagens still allows the formation of a complete triple-helix, but that it slows down folding and results in overmodification of Lys residues N-terminal to the mutation site. Peptide models containing Gly mutations in a type I collagen sequence context have been designed to serve as models for this defective folding process, to better define the defect and its dependence on amino acid sequence. Circular dichroism and NMR spectroscopy indicate that when a favorable renucleation sequence is placed N-terminal to the mutation site, these peptides fold completely, but at a decreased rate. Differential scanning calorimetry scans indicate the presence of a folding intermediate, which accumulates at early times and then disappears as the complete triple-helix is formed. The kinetics of the disappearance of the intermediate is markedly slower when Gly is replaced by Ser, compared with Ala. It appears that the C- to N-terminal propagation process is terminated at the mutation site, and the length of time to reinitiate folding depends on the identity of the residue replacing Gly. Studies are in progress to characterize the effects of all residues which are found to replace Gly in OI on folding and to the define conformational and dynamic perturbations at the mutation site in the folded molecule.

Notes

P02

INVESTIGATION OF THE RELATIONSHIP BETWEEN TRIPLE-HELIX DESTABILIZATION BY Gly→Ser SUBSTITUTIONS AND CLINICAL SEVERITY IN OSTEOGENESIS IMPERFECTA

Anton V. PERSIKOV and Barbara BRODSKY
Robert Wood Johnson Medical School – UMDNJ, Piscataway, NJ

Statistical analyses of COL1A1 genomic sequence indicated that the least destabilizing Gly→Ala substitution is underrepresented in reported OI due to a mild phenotype, while the most destabilizing mutations (Gly→Val, Glu, Asp) are underrepresented because of their severe consequences (Persikov et al., 2004). It has been hypothesized that the level of triple-helix destabilization determines whether Gly mutation becomes clinically detected as OI. To test this hypothesis, an experimentally derived algorithm, Collagen Stability Calculator (Persikov et al., 2005), was used to predict the regions of different stability along collagen triple-helix. A weak correlation was observed between the clinical severity of Gly→Ser mutations observed in OI and local stability around the mutation sites. A set of peptides was designed to model collagen regions with Gly→Ser mutations found in OI, including those found in positions 382, 661, 862, 964 of the α1(I) chain and residues 238 and 661 in the α2(I) chain. The degree of destabilization resulting from a Gly→Ser replacement differed among the peptides, and a greater destabilization correlated with a more severe clinical outcome. In addition, it was found that the Gly→Arg substitution is much more disruptive than Gly→Ser at residue 382 in α1(I) chain, consistent with the more severe type II OI phenotype at Gly382Arg mutation and the milder type IV cased by Gly382Ser. Molecular dynamics study is being used to evaluate which physical chemical forces are crucial in this context.

References:

Persikov A.V., Pillitteri R.J., Amin P.A., Schwartze U., Byers P., Brodsky B. (2004) "Stability Related Bias in Residues Replacing Glycines within the Collagen Triple Helix (Gly-Xaa-Yaa) in Inherited Connective Tissue Disorders". *Hum. Mutat.* **24** (3): 330-337.

Persikov A.V., Ramshaw J.A.M., Brodsky B. (2005) Prediction of collagen stability from amino acid sequence. *J. Biol. Chem.*,electronically published ahead of print on March 7.

Notes

P03

UNRAVELING THE MYSTERIES OF TYPE I COLLAGEN STRUCTURE-FUNCTION RELATIONSHIPS: BRITTLE BONES BREAK THE SILENCE.

Gloria DILULLO[1], Shawn M. SWEENEY[1], Leena ALA-KOKKO[2], Antonella FORLINO[3], Wayne A. CABRAL[3], Aileen M. BARNES[3], Joan C. MARINI[3], and James D. SAN ANTONIO[1].
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Type I collagen is ubiquitous in the human body and helps to maintain the integrity of bones, skin, tendons and other tissues via its interactions with cells, extracellular matrix molecules, and growth and differentiation factors. We recently created a map of the type I collagen D-period that included all of its reported ligand binding sites and mutations and examined their spatial inter-relationships (DiLullo et al., 2003). Here we present an updated map including hundreds of new mutations collected by the osteogenesis imperfecta (OI) consortium (Marini et al, 2005), as well as additional ligand binding sites and functional domains. The map reveals that of the three hot spots for ligand interactions originally identified on type I collagen, only the central and most C-terminal sites co-localize with regions rich in lethal OI mutations, consistent with their critical function in ligand-binding. Of the eight gaps in mutations on the α1 and α2 chains, three overlap with sequences for $\alpha 1\beta 1/\beta 2\beta 1$ integrin receptor binding, reported to mediate cell-collagen attachment, endothelial activation, and angiogenesis, thereby implicating $\beta 1\beta 1/\beta 2\beta 1$ integrin-type I collagen interactions as essential for embryonic development and survival. Non-random distributions of certain mutations associated with OI and other diseases exist within the monomer and fibril, implying that mutation position correlates with disease phenotype. Potentially relevant relationships between binding sites include: integrin-binding or vertebrate collagenase cleavage of collagen may be modulated by fibronectin-collagen interactions; proteoglycan (PG) binding may regulate collagen fibrillogenesis and cell-collagen adhesion, and collagen interactions with $\beta 1\beta 1/\beta 2\beta 1$ integrins and PGs may be impacted by non-enzymatic glycation of collagen seen in diabetes and aging. These and other observations presented here provide novel insights into evaluating type I collagen structure and function, and the relationships between its binding partners and mutations at the monomer and fibril levels (Supported by NIH HL053590 and AR049604 to J.S.A.).

Notes

P04

PROBING THE PROTEOGLYCAN-BINDING FUNCTION OF THE LETHAL OI MUTATION-RICH REGIONS IN THE α2(I) CHAIN OF TYPE I COLLAGEN.

Kristin L. REIGLE[1], Luke SERGOTT[1], Janelle L. LAUER-FIELDS[2], Gregg B. FIELDS[2], Elena MAKAREEVA[3], Sergey LEIKIN[3], Joan C. MARINI[4], and James D. SAN ANTONIO[1].
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Hundreds of mutations in type I collagen are associated with heritable connective tissue disorders in humans, characterized by altered matrix assembly, stability, and function. Proteoglycans (PGs) including decorin, keratocan and lumican reside on type I collagen fibrils in various tissues, and are proposed to regulate fibril assembly and lateral association. Their disruption may be the mechanism underlying certain connective tissue diseases. Moreover, Marini and co-workers determined that six regions containing lethal osteogenesis imperfecta (OI) mutations on the α2(I) chain overlap in distribution with proposed sites of PG binding. To investigate the role of these regions in collagen-PG interactions and OI we examined interactions of radioiodinated human recombinant decorin with type I collagens purified from fibroblast cultures from healthy humans or from those with OI mutations that map to PG-binding domains of collagen. Collagen samples were prepared by ammonium sulfate precipitation, pepsin digestion, and selective salt precipitation to > 95% purity. Affinity coelectrophoresis (ACE) revealed that decorin bound strongly to normal collagen (K_d 67 ± 8 nM), moderately to non-lethal (K_d 110 ± 52 nM) ,and weakly to lethal OI (K_d 451 ± 312 nM) samples. Collagen model triple helical peptides (THPs) were synthesized (T_ms ≥ 37°C) which included proposed PG-binding sites at residues 343-364, 451-463, 622-640, 694-706, 853-868, and 886-896. No detectable binding of the THPs to various PGs were observed by ACE, but in a spectrophotometric assay, five of the peptides affected the time course and/or extent of fibrillogenesis. Binding of the human collagens to biotinylated fibronectin (FN) was assayed by ELISA, and all but one sample bound FN similarly. Thus the affinity of decorin, but not of FN, for collagen diminishes with increased OI severity. Whether regions of the α2(I) chain rich in lethal mutations correspond to PG-binding sites or to domains involved in other aspects of collagen function such as fibrillogenesis requires further investigation (Supported by NIH AR049604 to J.S.A.)

Notes

P05

SIBLINGS WITH SEVERE FORM OF OSTEOGENESIS IMPERFECTA AND NORMAL FIBROBLAST ANALYSIS.

Natalia A. BELOVA, Moscow Research Pediatric Institute, Moscow, Russia

Two siblings of healthy parents have clinical manifestation of severe diffuse osteoporosis, multiple bone fractures, scoliosis. Fibroblast analysis was performed in Simon Winter Institute for Human Genetics (Director – Dr. Zwi BOROCHOWITZ, Bnai-Zion Medical Center, Haifa, Israel) and did not show abnormalities of types I and types III procollagenes.

Family: The parents are Caucasian of Russian origin, healthy, non-consanguineous. Mother is 30 y\o with height of 172 cm, and father is 32 y\o with height of 191 cm. I.K. and N.K. are their only children. Parents are known to be healthy, with no apparent bone fractures, dental or hearing problems. No other family members are known to have any known genetic disorders, malformations or bone fractures.

I.K. a 7 y\o girl was born after an uneventful pregnancy. Delivery was normal, however a fracture of a clavicle was thereafter noted. She has had about 25 bone fractures by present time. Height 120 cm (Z score : -0.22), weight 19 kg (Z score : -2.22). Sclerae are white, teething is normal. There are mild deformities of lower extremities, partially corrected after repeated rodding surgery (at 2002-2004); walkswith orthesis.

Dencitometry: Right femur BMD (total) 0.476 g/cm^2, BMC 11.38gSpine: BMD (total) 0.353 g/cm^2, BMC 8.519g (Z-score: -2.52).

N.K. a 6 y\o boy was born after 2nd pregnancy, complicated with pre-eclampsia. Delivery was normal, multiple bone fractures have been noted at birth. He has had about 35 bone fractures by present time. Height 110 cm (Z score: -1.02), weight 15 kg (Z score : -3.97). Sclerae are white, teething is normal. There are moderate deformities of lower extremities, partially corrected after repeated rodding surgery.Does not walk.

Dencitometry: Right femur BMD (total) 0.550 g/cm^2, BMC 5.541g Spine: BMD (total) 0.273g/cm^2, BMC 7.871g (Z-score: -3.92).Skin biopsy from I.K. was performed for fibroblasts analysis.

Fibroblast analysis: The cells synthesized and secreted types I and III procollagens normally, and the electrophoretic mobility of the chains of these procollagens, the efficiency of secretion and the efficiency of conversion of procollagens to collagen were similar to those in the control cells.

Conclusion: Clinical picture is more likely to OI type III or type IV, but structure of the proα1(I) or proα2(I) chains of type I procollagen seems to be normal. DNA testing is necessary; new form of OI (type V) cannot be excluded at the present time.

Notes

P06

Y-POSITION CYSTEINE SUBSTITUTION IN TYPE I COLLAGEN (α1(I) R888C) IS ASSOCIATED WITH TYPE IV OSTEOGENESIS IMPERFECTA

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The most common mutations in type I collagen causing types II-IV OI result in substitution of a glycine in a Gly-X-Y triplet by another amino acid residue. Only one non-glycine substitution in type I collagen has been described; it is an X-position change, α1(I) arg134cys, which causes classical Ehlers-Danlos syndrome. We have delineated a Y-position substitution in a father and son with mild type IV OI. The 12 yr old proband sustained 4 fractures and has moderate joint laxity. He is osteopenic with L1-L4 DEXA z-score of –1.3. Skeletal survey revealed straight limbs. His father sustained 2 fractures and has large-joint hyperextensibility. They have an α1(I) arg888cys substitution in one COL1A1 allele. The C→T nt change predicts the elimination of an MboI restriction site and heterozygosity was confirmed in gDNA. SDS-urea-PAGE of [^{3}H]-proline labelled steady-state collagen reveals only slight overmodification of the α1(I) monomer band, much less than expected for a glycine substitution at this position, and a faint α1(I) dimer. [^{35}S]-cysteine labelling of steady-state collagen demonstrated dimers in about 10% of heterotrimers in media and cell layer of fibroblasts. Since a maximum of 25% dimers can occur, dimer formation is inefficient, probably due to decreased proximity of the cysteines in adjacent chains. *In vitro* processing with N-propeptidase at 37ºC yielded 15-20% pN-collagen. The unprocessed pN-collagen was enriched for heterotrimers containing α1(I) dimers, suggesting that dimers result in a register shift that propagates along the helix. Immunofluorescence studies detected no accumulation of mutant procollagen in the ER on ascorbate stimulation, confirming efficient secretion of mutant procollagens from fibroblasts. Differential scanning calorimetry revealed only local helix destabilization. In matrix deposited by cultured fibroblasts, [^{3}H]-proline labelled dimers were seen in immaturely and maturely cross-linked fractions. *In vivo*, proband dermal fibril diameters have a wider range than controls. This Y-position arg→cys substitution may cause bone matrix abnormalities by (a) loss of an arginine residue important for staggered helix conformation and interaction with adjacent helices during fibril growth, and/or (b) gain of a reactive -SH moiety in this position. Hyperextensibility may be due to N-propeptide retention.

Notes

P07

MUTATION ANALYSIS OF COL1A1 AND COL1A2 IN A COHORT OF PATIENTS WITH OSTEOGENESIS IMPERFECTA TYPE I-IV

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Osteogenesis Imperfecta (OI) is a heterogeneous group of genetic disorders clinically characterised by increased bone fragility. The genetic defect in >90% of patients with a clinical diagnosis of OI type I -IV is a mutation in one of the two genes that encode the alpha chains of collagen type 1, COL1A1 and COL1A2.

In this study the entire coding region and intron/exon boundaries of COL1A1 and COL1A2 genes were screened by genomic DNA sequencing in a cohort of 58 unrelated patients with varied clinical severity. Of the 58 patients analysed 16 were clinically diagnosed as OI type 1, 13 type II, 6 type III, and 14 type IV. Seven patients had an unusual or unclear clinical phenotype. Two patients were identified as being borderline type I/IV. Fifty one mutations were identified, 42 in COL1A1 and 9 in COL1A2. Thirty five mutations appear to be novel and these are described briefly below.

In severe OI (type II and III) 6 patients were shown to have missense mutations resulting in substitution of glycine in the collagen triple helical domain, disrupting protein folding and structure. A doublet GC deletion, an unusual 398 base deletion predicted to completely remove exon 20 of COL1A2, a splice junction mutation and an aspartic acid substitution were also identified in this group. In moderate OI (type IV and I/IV) 6 glycine substitution were identified. Four patients were shown to have a single base insertion/deletion resulting in a frame shift. A splice junction mutation and nonsense mutation were also identified. In Type I (mild OI) we identified 2 splice junction mutations, 4 single base insertion/deletion resulting in a frame shift, and 5 missense mutations resulting in amino acid substitutions other than glycine. A further missense mutation resulting in substitution of a conserved cysteine in the C-terminal propeptide domain and predicted to affect procollagen assembly was also detected. Glycine substitution was identified in one patient with mild OI. In three patients (types I,I and II) we identified more than one sequence change which was potentially pathogenic.

The variety of mutations identified reflects the heterogeneity of the disease and is consistent with the causative mutation being family specific in the majority of OI patients.

Notes

P08

AUTOSOMAL RECESSIVE OSTEOGENESIS IMPERFECTA POPULATION GENETICS AND NOMENCLATURE

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The delineation of a group of OI syndromes with congenital joint contractures (Bruck syndromes type I, II,?III)[1] and the delineation of OI type VII in North American First Nations[2] raises the potential for a continuum of distinct but phenotypically related autosomal recessive (AR) forms of OI. Some 25% of subjects in Southern Africa have a form of AR OI type III not linked to type I collagen structural loci[3]. We have observed similar families from Italy and Lebanon where OI does not appear to result from mutations in type I collagens. Should these AR syndromes be named OI type III (North American) or (African) or (Libyan) or (Pakistani) type etc. depending on their population of origin or should we continue to extend the nomenclature to OI type VIII, IX, X etc. Within inbred isolated populations, the technique of Autozygosity mapping has great power to resolve this heterogeneity. There is thus an urgent need to identify and characterise such families with OI. Finally the AR Fragilitas Ossium (fro) mutation in the mouse produces offspring with either autosomal/recessive OI type II (20%) or OI type III (80%)[4] How should we report phenotype versus genotype in OI syndromes.

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4. Sillence DO, Ritchie HE, Dibbayawan T et al…Am J Med Genet 1993; 45(2):276-283.

Notes

P09

ATYPICAL MUTATION CAUSING OI: A FAMILIAR CASE OF A NEW CARBOXYL-TERMINAL PROPEPTIDE SINGLE AMINOACID SUBSTITUTION (L1437Q) OF THE PROα1(I) CHAIN OF TYPE I COLLAGEN.

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During mutation screening of the two genes encoding type I collagen protein (COL1A1 and COL1A2) by capillary electrophoresis sequencing, in a female infant with osteogenesis imperfecta II/III we identified a new heterozygous single base change in exon 52 of COL1A1, which converted the codon 1437 from Leucine (CTG) to Glutamine (CAG) along the proα1(I)carboxyl-terminal propetide. Since the complete sequencing of the two genes didn't reveal any other variation in the proband's DNA, we deduced that this mutation is causative of her pathological phenotype.
The particular location of this mutation underlines the pivotal role of this proteic region in causing severe to lethal OI (III/II) as already reported by only a few literature data. As a matter of fact, the correct folding of collagen protein starts from the carboxyl-terminal propeptide and here are located the cysteine residues involved in the formation of intra-inter-chain disulphide bonds essential for the triple helix formation and stabilization. Furthermore, some molecular chaperones as PDI and BiP (GRP78) associate with this region.
Biochemical experiments, demonstrating a remarkable overmodification along the full length of both the collagen type I chains, further confirmed our findings.
We identified the same substitution in her newborn sister affected by OI II, who died at age 10 months for respiratory failure. Since both the healthy parents don't show this substitution in their DNA extracted from peripheral blood, the genotypic data seem in agreement with a parental germinal mosaicism that should be investigated further.

Notes

P10

MOLECULAR SCREENING IN OI PATIENTS AT VERONA REFERENCE CENTER: "TYPICAL" MUTATIONS

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Identification of COL1A1/COL1A2 causal mutations in our OI patients is achieved by direct sequencing of genomic PCR products (Beckman CEQ Ver.8.0.52 system).We amplify routinely 27 fragments for COL1A, encompassing exons 6-52 and 38 fragments for COL1A2 gene, encompassing exons 1-52. Intronic sequences are included as well in our screening procedure The following Gly substitutions were found in unrelated patients:

Gene	Gly	helix#	NT subst.	AA subst.	OI type	comments
COL1A1	79		G.>A	Arg	I	recurrent (14 reported)
	505		G.>T	Ser	IV	new
COL1A2	421		G.>T	Ser	IV	new
	664		G.>T	Cys	III/IV	new

An **exon 13-skipping** mutation (**COL1A1**) : IVS12-2A.>C was found in a type IV OI patient.

Finally, a **COL1A1 frameshift** mutation: gDNA 9251-9252 insC (#AF017178) caused type I OI in two sibs born from healthy parents. The insertion falls within Ex28 and it predicts a PTC in Ex29. It was not detected in white blood cells DNA obtained from the parents and two unaffected sibs. Paternal sperm cells DNA will be investigated.

In summary, all the mutations we found show coherent genotype-to-phenotype correlations, when compared with data reported previously. The G505S substitution in COL1A1 may represent an exception, since it falls within more severe (type II/III OI) analogous substitutions nearby. We believe our data can contribute to the completion of the mutation map of type I collagen genes and to the genotype-to-phenotype correlation task.

Notes

P11

NON-ENZYMATIC GLYCATION OF TYPE I COLLAGEN DIMINISHES COLLAGEN-PROTEOGLYCAN BINDING AND WEAKENS CELL ADHESION.

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Non-enzymatic glycation (NEG) of type I collagen occurs during aging and in pathological states including diabetes. This modification may affect collagen solubility, charge, and polymerization. Proteoglycans (PGs) bind type I collagen *in vivo* and are proposed to regulate fibril assembly and function. Moreover, our ligand-binding map of type I collagen revealed that a keratan sulfate (KS) PG binding region on the collagen fibril overlaps with all of the preferred NEG sites on collagen. Thus, here we examined the effect of collagen modification by simple glycation (glycosyl-lysine) adducts on PG-collagen interactions. By affinity coelectrophoresis (ACE), we found reduced affinities of heparin and native KSPGs for glycated but not normal collagen, whereas native decorin and biglycan PGs bound similarly to both. ACE of heparin-collagen binding at pH 6.0, 7.0 and 8.0 showed that the affinity decreased with increasing pH. CD spectroscopy revealed both collagens to assume triple helical conformations, but heparin addition caused precipitation and decreased triple helical content - effects that were more marked with NEG collagen. A spectrophotometric assay revealed slower polymerization of the NEG collagen, and mature NEG or normal fibrils formed with or without heparin appeared similar by electron microscopy. B-cells transfected to express the cell surface heparan sulfate PG syndecan-1 adhered well to normal collagen but poorly to NEG collagen. We speculate that in glycated collagen, neutralization of basic hydroxylysine residues may reduce the electrostatic component and therefore the strength of PG-collagen interactions, and may directly interfere with core protein-collagen interactions for KSPGs but not decorin or biglycan. Therefore *in vivo*, glycation may disrupt the integrity of PG-matrix interactions and affect processes dependent upon cell surface PG-collagen interactions such as cell migration, and cell-mediated ECM restructuring and turnover. (Supported by NIH HL053590 and AR049604 to J.S.A.)

Notes

P12

INTERACTION BETWEEN BM-40 AND OI PROCOLLAGENS

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BM-40 is a multifunctional glycoprotein belonging to the matricellular group of proteins. It modulates cellular interactions with the extracellular matrix by its binding to structural matrix proteins, such as collagens (1). The importance of this interaction is clearly demonstrated in BM-40-null mice, where the collagen fibrils are smaller and more uniform in diameter and fibrillogenesis is affected (2). Furthermore, in mov-13 mice, which harbour an embryonic lethal mutation in the α1(I) collagen gene, the lack of collagen type I significantly impairs the deposition of BM-40 in the ECM (3), indicating a specific correlation between these two components.
In order to know whether the interaction of BM-40 with collagen is also affected in pathological conditions, such as OI, we studied in solid phase assay the interaction between BM-40 and several type I procollagens isolated from OI patients carrying different point mutations, all of them involving substitution of a Gly in the α2 (I) chain. We found that two OI procollagens with a different amino acid substitution have a dramatically decreased affinity for BM-40: i.e. Gly421→Asp (lethal) and Gly688→Ser (non-lethal). Other lethal mutations (Gly457→Arg and Gly706→Ser) do not show any decreased affinity and another one (Gly622→Asp) has a milder effect as compared to the control, indicating that the interaction is not correlated with the clinical severity of the patient. We could not observe any correlation with the gradient of severity often observed from the C- to N-terminus and due to over-glycosylation and consequently to abnormal folding of the collagen chain. Interestingly, the two mutations responsible for the decreased binding (i.e. Gly421→Asp and Gly688→Ser) are contained in the collagen region that we identified to be involved in the binding with BM-40.

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(3) Iruela-Arispe M.L., Vernon R.B., Wu H., Jaenisch R., Sage E.H. (1996), *Dev. Dyn.*, **207**, 171-183

Notes

P13

BINDING OF MUTANT TYPE I PROCOLLAGENS AND COLLAGENS TO SMALL LEUCINE-RICH PROTEOGLYCANS.

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We studied the effect of mutations in the α2(I) chain on the interactions of type I procollagen and collagen from patients affected by Osteogenesis Imperfecta (OI) with decorin (DCN), biglycan (BGN) and fibromodulin (FM), all small leucine-rich proteoglycans (SLRPs).
Mutated procollagens I and collagens I were purified from culture media of skin fibroblasts with the following *lethal or °non-lethal mutations: °G250S, *G421D, °G436R, *G622D, °G646C, °G688S and *G706S. DCN and FM were purified from bovine tendon, recombinant BGN from a clone of stable transfected CHO cells. FM and BGN were purified without any use of chaotropic agents. The interaction was determined by ELISA using biotinylated SLRPs (1).
Our data indicate that mutations affect type I collagen interactions with the three SLRPs tested. Lethal samples G421D and G622D showed a very low binding to DCN and FM and a low binding also to BGN. The former mutation falls in the binding region for chondroitin/dermatan sulfate PGs, the latter in the binding region for keratan sulfate PGs (2). Different affinities for the three SLRPs were obtained for the collagen samples with the two most C-terminal mutations, both falling in the binding region for keratan sulfate PGs; in particular, a very low binding was found for FM. With the exception of the two cited lethal collagens, the affinity for SLRPs of the other mutated collagen samples seems to increase as the mutation is more N-terminal. This last result can be explained, at least for DCN and FM, by the presence in collagen of multiple binging sites for these two SLRPs and the fact that Lys/Hyl are essential for collagen binding to DCN (1) and FM (unpublished results). In particular, post-translational Hyl overglycosylation - in the portion of the triple helix that is N-terminal with respect to the mutation site - probably causes steric hindrance for SLRP binding.

(1) Tenni R, M Viola, F Welser, P Sini, C Giudici, A Rossi, ME Tira. Eur J Biochem 269, 1428-37, 2002. (2) Di Lullo G, AM Sweeney, J Korkko, L Ala-Kokko, JD San Antonio, J Biol Chem 277, 4223-31, 2002.

Biophysical Insights

Invited Speaker 4
Posters 14 - 17

S04

DOMAIN ORGANIZATION OF COLLAGEN TRIPLE HELIX AND ITS ROLE IN OI

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In this presentation we report the results of an ongoing systematic study of the structure and stability of type I collagen from OI patients with glycine substitutions. To date about three dozen mutations were screened by differential scanning calorimetry, circular dichroism, differential fluorescent labeling and other physical and biochemical techniques. We found that the effect of a substitution on the stability of full-length collagen depends on the location of the mutation within different structural domains, but not on the identity of the substitution or whether it is located within α1(I) or α2(I) chain. Regions of the triple helix which fold and unfold cooperatively are likely to play an important role in OI, e.g., a mutation causing a structural defect might affect the whole domain. Their existence was long suspected, but no specific domains were found before. The present study mapped a highly stable “N-anchor” domain containing the first 85 N-terminal amino acids of the triple helix and revealed evidence for at least few more such domains. Mutations in the N-anchor domain cause a distinct phenotype with combined OI and Ehlers-Danlos Syndrome (EDS) symptoms. The structural changes caused by these mutations and the molecular mechanism of EDS symptoms in the corresponding patients are discussed in detail in an accompanying poster. Mutations within other structural domains might also give rise to distinct OI features. However, comparison with published studies of binding regions for matrix molecules suggests that structural and functional domains within the collagen triple helix do not coincide, contributing to the apparent complexity of genotype – phenotype relationship in OI. The example of OI/EDS suggests that further detailed mapping of structural domains is crucial for understanding this relationship and uncovering the molecular mechanisms of OI and factors determining its severity.

Notes

P14

MORPHOLOGICAL COMPARISON OF NORMAL AND OI COLLAGEN FIBERS VISUALIZED BY SECOND HARMONIC GENERATION IMAGING MICROSCOPY

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We demonstrate that the nonlinear optical imaging modality of Second Harmonic Generation (SHG) can be utilized to elucidate morphological differences between collagen fibers from normal tissues and those from Osteogenesis Imperfecta diseased states. SHG is a powerful method for tissue imaging, since, like the more well-known two-photon excited fluorescence microscopy, it provides intrinsic 3-dimensionality, and due to the use of near-infrared excitation wavelengths, affords the ability to penetrate deep into tissue. For example, we have achieved depths of 100 and 600 microns into bone and muscle, respectively. Unlike fluorescence, SHG imaging of collagen requires no exogenous labels, and provides great contrast with excellent viability. SHG requires a non-centrosymmetric environment and highly ordered structures (e.g. fibers) to produce signal. Thus molecular arrays consisting of collagen including tendon, skin, and bone are well-suited for investigation. SHG provides direct data on the molecular organization not possible by other methods such as fluorescence and polarization microscopy, including crosslink density, supra-molecular symmetries, and orientation of the helical pitch angle with respect to the fiber physical axis. Here we probe structural aspects of OI and normal collagen in several systems by examination of the fiber morphology and SHG intensity. We have imaged secreted collagen from confluent fibroblasts from wild type and OI mouse models and find that the collagen fibers from the latter are sparser, less organized, longer in length, and much weaker in intensity than those secreted by normal cells. These trends are also observed inthe collagen fibers secreted by human fibroblasts from patients with normal, mild, and severe forms of OI. We have also used SHG to image explanted bone from OIM mouse and that while there are no clear morphological differences relative to the wild type, the intensity from the Wt/Wt is approximately five fold greater than the OI/OI mutant. Finally, we compared the forward to backwards (F/B) ratio of SHG intensities in wild type and OIM tendon and find clearly discernible values of 3.0 and 0.9 respectively. Furthermore, the images from of the backwards directed SHG from the OIM displayed banding perpendicular to the direction of the fibers, whereas these were absent the in the wild type. These findings indicate that the SHG approach reveals structural aspects of OI collagen that are not discernible by other methods. We anticipate that SHG will be a powerful tool in the diagnosis of OI.

Notes

P15

CHARACTERIZATION OF SKIN ABNORMALITIES ASSOCIATED WITH OSTEOGENESIS IMPERFECTA VIA HIGH-RESOLUTION MAGNETIC RESONANCE IMAGING

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We hypothesized that abnormalities in collagen I associated with osteogenesis imperfecta (OI) would be manifest in the skin, and that further, MRI evaluation of hydration and collagen packing alterations in the dermal layer would be feasible. This could lead to improved diagnostic capabilities in clinical settings where rapid diagnosis is of value. Heterozygous o*im*/+ and homozygous *oim/oim* mice have been used as models of type I and type III OI, respectively, for evaluation of both bone pathology and therapeutic interventions. Here, we utilized high-resolution MRI and histology to investigate dermal abnormalities in these mice. The MRI parameters of T_2 and MT, sensitive to hydration and collagen packing, respectively, were evaluated in excised skin samples from 3 mice of each genotype. Histologically, both *oim*/+ and *oim/oim* mice had a somewhat extended dermal layer containing collagen on the upper part of the layer, albeit very much reduced in depth, compared to the normal wild type (+/+) controls, and a much thicker inferior dermal layer showing properties very similar to those exhibited by the adipose tissue in the hypodermal fat layer. The dermal layer shows a 75% decrease in dermal collagen within the homozygous (*oim/oim*) mouse skin with respect to wild type control (+/+)and is accompanied by an approx. 60% decrease in the normal dermal depth. The 50% decrease in dermal collagen associated with a third of the heterozygous (*oim/*+) skin samples is shown by a 50% decrease in dermal thickness. In addition, the *oim/oim*s had an unusual abundance of melanin-containing hair follicles in the extended lower dermal layer. Differences in water mobility, collagen packing, and fat distribution, were observed also by MR Imaging. We conclude that characterization of phenotypic differences in the skin of *oim/oim* and *oim*/+ mice by high-resolution MRI is feasible, and that extension of these techniques to a clinical environment should be possible.

Notes

P16

NANOINDENTATION CAN BE USED TO MEASURE INTRINSIC MECHANICAL PROPERTIES OF OI BONE

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OI bone has been known for its poor quality, but the knowledge of intrinsic mechanical properties is limited. Intrinsic mechanical properties measurement could provide valuable information on how OI affects the bone matrix material properties isolated from any structural influence. Nanoindentation is a technique used to probe a sample's intrinsic mechanical properties with minimum sample amount required. A promising feature of this technique is its ability to map the mechanical properties and microstructural feature within 10 μm resolution. This capability makes it possible to obtain detailed mechanical mapping of structurally complex materials, such as OI bone. To test its feasibility, nanoindentation was used to measure elastic modulus and hardness of both osteonic and trabecular bone of eight (8) OI type III patients (age from 2.4 to 12.4 yrs old). The samples were harvested at Shriners Hospital at Chicago during routine orthopaedic surgery (IRB approved).

	Modulus (GPa)	Hardness (GPa)
Osteon	15.22 (1.94) (L)	0.42 (0.04) (L)
	13.92 (2.76) (T)	0.43 (0.05) (T)
Trabeculae	13.60 (3.38)	0.42 (0.06)

(): Standard Deviation; L: Longitudinal; T: Transverse

Unlike normal bone tissue, OI type III osteonic bone exhibited more isotropic material properties. Young's modulus and hardness values measured in the longitudinal direction did not show significant differences from the transverse measurements. No differences were observed in modulus or hardness in an analysis of the osteonic and trabecular measurement, which are consistent with a previous ultrasound study [1]. The increases in isotropic and homogeneous material properties of the OI bone may result from distortions in the collagen network. Both cortical and trabecular bone demonstrated decreased mechanical properties (modulus, hardness) when compared to normal (adult) bone. These decreases were greatest in the cortical bone. The deformation patterns of the OI type III bone during nanoindentation were similar to those of normal adult bone through an analysis of the elastic modulus/hardness ratio, which might suggest that OI bone is more brittle than normal children's bone. The study results are consistent with expectations and previous observations, which indicate that nanoindentation can be used to obtain intrinsic mechanical properties of microstructural features of OI bone.

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Notes

P17

UNFOLDING OF N-TERMINAL DOMAIN OF COLLAGEN TRIPLE HELIX IN OI/EDS

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We demonstrate that 85 N-terminal amino acids form a distinct, highly stable folding region, acting as the stabilizing anchor for the amino end of type I collagen triple helix. On the carboxyl end, this region is bordered by a stretch of 15 residues containing no proline or hydroxyproline and a chymotrypsin sensitive site. Glycine substitutions and amino acid deletions within the first 85-90 aa destabilize the N-anchor domain and induce its unfolding already at 34 ^{o}C, reducing the overall triple helix denaturation temperature by 5-6 ^{o}C. The thermal stability of mutant molecules is similar to truncated collagen without the N-anchor domain. N-propeptide partially restores the stability of mutant procollagen, but not sufficiently to prevent the N-anchor unfolding and a conformational change at the N-propeptide cleavage site. The ensuing failure of N-proteinase to cleave at the altered site leads to incorporation of pN-collagen into fibrils. Similar to EDS VIIA and VIIB, the fibrils containing pN-collagen become thinner and weaker causing skin hyperextensibility and joint laxity. However, unlike EDS VIIA/B, mutations in the N-anchor domain have a profound effect on the triple helix folding, resulting in a distinct phenotype with combined OI and EDS symptoms.

Bone and Cell Biology

Invited Speakers 5 - 8
Posters 18 - 19

BONE AND CELL BIOLOGY IN OSTEOGENESIS IMPERFECTA: THE BONE TISSUE LEVEL

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Bone is a living tissue that constantly undergoes formation and resorption, which are the functions of osteoblasts and osteoclasts, respectively. These two types of effector cells can be involved in two very different metabolic activities, remodeling and modeling. In remodeling, osteoclast and osteoblast actions are linked in space and time so that the bone removed by osteoclasts is soon afterwards replaced by osteoblasts. Remodeling thus renews bone tissue, but has little effect on the amount of bone. Conversely, in modeling there is no obvious link between osteoblast and osteoclast action. Bone formation can proceed without interruption by bone resorption, which makes modeling an efficient mechanism for increasing bone mass. Whereas remodeling is the main type of metabolic activity in trabecular bone, modeling occurs mostly on the inner (endocortical) and outer (periosteal) surfaces of the bone cortex as long as growth continues.

In OI, individual osteoblasts secrete a lower than normal amount of collagen. Nevertheless, the total activity of bone formation in trabecular bone is increased, because the low activity of individual osteoblasts is more than compensated by a very high number of these cells. Despite the elevated bone formation activity, trabeculae do not thicken normally in children with OI. This shows that the activity of bone resorption is increased to a similar extent as bone formation, as expected in the remodeling process.

Bisphosphonate treatment decreases osteoclast activity. As far as the remodeling process is concerned, this automatically leads to a similar decrease in osteoblast activity and therefore the thickness of trabeculae is not affected by the treatment. However, the effect of bisphosphonates on the modeling process is very different, as the drug can target osteoclasts without interfering with osteoblast action. This explains why pamidronate treatment mostly increases the amount of cortical bone, and why the treatment is much less effective after growth has stopped.

Thus, although the difference between bone remodeling and modeling has been largely ignored by basic science, knowledge of these tissue-level metabolic activities is key to understanding bone development in OI and the action of bisphosphonates in children with this disease.

Notes

P18

CHAPERONE-PROCOLLAGEN INTERACTIONS DIFFER WITH MUTATION LOCATION IN OSTEOGENESIS IMPERFECTA

Aarthi ASHOK, Aileen.M. BARNES, & Joan.C. MARINI BEMB, NICHD, NIH, Bethesda, MD.

Osteogenesis imperfecta (OI) or brittle bone disease, is caused by mutations in type I procollagen. The heterotrimeric procollagen molecule consists of a central triple helical domain flanked by C and N-terminal propetides and mutations in these regions results in OI. As both fibroblasts and osteoblasts secrete mutant collagen, the bone-specific pathophysiology of OI has not yet been delineated. However, in OI cases with mutations in the helical region of collagen, osteoblasts secrete a greater proportion of the mutant collagen forms than fibroblasts. As interactions with ER chaperones can direct the fate of proteins between secretion and degradation, we hypothesized that differential interactions of mutant procollagens with chaperones in osteoblasts and fibroblasts may be responsible for the 'permissiveness'of osteoblasts to mutant collagen survival.

Using confocal microscopy, collagen and chaperone-specific antibodies, we compared the intracellular localization of procollagens and chaperones in control versus OI fibroblasts. Normal procollagen and procollagen with a helical mutation displayed a distinct reticular pattern of immunofluorescence in the ER that overlapped with calnexin, but not with Hsp-47, PDI and BiP. In contrast, procollagens with C-propeptide mutations displayed a diffuse pattern of ER localization that co-localized with Hsp-47, PDI and BiP, but not with calnexin. These chaperone interactions are maintained in normal and OI osteoblasts.

Our novel findings demonstrate a clear correlation between the type of mutation and both the subcellular localization pattern of procollagen and the nature of chaperone interactions in both fibroblasts and osteoblasts. We are currently exploring the hypothesis that differential chaperone interactions lead to segregation of nascent proteins into different sub-domains of the ER.

Notes

BONE CELL INTERACTIONS IN OSTEOCLAST RECRUITMENT, DEVELOPMENT, AND SURVIVAL

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Osteogenesis Imperfecta (OI)(Type I-IV) is an inherited, autosomal dominant, bone disease often caused by defects within type 1 collagen. Type V-VII OI individuals do not have type I collagen mutations and these forms of OI may involve dysfunctional crosstalk between osteoblasts (OB) and osteoclasts (OC). OI patients frequently exhibit lower bone mass, skeletal deformities, and recurring fractures due to the fragility of the bone tissue. The bone fragility is, in part, the outcome of diminished OB bone formation and enhanced OC-mediated bone loss. Bisphosphonates have proven to be effective anti-resorptive therapeutic agents that alleviate the severity of OI by suppressing elevated OC activity. However, much is still unknown relative to the cellular mechanisms responsible for OI associated bone loss. Here, we review our current knowledge of OC/OB interactions and discuss how such interactions may be altered so as to contribute to the OI phenotype. Ocs originate from hematopoietic cells of the monocyte-macrophage lineage found in the peripheral circulation and bone marrow, and their development into functional bone-resorbing cells is strictly controlled by the essential stromal cell- and OB-derived signals macrophage colony stimulating factor (M-CSF) and receptor activator of NF-kB ligand (RANKL). Various hormones, growth factors and cytokines influence OC bone resorption, in part, via regulating M-CSF, RANKL, and/or a soluble antagonist of RANKL, osteoprotegerin (OPG), particularly in OB and bone marrow stromal cells (BMSC), but also in vascular endothelial cells (VEC). Inflammatory cytokines (e.g. IL-1, TNFα) stimulate both OB and VEC RANKL expression. Thus, one could hypothesize that mutated collagens might elicit immune or inflammatory responses that cause enhanced RANKL expression, leading to increased OC development and activity in OI. OC-mediated osteopenia in OI might also involveperturbations in pre-OC recruitment and/or OC survival. Pre-OCs can develop from CD34+ myeloid stem cells and CD14+ monocytic lineage cells, and their recruitment from the circulation may be carefully controlled by chemotactic peptides known as chemokines that critically regulate the trafficking of such cells. Chemokines are elevated under inflammatory conditions and certain ones may direct the movement of pre-OCs into or out of the bone marrow and circulation. Such processes involve chemokine modulation of their adhesion and penetration through the vascular endothelial barrier, as well as chemokine influences on their subsequent migration to bone marrow developmental niches and sites for their expansion, differentiation, active bone resorption, and survival. Thus, we also review the role of chemokines (eg. SDF-1 and MIP–1–) in OC development and survival, and consider their roles in OI.

Notes

P19

INCREASED OSTEOCLAST ACTIVITY IN OIM MOUSE OSTEOBLAST/ OSTEOCLAST IN VITRO CO-CULTURE SYSTEM

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Osteogenesis imperfecta (OI) is a disease which results from a variety of mutations in type I collagen. Dysregulated bone remodeling has been linked to OI, but the precise mechanisms are unclear. In particular, little is known about osteoclast (OC) activity, and how it is modulated by osteoblasts (OB) in OI patients or animal models. The goal of the current study is to investigate OC function in an in vitro OB-OC co-culture system from wild type (+/+) and OI (*oim/oim*) mice, a model moderate-to-severe OI. **Materials and Methods** Osteoblasts were harvested from 4-8 week old +/+ and *oim/oim* mice calvaria and cultured in dish (60 mm) the day prior to the co-culture day. Nucleated bone marrow cells (1×10^5) and osteoblastic cells (5×10^4) were co-cultured on bone slices (4×4 mm^2) in a 96-well plate in α--MEM containing 10% FBS in the presence of 10^{-8} M 1α,25-$(OH)_2D_3$ and 10^{-6} M prostaglandine E_2 at 37^0C for 14 days. Bone slices were TRAP-stained to identify multinuclear cells (MNCs). Confocal microscopy was used to assess osteoclast morphology, fusion (nuclei number), and resorption function. For each assay, 2 bone slices per group were evaluated, and N = 2 or 3 separate experiments run. **Results** There were a significantly greater number of TRAP-positive MNCs on the bone slices from the *oim/oim* group compared to the +/+ OB-OC co-culture group (58.2 ± 0.85/mm^2 vs 34.6 ± 4.38/mm^2, $p<0.05$). OCs from *oim/oim* co-culture fused to form larger MNCs with increased diameter (102.31 ± 12.70 nm vs 75 ± 7.34 nm), area (5576.8 ± 975 nm^2 vs 3461.2 ± 819 nm^2) and number of nuclei (17.93 ± 2.37/cell vs 11.1 ± 0.71/cell) compared to +/+. MNCs from *oim/oim* co-culture also showed increased resorption function, as evidenced by a greater number of F-actin rings (2.1 ± 0.14/cell vs 1.1 ± 0.14/cell) and resorption pits (4.5 ± 0.71/cell vs 2.2 ± 0.28/cell) compared to +/+. **Conclusions** Osteoclasts from the *oim/oim* OB-OC co-culture system showed increased number, size and and resorption function compared to osteoclasts from +/+ OB-OC co-culture system. The specific role of the osteoblast in signaling the osteoclast towards increased resorptive characteristics will be further elucidated in this in vitro OB-OC system by co-culture of *oim/oim* OBs with +/+ OCs.

Notes

S07

THE ABNORMAL MINERAL IN OSTEOGENESIS IMPERFECTA: HOW COLLAGEN AND NONCOLLAGENOUS PROTEINS AFFECT MINERAL PROPERTIES

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The mineral crystals in bones of humans and animal models of osteogenesis imperfecta (OI) are smaller and different in composition from those of age- and sex-matched healthy individuals. While in all bones the mineral is an analogue of the naturally occurring mineral hydroxyapatite, in some cases of OI the hydroxyapatite mineral crystals unexpectedly form outside the collagen matrix rather than within and upon this matrix. It is our hypothesis that the abnormal collagen fibrils in OI have both direct and indirect effects on mineral deposition and mineral crystal proliferation and growth in OI bones. This in turn may contribute to the bone fragility.

Type I Collagen provides the template within and upon which mineral crystals deposit. Abnormalities in the collagen fibril structure associated with OI will directly affect the organization of the mineral. This in turn may alter the mechanical properties of this abnormal bone. Non-collagenous matrix protein distribution and organization is also altered in OI bone, perhaps because of the altered collagen structure, but perhaps because of alterations of non-collagenous matrix protein synthesis. The non-collagenous proteins regulate collagen fibril organization, but they are also involved in the nucleation and growth of mineral crystals upon the collagen surface. From in vitro experiments, confirmed by analysis of transgenic animals, we have shown direct impacts of two families of matrix proteins on bone mineral formation and growth. Members of the SIBLING (Small Integrin Binding LIgand N-Glycosylated proteins and the SLRP (small leucine rich proteoglycan) families bind to collagen, regulate collagen fibril size, and can regulate hydroxyapatite mineral crystal growth and formation in vitro. Each of these proteins can act both as nucleators of hydroxyapatite and inhibitors of hydroxyapatite growth and proliferation depending on concentration, post-translational modification, and confirmation. In general, in the SIBLING protein family, dentin matrix protein-1 and bone sialoprotein appear to function as hydroxyapatite nucleators, while osteopontin and MEPE act as inhibitors. Among the SLRPs, preliminary data suggest that biglycan is a nucleator and decorin an inhibitor of hydroxyapatite formation and growth, however their effects may be dependent on their interaction with collagen fibrils.

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Notes

S08

USE OF MICROARRAY AND PROTEOMIC TO STUDY THE PHENOTYPIC VARIABILITY IN BRTLIV MICE.

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Introduction: In the BrtlIV mouse model for Osteogenesis Imperfecta (OI), the presence of the Gly349Cys substitution in the 1 chain of type I collagen causes a moderate/severe or a lethal OI outcome. To understand the molecular basis of this variability we investigated the mRNA expression of a pool of extracellular matrix proteins (ECM) by microarray. We also studied the bone protein profile by a proteomic approach.
Methods: Total RNA was extracted from calvarial bone of surviving and lethal BrtlIV and wild-type mice. Custom array EC Matrix (MEMOREC) containing 188 relevant ECM genes was used. Validation was performed by competitive and Real Time RT-PCR. Proteins were extracted from calvarial bone of mutant BrtlIV and wild-type mice and separated by 2D gels. Mass spectrometry was used for spot identification, PDQuest software (BioRad) for data analysis and western blotting for validation.
Results and Discussion: Five transcripts had consistently higher expression in the lethal than in the surviving BrtlIV mice: GADD153, Bmp6, Bmp7, 53bpI, PRELP. The transcription factor GADD153, activated by ER stress, was also determined to be increased at the protein level, suggesting that a different response to ER stress could be involved in the variable phenotypic outcome in Brtl. We generated a reference 2D map for newborn calvarial tissue since it was not yet available on international database. The comparison between protein patterns of lethal, surviving and wild type mice showed that mutant mice have an increase of and fibrinogen and that lethal BrtlIV have an increase of dihydropyrimidinase related protein 2, PGK-1 and B crystallin.

Notes

Insights from Murine Models

Invited Speakers 9 - 11
Posters 20 - 24

S09

MATURATION OR HOMOZYGOSITY MODULATES OI PHENOTYPE IN BRTL MOUSE.

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The Brtl mouse is a murine OI model generated by knocking a G349C substitution into one col1a1 allele. Brtl/+ pups have 30% perinatal lethality. Surviving Brtl mice are small, with weaker and more brittle femurs than WT.

A detailed study of bone architecture, strength and composition, together with histomorphometric analysis was undertaken at different ages. Improvement of bone strength (ultimate load) and stiffness were found with maturation (6 versus 2 months). Improvement in bone mechanics occurred despite continued weak femur geometry and moi, suggesting an improvement in matrix material composition. Static histology showed a reduction in bone volume per total volume (BV/TV) and a decrease in trabecular number (TbN) and trabecular thickness (TbTh) both at 2 and 6 months in Brtl mice, compared to WT littermates. Dynamic histology revealed a significant decrease in mineral apposition rate (MAR) and bone formation rate (BFR/BS) in 6 month Brtl/+ mice, compared to WT. Osteoclast surface was also significantly increased in 6 month Brtl/+, but osteoblast surface was normal.

We also made the unexpected finding that OI phenotype was milder in Brtl/Brtl homozygous mice. Brtl/Brtl have normal perinatal survival rate. Their weight is intermediate between WT and Brtl/+. They lack the rib fractures and flared thorax, as well as the vertebral and calvarial demineralisation seen in Brtl/+. Brtl/Brtl 2 month femurs have normal BMD and intermediate CSA, BV/TV and TbTh, withstand normal loading to fracture and are less brittle than Brtl/+. Cell numbers, MAR and BFR/BS were unchanged at 2 and 6 months, compared to WT.

Matrix insufficiency and collagen chain composition may contribute to the difference in phenotype between homozygous and heterozygous animals. In Brtl/+, type I collagen with one mutant chain is selectively retained by the cells, whereas the collagen with two mutant chains is better secreted. Since Brtl/Brtl mice make exclusively collagen with two mutant 1(I) chains, this results in a 33% matrix insufficiency in homozygotes vs the 40% insufficiency seen in heterozygous mice. Additionally, the reactive -SH group in Brtl/+ type I collagen might form illegitimate cross-links with intracellular or matrix components, while Brtl/Brtl collagen contains only disulfide-linked 1 dimers. We are investigating the relative importance of matrix insufficiency and collagen composition to phenotype modulation.

Notes

COL1A2 G610C MICE: A KNOCK-IN MOUSE MODEL BASED ON A LARGE HUMAN OI KINDRED WITH PHENOTYPE VARIATION

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We identified a COL1A2 G610C mutation in a large Amish kindred. Genotype assessment of 200 of the approximately 850 living descendents of the putative founder couple identified 62 carriers of the G610C variant. Hip and spine bone mineral density measurements of carriers and non-carrier relatives demonstrated large phenotype variation among the carriers. To determine the molecular and genetic mechanisms of phenotype variation in individuals of the same OI genotype, we generated the G610C knock-in mouse model. The G610C mice were created using a 13 kb genomic clone containing the relevant segment of the murine COL1A2 constructed with genomic DNA of 129Sv/Ev Taconic. The desired exon 35 base change was created using PCR based site-directed mutagenesis and a neomyosin resistance cassette flanked by loxP sites was placed in intron 34. A single male founder +/- mouse was mated with 2 founder +/- female mice. Mice of all 3 possible genotypes were obtained from this mixed background cross. Phenotype assessment by μCT did not reveal any differences in femur geometry, but ultimate torsional strength (T_{max}), shear modulus of elasticity (G), and tensile strength (Su) were significantly decreased in G610C -/- mouse femurs as compared to wild type; +/- animals appeared to have intermediate values though not significant. Torsional stiffness (Ks) did not appear significantly different between the genotypes. Statistical evaluation by polynomial orthogonal contrast analysis demonstrated the G610C allele exhibits a linear gene dose effect. Mutant heterotrimeric collagen (containing the G610C substitution) was demonstrated in tail tendon by SDS-PAGE analysis with Cys-specific fluorescent labeling. Differential scanning calorimetry thermograms confirmed deposition of mutant type I collagen heterotrimers, but also demonstrated the presence of α(I) homotrimers in G610C mice. The mechanism responsible for the appearance of α(I) homotrimers is unknown, but is likely related to the retention of the neomyosin cassette in intron 34. In summary the G610C knock-in mouse promises to provide new insight into genetic modifiers and the role of proα2((I) collagen in the pathogenesis of OI.

Notes

S11

TESTING THERAPEUTIC APPROACHES FOR OI USING COL1A1 TRANSGENIC MICE.

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We have developed mice transgenic for Col3.6GFP and Col2.3GFP and characterized them as visual markers of distinct stages of osteoprogenitor maturation. These mice were crossed with OIM model and we evaluated the differentiation capability of the osteoblast lineage. An analysis was performed on MSC cultures derived from the OIM/GFP mice and on histological sections of bone. Our results showed a marked impairment in the number of cells with GFP expression in the 2.3/oim/oim mice relative to 2.3/+/+. The 3.6/oim/oim cultures showed a similar number of low intensity GFP+ cells (preosteoblasts) but a lower number of high expressing GFP cells (early osteoblasts). Conventional markers of osteoblast differentiation confirmed the GFP data. Histological analysis of bones from the 3.6/oim/oim mice showed a dramatic increase in the number and strength of GFP positive cells lining the endocortical, periosteal and trabecular surfaces compared to 3.6 +/+ mice. We are currently using Col1a1GFPtransgenic/OIM mice to evaluate the effects of bone directed overexpression of insulin-like growth factor I. IGF-1 directed to bones of the oim/oim mice significantly increased bone density and mineral content.
Transplantation of osteoprogenitor cells has been another promising therapeutic avenue for OI. Although there have been numerous reports of successful osteoblast engraftment after transplantation of various progenitor cell populations, the extent of engraftment and differentiation of the donor cells within bone has been difficult to assess. We have developed a method that utilizes previously described properties of the GFP transgenic mice to assess the success of transplantation. Our model requires that recipient mice undergo lethal total body irradiation followed by rescue with total bone marrow. Under these conditions, the endogenous bone cells survive the acute effects of irradiation and undergo a wave of new bone formation 14-28 days after recovery. Following rescue with total bone marrow from Col1a1GFP transgenic mice, GFP positive donor cells can be observed on endocortical and trabecular surfaces. Despite the expression of Col1a1GFP they do not appear to be osteoblasts. When bone marrow stromal cells were injected systemically (i.v., i.p. or intracardiac, no evidence of osteoblast engraftment of Col1a1GFP or DIL labeled cells was found. However, when the marrow stromal cells or calvarial progenitor cells were injected into the intramedullary space, osteogenesis by the donor cells was observed. The injected cells form de novo trabecular bone along the injected needle tract and at sites distant from the injection site. The ability of the donor bone cells to persist as a regenerative bone cell pool will be a major determinant for success in correcting an underlying defect in the recipient bone.

Notes

P20

A DELETION IN SMPD3 CAUSES OSTEOGENESIS IMPERFECTA IN THE MOUSE

Isabelle AUBIN[1], Carolyn P ADAMS[2], Colin E BISHOP[2], Jean-Louis GUENET[1], Christophe POIRIER[2] [1] Pasteur Institute, Paris, France [2] Baylor College of Medicine, Houston, TX

So far, the type I collagen genes, *COL1A1* and *COL1A2*, are the only identified genes that cause *osteogenesis imperfecta*; however, in about 10% of human cases the disease is not caused by type I collagen defects. We report that in the *fragilitas ossium* mouse, *osteogenesis imperfecta* is caused by a deletion within *Smpd3*. *Smpd3* encodes an enzyme involved in lipid metabolism, the neutral sphingomyelinase 2, nSMase2. This represents the first report of a mutation in a non-type I collagen gene which causes *osteogenesis imperfecta*.

Notes

P21

EFFECTS OF SOLUBLE muRANK ON FRACTURE HEALING IN THE OIM MOUSE MODEL

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Osteogenesis imperfecta (OI) is a heritable disorder characterized by significant tissue fragility. The apparent success of anti-resorptives such as bisphosphonates in reducing fracture rate provides evidence that osteoclastic activity is involved in the pathophysiology of this disease. The most potent activator and differentiator of osteoclasts is receptor-activator of nuclear factor-κB ligand (RANK-L), expressed on osteoblasts, which binds to the receptor-activator of nuclear factor-κ B (RANK) receptor on osteoclasts as well as their precursors. Recently, a soluble form of the murine receptor RANK has been developed, which can bind to RANK-L, thereby rendering it inactive. In this study, we sought to determine the effects of soluble muRANK treatment (generously supplied by Amgen Inc.) on the fracture healing process in osteogenesis imperfecta mice (*oim/oim*), a model of moderate-to-severe OI. Twice-weekly RANK therapy, at a dose of 1.5mg/kg, or control saline therapy commenced at 6 weeks of age. At 8 weeks of age transverse fractures were generated in the mid-diaphysis of the right femora. The animals continued to receive either RANK or saline treatment until sacrifice at 2, 3, 4, or 6 weeks post-fracture, with N = 6 mice per group at the 2,3,and 4 week timepoints, and N = 15 mice per group at the 6 week timepoint. Radiographs of the harvested femurs in the AP and ML planes were obtained for all mice to obtain information on the callus density and area. Four point bend mechanical tests were performed on both fractured and non-fractured femurs harvested from mice sacrificed at the 6 week post-fracture time point. Image analysis of the radiographs demonstrated greater callus area ($p=0.004$), and increased density ($p=0.017$) in the RANK treated group compared to saline control at 6 weeks post-fracture. Four-point bend tests revealed significantly increased yield displacement ($p=0.049$) with trends to greater ultimate displacement ($p=0.086$) and energy absorption (0.071) in the RANK treated *oim/oim*s compared to saline controls. These results provide evidence that RANK therapy, while delaying the remodeling process, can strengthen the healing bone in the *oim/oim*, mice, leaving it less brittle.

Notes

P22

CRTAP IS REQUIRED FOR PROLYL 3-HYDROXYLATION OF FIBRILLAR COLLAGENS AND LOSS OF ITS FUNCTION CAUSES SEVERE KYPHOSCOLIOSIS AND OSTEOPOROSIS IN MICE

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Recently we described the isolation and characterization of a novel protein, Crtap, differentially expressed in chicken hypertrophic chondrocytes compared to proliferating chondrocytes in vitro. We isolated the mouse ortholog and demonstrated its expression in chondrocytes, osteoblasts and osteoclasts by in situ hybridization and RT-PCR. To understand its function, we generated mutant mice by homologous recombination. Heterozygous Crtap+/- mice were asymptomatic. Crtap null mice were generated and northern hybridization analysis of total embryo RNA confirmed the absence of Crtap mRNA. The null mice are born viable at the expected Mendelian ratios and, although smaller, they show no obvious abnormalities at birth. However, by 3-4 months of age they begin to develop a moderate kyphosis that becomes pronounced at 6 months of age. Faxitron analyses confirmed the deformity of the spine at the thoracic level and, most importantly, it revealed severe generalized osteoporosis affecting both cortical and trabecular bone. Histological analysis confirmed the severe osteoporosis and showed abnormal proliferating chondrocytes in the growth plate. Bone histomorphometry demonstrated approximately 50% decrease of axial and appendicular BV/TV compared to wild type mice, normal osteoblast and osteoclast numbers, but low mineral apposition and bone formation rates. Importantly, Crtap null mice have defective osteoid formation. Moreover, because Vranka JA et. al (JBC 2004) showed that Crtap co-purifies with prolyl 3-hydroxylase, we analyzed tryptic digested, cyanogens bromide derived peptides from types I and II collagen isolated from Crtap null mice tissues using tandem mass spectrometry. Interestingly, the single prolyl 3-hydroxylation modification known to exist in these collagen chains was completely absent. The altered post-translation modification of fibrillar collagens together with defective osteoid formation suggest a critical role of prolyl 3-hydroxylation for proper bone matrix formation and point to a key role for Crtap during skeletal development and bone mass acquisition. Moreover, these data point to a novel pathophysiologic mechanism leading to a new type of collagenopathy perhaps in the osteogenesis imperfecta spectrum of disorders.

Notes

P23

SELECTIVE RETENTION AND DEGRADATION OF MOLECULES WITH A SINGLE MUTANT α1(I) CHAIN IN THE BRTL IV MOUSE MODEL OF OI

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Our previous study of type I collagen from Brtl IV mouse with α1(I) Gly349Cys substitution found no significant changes in the physical and chemical properties of the mutant protein and no abnormalities in collagen-collagen interactions. We now report measurements of the composition and secretion kinetics of mutant collagen, which reveal selective intracellular retention and degradation of molecules with a single mutant α1(I) chain. We analyzed the composition of type I collagen from bone, skin and lung by CNBr peptide mapping, utilizing the Met356 introduced into mutant α1(I) chains in addition to the glycine substitution. We also studied the composition and secretion kinetics of mutant collagen from cultured fibroblasts and osteoblasts by ^{35}S-Cys labeling. Our results suggest that only ~1/3 of generated molecules with a single mutant chain and ~2/3 of molecules with two mutant chains are secreted and incorporated into animal tissues. The remaining mutant molecules appear to be retained and degraded by cells, potentially causing ER stress and cell malfunction. The reduced severity of this problem in homozygous cells, which produce only the molecules with two mutant chains, might be one of the causes of the unusual OI phenotype attenuation observed in the corresponding animals.

Notes

P24

GROWTH HORMONE INJECTIONS IMPROVE BONE QUALITY IN HETEROZYGOUS OIM MICE.

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Heterozygous Cola2^{oim} mutant mice experience a mild OI phenotype, with femurs and vertebrae that require less force than normal to break in a biomechanical test. Subcutaneous injections of recombinant human growth hormone (rhGH) or saline were given to *oim/+* mice 6 days per week between ages 3 and 12 weeks, in a protocol designed to simulate a trial on OI children. Injections of rhGH promoted significant weight gain and skeletal growth (femur and spine lengths) compared to saline-treated control animals. Micro-CT analysis on femur distal metaphyses revealed significant gender differences: male oim/+ mice had greater bone volume fraction, trabecular number, and trabecular thickness than females. On the other hand, female oim/+ mice had significantly greater tissue density than males. Different maturation rates for male and female mice is an insufficient explanation for these differences, as micro-CT revealed no gender differences in an age-matched cohort of wild-type mice. Female oim/+ mice had significantly higher osteoblast and osteoclast densities on trabecular surfaces than males, and these were unaffected by treatment. There were no significant gender differences in the pQCT and biomechanical testing results that follow. With rhGH treatment there were significant increases at the femur midshaft in cortical bone density (2.2%), mineral content (15.5%), and cross sectional area (13%) as determined by pQCT. Increases in the same cortical bone parameters were also measured in the distal metaphyseal region of the femur and in tail vertebrae, but lumbar vertebrae showed significant trabecular increases in mineral content (9.6%) and cross sectional area (10.1%). Three-point bending testing documented functional improvements to the femur midshafts. Growth hormone treatment produced significant increases in bone stiffness (23.7%), maximum load (30.8%), the energy absorbed by the femurs to the point of maximum load (44.5%), and the energy to actual fracture (40.4%). The ultimate stress endured by the bone material was increased by 14.1%. A spectroscopic study is in progress to evaluate whether differences in mineral crystal size or distribution correlate with the biomechanical structural or material enhancements in rhGH treated femurs. Gains in bone length, cross sectional area, density, mineral content, structural biomechanical properties, and strength were achieved without directly addressing the genetic collagen defect in the mice. Results support expanded clinical testing of rhGH injections in children with OI.

Notes

Orthopedics

Invited Speakers 12 - 14
Posters 25 - 29

ORTHOPAEDIST + OI: ADVANCES OR NEW PROBLEMS

Cathleen L. RAGGIO, MD

OI has been treated surgically for many years. Techniques have been fairly unchanged – osteotomy, IM rodding and splinting. The new advances are different rods which allow a simpler surgery. In addition, surgical and fracture complications due to bisphosphonate treatments are becoming more apparent. Biology must always win over technology and technology must address A need – not just be “new”.

Notes

S13

EXPERIENCE WITH THE FASSIER-DUVAL ROD: EFFECTIVENESS AND COMPLICATIONS

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Deputy Chair, Division of Orthopedic Surgery
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Notes

S14

THE INFLUENCE OF BISPHOSPHONATES ON ORTHOPAEDIC MANAGEMENT IN OSTEOGENESIS IMPERFECTA

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Choices in how to treat fractures of long bones in children with osteogenesis imperfecta (OI) are limited due to bone fragility. Plate osteosynthesis causes stress risers at the ends of the plate with subsequent fracturing of the bone at these locations. Longer periods of casting induce secondary osteoporosis resulting in a further decrease of the already compromised bone strength. Intramedullary rodding has become the golden standard for both prevention and treatment of fractures and misalignments. Sometimes additional casting is necessary, however usually not longer than 2 weeks.
With the introduction of bisphosphonate therapy in OI, bone quality in these children has improved. In a placebo-controlled RCT with olpadronate we found a fracture risk reduction of 31%. Our treatment protocol has changed since then. In our hospital, all children with OI with fractures and a low DXA score (BMC, BMD) are treated with bisphosphonates (age 0-4 intravenously 2 mg/kg/quarterly, > 3 years of age alendronate 10 mg or risedronate 5 mg + Calcichew D3).
Bisphosphonate treatment has changed orthopedic management in children in OI. The incidence of popcorn epiphysis has decreased. Bowing of the distal end of the long bone beyond the tip of the intramedullary nail during growth has ceased to occur in the majority of cases. Therefore, the frequency of replacement of too short intramedullary nails due to growth of the bone has decreased. Fracture incidence has decreased resulting in less out-patient clinic visits per patient. Realignment of long bones has become easier since plate osteosynthesis, often necessary for a stable fixation, no longer results in fractures due to stress risers. Often a combination of a plate osteosynthesis and an intramedullary rod is used. The effects of bisphosphonates on the incidence and progression of scoliosis are not yet clear. However, considerably less failure of spinal instrumentation is seen in case of operative scoliosis treatment.

In conclusion, increased bone strength due to bisphosphonate therapy in OI does not only lower fracture risk but also allows for improved surgical realignment of limbs and adequate spinal instrumentation. Bone lengthening has also become an option in case of leg length discrepancy.

Notes

P25

RADIAL HEAD DISLOCATION IN OSTEOGENESIS IMPERFECTA

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This retrospective work studied radial head dislocation (RHD) in patients with osteogenesis imperfecta (OI). We assessed 489 upper limb radiographs from 254 OI patients (mean age 9.57) for presence and direction of RHD, calcification of the interosseous membrane (IOM), capitellum and radial head or neck dysplasias and location, direction and magnitude of humerus or forearm bone bowings. The grip force was also measured. We observed 44 RHD. The frequency of this anomaly was significantly higher in OI type V (58% in 36 analyzed limbs) than in the other OI types (1.5% in 142 type I, 8.5% in 116 type III, 6.5% in 166 type IV, none in 29 type VI or VII). The direction of RHD was statistically different for OI type V (90.4% anterior, lateral or antero-lateral) compared to the other types (69.6% posterior or postero-lateral). The RHD was statistically linked with calcification of the IOM in type V, with studied dysplasias in all types, with direction of ulnar bow in type V and with direction and location of radial and ulnar bow in other OI types. Radial bow magnitude was significantly higher in RHD for type V, idem for both forearm bones in the other types. RHD in types III, IV and V were associated with lower grip force. The RHD was very common in OI, especially in type V. Several bowing characteristics were significantly linked to it. Grip force was impaired in those children. This may lead to propose a surgical correction of forearm bone bowings in OI patients.

Notes

P26

SURGICAL TREATMENT OF FRACTURES AND DEFORMITIES IN PATIENTS WITH OSTEOGENESIS IMPERFECTA

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INTRODUCTION: Fractures in patients with OI usually heal rapidly, and conservative treatment is successful. However, in displaced and unstable fractures surgical treatment is the only option.
AIM: To present our experience in surgical treatment of fractures and deformities as a consequence of OI.
PATIENTS AND METHODS: There are 42 individuals with OI in Croatian OI Register. We analyzed 19 patients retrospectively (12 males, 7 females) who were surgically treated from 1979 to March 2005 due to fractures and deformities of the long bones. The youngest patient was surgically treated at the age of 23 months, and the oldest patient was 34 years old (average: 9.3 years). There were 68 operative procedures, with the range from one to eight procedures per patient. We performed 37 reoperations mostly due to fractures of overgrown bone on solid intramedullary nail or after fixation with plate and screws.
RESULTS: Different intramedullary rods were used on 39 occasions. We used solid intramedullary nails (Küntchner's nail, Rush's nail, interlocking nail) in 15 operations, Kirschner wires in 13 operations, and expandable intramedullary rod (Sheffield, Fassier-Duval) in six operations. Elastic titanium nail (Nancy) was used in five operations. Other modes of fixation i.e. plates and screws, ASIF external fixator, and Ilizarov system (one patient) were used in total 29 operative procedures. Operations were mostly performed on femur (46 operations, 68%) and tibia (15 operations, 22%). There were 7 procedures (10%) on the upper extremities. We observed delayed union in three patients who were treated with bisphosphonates, and in two patients on proximal ulna. At the last follow-up ten patients were outdoor walkers, with or without hand aid and nine patients are wheelchair bound.
CONCLUSION: The correct indication, surgical technique and appropriate fixation device will lead to the best possible results of surgical treatment in patients with OI. The comprehensive approach i.e. surgery, rehabilitation, and medical treatment may significantly improve mobility and function in OI patients. Small numbers of operations performed per a year due to rarity of the disease, and the variable and complex deformities, support conclusion that grouping OI patients and performing surgeries in special centers could enable optimal outcomes.

Notes

P27

INTERLOCKING TELESCOPIC ROD FOR OSTEOGENESIS IMPERFECTA PATIENTS

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The purposes of this study are to introduce an interlocking telescopic rod system that does not invade the distal joint nor the distal articular cartilage of the operated limb segment, and to report its early outcome. We modified the Sheffield telescopic rod system. The T-piece was removed from the male rod, and a hole for interlocking pin was made at its tip. A female rod from the Sheffield system was inserted from the tibial plateau or the greater trochanter of the femur through multiple osteotomies or fracture site down to the distal metaphysis. The male rod was inserted antegrade through the female rod down to the distal epiphysis. The male rod was transfixed at the distal epiphysis by a Kirschner wire using free hand technique. Twenty-three tibiae and nine femora in sixteen patients underwent tibial or femoral stablization using this telescopic rod. The mean age at the time of surgery was 7.3 years (range, 1.9 to 11.9 years). They were followed for average 2.7 years postoperatively. Telescoping took place in all the operated limb segments. In early four patients (3 tibiae and 1 femur), the interlocking pins (smooth Kirschner wire) backed out in 1.9 years of follow-up at average. After threaded-tip Kirschner wire was inserted deeply into bony epiphysis, no interlocking pin had backed out until latest follow-up. In three patients the female rod migrated proximally requiring surgical repositioning. In 4 tibiae, the interlocking pin cut through the physis into distal metaphysis in 3 years of follow-up at average, two of which were surgically replaced.The interlocking telescopic rod is effective and less invasive for tibial and femoral stabilization in osteogenesis imperfecta patients.

Notes

P28

STRUCTURAL ALLOGRAFT TO SOLVE MECHANICAL PROBLEMS IN OSTEOGENESIS IMPERPECTA

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Background: Patients with Osteogenesis imperfecta sometimes require orthopaedic surgery to enhance their function. Intramedullary rods work well as load sharing devices. However, those lacking axial continuity and rotational stability sometimes require additional strategies. In addition, periarticular fractures are not well stabilized by rods.
Patients and Methods: We investigated the results of cortical allograft use in OI patients to provide external bone-bridging. Six patients with OI had skeletal problems treated in this fashion. Two had proximal femoral, one distal femoral, one tibial diaphyseal, one distal humeral defects and one had basilar invagination. Ages ranged from 12-74.
Results: Four of the allografts were used in a peripheral "sandwich" construct and two in a "flying buttress" construct. The Sandwich construct was held by circumferential bands. The buttress constructs were held by press fit. Minimum follow-up was two years. There were no infections. All allografts incorporated and all reconstructive problems were solved by this technique.
Conclusions: Allograft seems to function well in Osteogenesis Imperfecta. It brings normal collagen into the region. It incorporates well. It provides rotational control as well as axial stability over a broad area of contact. It has mechanical and biological properties which a plate and screws cannot offer. Further investigation of this technique is warranted.

Notes

P29

POSTERIOR SPINAL FUSION IN KYPHO-SCOLIOSIS ASSOCIATED WITH OSTEOGENESIS IMPERFECTA. LONG TERM RESULTS.

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A survey was conducted at Hôpital Necker-Enfants-Malades in Paris and Centre Helio-Marin in Roscoff to document the results of posterior spinal fusion for scoliosis associated with Osteogenesis Imperfecta. Observations were made of 27 patients who underwent treatment by posterior spinal fusion and Harrington (n=2), or C.D. (n=23) instrumentations by one senior orthopaedic surgeon, two patients had posterior arthrodesis without instrumentation and were left in halo-traction together with a plaster cast till consolidation was achieved. Eleven of these patients were males and sixteen females. The average age at fusion was 13 years 4 months, the average curve before operation measured 77 degrees and the average correction was 37 %.The mean follow-up was of 6 years. Minor complications due to halo pin infection or migration were observed in two cases and loss of correction with rod fracture occurred in 4.We believe that spinal surgery and arthrodesis for spinal deformity should be considered after a preparation period by halo-traction with exceptional precautions to correct major curves and prevent complications that could succeed in patients with osteogenesis imperfecta because of physical and respiratory incapacities that could result. Correction and stabilisation of the scoliotic spine in osteogenesis imperfecta has yet to be determined since we have noticed that the deformation site is transferred at the sacroiliac region once spinal fusion is achieved.

Notes

Bisphosphonates

Invited Speakers 15 - 17
Posters 30 - 41

S15

BISPHOSPHONATE THERAPY IN OI: UPDATE ON EFFICACY AND SAFETY

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Bisphosphonate therapy has brought about significant clinical gains for infants, children and adolescents suffering from moderate to severe forms of OI. Benefits include decreased bone pain, lower fracture incidence, and improved mobility. Vertebral body size and shape improve, sometimes dramatically. At the bone level, there is evidence of thickening of cortical bone with an increase in trabeculae number (but not thickness), which explains the gain in bone mass. The magnitude of the response is clearly growth dependent, it is thus important to initiate treatment as early as possible after diagnosis is established, even in infancy. On x-rays, transversal lines, akin to growth arrest lines, appear as signatures of the treatment cycles. These transverse trabeculae contain calcified cartilage, progressively replaced by bone. A mild modeling defect that does not seem to have mechanical consequences is sometimes visible in the metaphyses, particularly in the distal femur. Despite a frequent acute phase reaction – which may include high fever, a transient drop in serum calcium and WBC count, as well as a rare bronchospasm in babies – at first exposure to the drug, treatment is well tolerated. After several years of treatment, healing of surgical osteotomies may be slow, but fracture repair proceeds normally. Renal function is not affected. Growth rate is not reduced, it might actually be stimulated. Bone remodeling (which is high in the untreated state) is decreased to a fraction of normal. Whether this will affect bone integrity in the long term is not known. Other unresolved questions concern the optimal duration of drug treatment, dosages and routes of administration of the various forms of bisphosphonate, and what happens when treatment is stopped. Preliminary observations indicate that bone formed after therapy was withheld is essentially untreated and osteoporotic. Because of these uncertainties, it appears unwarranted at this time to treat mild forms of OI. It is also important that treatment continues to be carefully monitored by physicians experienced in the use of bisphosphonates, preferably within the framework of an interdisciplinary approach (pediatrics, orthopedics, OT/PT, dentistry, genetics, nutrition, and social work).

Notes

S16

BISPHOSPHONATE TREATMENT FOR CHILDREN : ASSESSING FOR TOXICITY

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Bisphosphonate (BP) therapy for pediatric forms of osteoporosis, including osteogenesis imperfecta (OI), is increasingly common worldwide. We have treated 55 children with bisphosphonates (38 with OI, and 17 typically with other forms of juvenile osteoporosis) primarily using oral BP preparations and find that many of our patients benefit. However, optimal duration of treatment, complications from excessive BP exposure, and timing of cessation of therapy remain important challenges. Accordingly, we have begun a study aimed ultimately to detect early-on and non-invasively excessive suppression of osteoclast function in children treated with BPs. In part the impetus for this effort is the boy we reported with pamidronate-induced osteoclast failure causing osteopetrosis (NEJM 349: 457-63, 2003). However, we are increasingly being referred similar, but less dramatic, cases. Tartrate resistant acid phosphatase (TRAP) and “brain” creatine kinase (BB-CK) are the isoforms of these enzymes found in osteoclasts. We hypothesize that high serum levels of TRAP and the appearance of CK-BB in the circulation reflect osteoclast toxicity. To monitor BPs, we also follow radiographs of wrists and knees for changes in metaphyseal modeling (shaping) − searching for loss of concavity at metaphyseal surfaces. Our study also includes transapophyseal iliac crest biopsy and computerized tomography (CT) of the knees to assess for accumulation of primary spongiosa (calcified cartilage). Cessation of resorption of primary spongiosa documented using bone biopsy and CT and radiographic assessment of metaphyseal modeling will be contrasted to TRAP and BB-CK levels and biochemical markers of bone turnover. Hence, we hope for improved safety, efficacy, and understanding of the effects of BP therapy in children clarifying complication-free, target outcomes for BP treatment.

Notes

P30

CONTROLLED TRIAL OF PAMIDRONATE IN CHILDREN WITH TYPES III AND IV OSTEOGENESIS IMPERFECTA CONFIRMS VERTEBRAL GAINS BUT NOT SHORT-TERM FUNCTIONAL IMPROVEMENT

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Introduction
Bisphosphonates, anti-resorptive drugs for osteoporosis, are widely administered to children with osteogenesis imperfecta (OI). Uncontrolled pamidronate trials in OI reported increased bone density, vertebral coronal area, and mobility, and decreased pain. We conducted a randomized controlled trial of pamidronate in children with types III and IV OI.
Methods
This randomized trial included 18 children (age 4-13 years) with types III and IV OI. The first study year was controlled; 9 children received pamidronate (10 mg/m2/day iv for 3 days q3mos). Four children in each group also received rGH injections (0.06 mg/kg/day sq 6d/week). Seven children in the treatment group received pamidronate for an additional 6-21 months. All patients had L1-L4 DEXA, spine qCT, spine radiographs, musculoskeletal and functional testing.
Results
In the controlled phase, treated patients experienced a significant increase in L1-L4 DEXA z score ($p<0.001$), increased L1-L4 mid-vertebral height ($p=0.014$) and total vertebral area ($p=0.003$), as compared to controls. During extended treatment, DEXA z scores, vertebral heights and areas did not increase significantly beyond the 12 month values. Fracture rate decreased significantly in the upper extremities ($p=0.04$) but not the lower extremities ($p=0.09$) during the first year of treatment. Gross motor function, muscle strength and pain did not change significantly during the controlled or extended treatment phases.
Conclusions
A controlled trial confirmed the spine benefits of short term pamidronate treatment in children with types III and IV OI. Pamidronate increased L1-L4 vertebral DEXA and decreased vertebral compressions and upper extremity fractures. Vertebral measures did not improve during the extended treatment phase. The treatment group did not experience decreased lower extremity long bone fractures, significant improvement in growth, ambulation, muscle strength or pain. There was substantial variability in individual response to treatment.

Notes

S17

BIOMECHANICAL CHARACTERIZATION OF THE OI PHENOTYPE: EVALUATION OF BRTL IV AND THE EFFECT OF BISPHOSPHONATE THERAPY

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The hallmark of genetic disorders resulting in Osteogenesis Imperfecta is a decrease in the resistance of bone to failure. From a material science perspective, the disorders are appropriately characterized as “brittle bone” disease, since they involve a significant reduction in the ability of the bone to absorb energy during the process of failure. The purpose of this paper is to review the phenotypic aspects of bone quality that may be altered by mutations in collagen. The biomechanical parameters will be discussed within a hierarchical framework ranging from structural and density measures to fundamental alterations in constituent material properties at the level of the extracellular matrix. Using Brtl IV as a model, the effect of a single amino acid substitution on structural and material properties will be presented as well as an apparent physiologic adaptation. The assays include whole bone mechanical testing, geometric and morphologic analysis using Micro CT, material tests from microbeams and nanoindentation and Raman spectroscopy of ECM constituency. The short term and potential long-term consequences of bisphosphonate therapy will also be discussed. Interestingly, 12 weeks of treatment with alendronate (age 2 – 14 weeks) increased the amount of bone and the overall structural properties in both wild-type and Brtl. IV mice while having little effect on brittleness and raising the potential need for further study.

Notes

P31

ALENDRONATE INCREASES BONE STRENGTH AND BONE VOLUME BUT FAILS TO IMPROVE BRITTLENESS OR MINERALIZATION IN BRTL MOUSE MODEL FOR TYPE IV OSTEOGENESIS IMPERFECTA

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Bisphosphonate are widely administered to children with osteogenesis imperfecta (OI) in spite of the fact that data from controlled trials has only recently become available. To evaluate the direct effect of alendronate (Aln) on the properties of the long bones, we have undertaken a controlled trial in male Brittle (Brtl) mice, a murine model for type IV OI, and wild-type (WT) littermates. Brtl and WT mice were treated with Aln (0.219 mg/kg/wk, gift of Merck) or saline placebo from 2-14 wks of age. Brtl and WT bone have similar responses to Aln. Total weight and femoral length were unchanged. Areal BMD of femurs and lumbar vertebrae were significantly increased in all treated mice. Aln improves Brtl bone geometry, with increased diaphyseal cross sectional area, a more rounded cross-sectional shape, and dramatic doubling of trabecular number in distal femurs. Mechanically, Aln did not rescue the decreased pre-treatment stiffness of Brtl femurs, although it significantly increased normal WT stiffness. Aln treatment increased the ultimate load at which both Brtl and WT femurs fractured. However, Aln has a negative impact on several aspects of bone quality. First, the predicted material strength and modulus of Brtl and Wt bone is decreased by treatment. Second, the brittleness (post-yield displacement) of treated Brtl femurs was unimproved and treated WT femurs became more brittle. Third, the metaphyses of treated Brtl femurs have increased remnants of mineralized cartilage. The matrix discontinuities caused by the presence of mineralized cartilage may increase the risk of fracture initiation and contribute to the observed increase in BMD. Fourth, there was a detrimental effect on bone cells. After 12 weeks of Aln treatment, BFR/BS, MAR and MS/BS are decreased to less than 25% of pre-treated values in Brtl and WT. At that time point, percent ObS is decreased in both genotypes, significantly in WT, while percent OcS is stable. Additionally, Brtl osteoblast morphology changed from the plump cuboidal cells seen in untreated femurs to an intermediate morphology, supporting a direct effect on the cells. Thus, Aln treatment of Brtl bone improves bone geometry and increases loading before fracture, but decreases predicted bone material quality and alters osteoblast surface and morphology.

Notes

P32

EFFECTS OF ORAL ALENDRONATE ON BONE MINERAL DENSITY IN ADULT PATIENTS WITH OSTEOGENESIS IMPERFECTA. A three-year randomised placebo-controlled trial.

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Objective. To evaluate oral alendronate on the bone mineral density of adult patients with osteogenesis imperfecta.
Methods. We carried out a 3-year, randomised, double-blind, placebo-controlled trial of oral alendronate in 64 adult patients with osteogenesis imperfecta. The primary end point was the difference between the groups in the mean percent change in lumbar-spine bone mineral density at 3 years. Secondary outcomes included changes in bone density of total hip, vertebral and peripheral fracture incidence, pain, hearing loss, and bone turnover biochemical markers. Patients were treated daily with either placebo or 10 mg alendronate. All received 1 g of calcium and 800 IU of vitamin D daily.
Results. The mean (± SD) increases in the lumbar spine bone density were 10.1 ± 9.8 ($p<0.001$) and 0.7 ± 5.7 percent in the alendronate and placebo groups respectively. Hip bone density decreased in placebo group by 0.3 ± 0.6 percent and increased in alendronate group by 3.3 ± 0.5 percent ($p=0.001$). Alendronate had no significant influence on the fracture rate. A significant increase of the pain score was noted in the alendronate group ($p=0.04$). The hearing loss was not modified in both groups. Bone resorption and formation markers were significantly decreased in the alendronate group ($p<0.001$). There were no differences in severe adverse effects between the groups, but there was an increase in non-severe upper gastrointestinal effects in the alendronate group ($p=0.003$).
Conclusion. Oral alendronate increases bone mineral density but does not decrease fracture rate in adult patients with osteogenesis imperfecta.

Notes

P33

RESULTS OF BISPHOSPHONATE TREATMENT IN ADULT OSTEOGENESIS IMPERFECTA.

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Bisphosphonate use is widespread in the adult OI population. In contrast to bisphosphonate effects on bone density and fracture rate in children, uncertainty exists about the effectiveness of IV or oral bisphosphonate preparations in adults. Baseline data was obtained on 49 adults ages 16-72 years: 29 type I, 9 type III, 9 type IV and 2 type V. 64% were female and 36% were male. A total of 46 individuals, have been treated with pamidronate (n=24), alendronate (n=16) or residronate (n=6). To date, 25 have completed 18 months with follow-up DXA. An additional 34 individuals were untreated. L1-L4 and total hip BMD were measured on the same DXA machines. Pamidronate was administered intravenously over 4 hours, at 3-month intervals, at 1.5 mg/kg, to a maximum of 60 mg. Alendronate dose: 70 mg /week. Residronate dose: 35 mg/week. BMD (gm/cm^2) and t scores were obtained. Pertinent biochemical studies included: serum alkaline phosphatase (SAP), serum creatinine, 25 (OH) vitamin D and urinary n-telopeptide (NTX) excretion.

Baseline L1-4 DXA data (gm/cm2 ± SD) was obtained on 29 type I (0.785 ± 0.16), 9 type III (0.590 ± 0.14), 9 type IV (0.652±0.19), and 2type V patients (0.821± 0.10). Total hip values were: 0.773 ± 0.11, 0.697±,0.21, 0.697 ± 0.21, 0.455 ± 0.34, and 0.996 ± 0.12 g/cm2 respectively. We report initial BMD and BMD at 18 months for L1-L4 and total hip in 25 individuals, types I, III and IV, treated with pamidronate or alendronate. Start to →18 month, L1-4 and hip values, + n (#) were: type I-pam: L1-4; 0.780→0.801(11), hip; 0.722→0.735 (4): type I alend: L1-4; 0.791(5)→-0.845 (5), hip; 0.664 (1)→0.748 (1): type III-pam: L1-4; 0.585 (4)→0.649 (4), hip: 0.774→0.879 (2); type III-alend: .750; hip0.636 (0.715 (1)→0.750 (1) hip; 0.636→ 0.609 (2); type IV-pam: 0.551 (1)→0.604 (1); hip; 0.212 (2)→0.289 (2); type IV-alend: 0.736 (1)→0.802 (1). Of these 18 month differences, statistical significance before and after after 18 months of treatment was seen only for type I subjects treated patients treated with alendronate. This increase in BMD later declined with continued treatment.

Baseline morning NTX values were 61 (± 80) nmol/nmCr in type1, vs. 72 (± 106) nmol/nmCr in type 3, and 38 (± 29) nmol/nmCr in type 4. Each bisphosphonate led to a continuous 50% decrease in urinary NTX excretion in types I and IV. In type III OI NTX values fell and rebounded remaining elevated for 18 months. Conclusions: Bisphosphonates do not significantly improve BMD in OI adults with the exception of alendronate treatment at 18 months in type 1 patients.

Notes

P34

EFFECT OF BISPHOSPHONATE THERAPY ON TOOTH ERUPTION IN CHILDREN AND ADOLESCENTS WITH OSTEOGENESIS IMPERFECTA.

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Introduction The rationale for the bisphosphonate treatment in OI is that the reduced osteoclast activity could lead to an increased bone mass. The exact mechanism of tooth eruption is not clear but is most probable depending of several factors. Before the tooth breaks through the oral mucosa the bone coronal to the tooth has to be resorbed. Therefore the question arises if tooth eruption is affected by the reduced osteoclast activity. **Aim** The aim of the study was to investi-gate if treatment with bisphosphonates affects the eruption of teeth in children and adolescents. **Subjects** 58 patients treated with intra-venous infusions with pamidronate were investigated. In 20 children the treatment started between the ages of three months to five years that is before eruption of the first permanent teeth. In 27 children the treatment started between the ages of five years to 14 years, that is in the mixed dentition, and in the remaining 11 patients after 14 years of age when all permanent teeth usually have erupted. Twenty-four of the patients exhibited dentinogenesis imperfecta. **Method** The dental status was registered every sixth months. The timing of tooth eruption was compared to matching controls. **Results** Tooth eruption was age-appropriate in all but 1 of the 47 patients younger than 14 years of age. In this patient only the lower medial incisors had erupted at 19 months of age although all expected teeth could be found on the radiographs. Impaction of second permanent molars was found in 3 out of the 11 patients older than 14 years of age at the start of bisphosphonate therapy. **Discussion** In this study the reduced osteoclasts activity had no effect on tooth eruption. Only one patient showed late eruption of primary teeth. In this patient the bisphosphonate therapy was started when she was one year old. Thus, the late eruption of the primary incisors was not an effect of the bisphosphonate therapy. Impaction of second permanent molars is a common finding in patients with OI (37% in patients >15 yrs), and found in patients not treated with bisphosphonate as well. **Conclusions** Our results indicate that bisphosphonate therapy does not affect tooth eruption.

Notes

P35

INTRAVENOUS PAMIDRONATE TREATMENT TO INFANTS WITH SEVERE FORM OF OSTEOGENESIS IMPERFECTA

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Objective Children with osteogenesis imperfecta (OI) have in several studies been treated with intravenous Pamidronate, but there are only few reports of the effects of early treatment. The aim of this study was to evaluate the effect of treatment started in infancy.
Methods In prospective observational study disodium pamidronate (APD) was given as monthly intravenous infusions to 11 children with OI aged 3-14 months (median 3.5 and mean 6 months) at the start of treatment. They had severe forms of OI (five with type III, four with type IV and two with severe form of type I) with congenital bowings of the femora and spinal compression fractures.
Results During treatment for 2.5 to 6 years (median 4.5 and mean 4.1 years) DXA measurements of the whole body and the lumbar spine showed a gradual increase in bone density. Bone metabolism parameters in serum (ALP, osteocalcin, P1CP, 1CTP) and urine deoxypyridinoline all indicated that there was a decrease of bone turnover. An improvement of mobility was seen and at the latest recording at the age of 2.8-6.5 years (median 4.7 years) they all had some walking ability, eight had a functional walking in secluded surroundings which for five of them was normal compared to age matched healthy children. Vertebral remodeling was seen and no one developed scoliosis, kyphosis or basilar impression during this time. All children were operated with femoral intramedullar rods due to fractures, eight with bilateral and three with unilateral rods. Additionally five children also needed tibial osteotomy and rodding due to extreme curvatures which prevented functional standing and walking. No adverse effects were seen on growth, fracture healing or on toxicity monitoring of blood chemistry for liver, kidney and bone marrow function. The median value of height was at the start of treatment -5.5 SD and at the latest recording the corresponding median value was -4.8 SD. In seven children a spondylolysis of the 5^{th} lumbar vertebra was seen.
Conclusions APD treatment is an efficient symptomatic treatment to infants with severe form of OI, but additional orthopedic surgery is often needed. Early treatment may prevent scoliosis and basilar impression. Long term follow up is important but so far the treatment has not shown any major toxicity.

Notes

P36

EARLY BISPHOSPHONATE TREATMENT IN INFANTS WITH SEVERE OSTEOGENESIS IMPERFECTA

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Background: Type III Osteogenesis imperfecta (OI) is characterized by high frequency of fractures from birth, severe bone deformities and extreme short stature. Bisphosphonates have demonstrated beneficial effects in children affected by this severe type of OI, but no studies at present were conducted in infants.
Objective: To evaluate the efficacy and the safety of bisphosphonate (Neridronate) treatment during the first months of life in infants affected by severe forms of OI, with the aim to positively modify the natural history of the disease.
Patients and Methods: Ten children (6 F) with diagnosis of OI type III at birth were studied. 5 patients (3 F) started treatment just after diagnosis (Group A), 5 were followed for 6 months and started treatment after this period (Group B). All patients were followed for 18 months. The control group consisted of ten children matched for age, sex and clinical severity of OI, who had not received treatment in the first 2 years of life. Neridronate was administered for two consecutive days at the dose of 1 mg/kg body weight, by slow i.v. infusion, every 3 months. At the start of the study and every 3 months weight, length, number of fractures were taken. Serum and urinary levels of calcium, phosphate, creatinine, serum IGF-I and osteocalcin and urinary NTx were measured every six months. Lateral X-rays of vertebral column were obtained at baseline and every 6 months to evaluate projected area (mean L2-L4) and shape of lumbar vertebral bodies.
Results: No adverse side-effects were noted, apart from the well known acute phase reaction during the first infusion cycle in 9 patients. Weight and length gain (in SDs) were significantly higher in Group A respect to Group B and controls. When Group B started treatment, a rapid catch-up growth was found. Fractures significantly decreased in frequency (n/yr) in treated infants and the treatment did not alter the rate of fracture healing. IGF-I increased significantly while NTx and urinary calcium decreased significantly in treated patients. An improvement in the morphology and a correction deformities of vertebral bodies were found.
Conclusions: Intermittent i.v. Neridronate treatment is safe, appears to have a positive impact on morbidity of OI and may be started in the first months of life, at a very important age for psychological and motor development.

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PRECOCIOUS PUBERTY IN A GIRL WITH OSTEOGENESIS IMPERFECTA TREATED WITH NERIDRONATE: EVIDENCE FOR NEGATIVE EFFECT ON BONE MINERAL DENSITY OF GNRH ANALOG TREATMENT.

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Background. Treatment of precocious puberty (PP) with GnRH analogues (GnRHa) leads to a situation of hypoestrogenism, that may have a theorethically detrimental effect on bone mass during pubertal development. However, GnRHa treatment in patients with PP does not seem to impair the achievement of a normal PBM at final height. On the other hand, bisphosphonates have demonstrated beneficial effects in children affected by osteogenesis imperfecta (OI), increasing bone mineral density and reducing the fracture rate and pain with no adverse effects.

Patient and Methods. We report a case of a girl affected by type I OI treated with Neridronate iv at the dose of 2 mg/kg body weight, every 3 months, by the age of 5.5 years. At the age of 7.8 yrs, a diagnosis of PP was made and a treatment with GnRH analog (triptorelin im, 3.75 mg every 28 days) was started at 8 years of age, for the parents' request and after informed consent. Neridronate treatment continued as usually. Bone mineral density (BMD mg/cm^2) at the distal and proximal radius, lumbar spine (L2-L4), and femoral neck was measured by dual energy x-ray absorptiometry (DXA) every 6 months.

Results. An increase in BMD levels were reported during Neridronate treatment between 5.5 and 8.0 yrs. Between 8 and 9 yeras of age, during GnRHa treatment we found a decrease in radial and lumbar BMD, but not in femoral BMD. At this time we decided to stop GnRHa treatment, continuing Neridronate. After stopping GnRHa, radial and lumbar BMD increased, while femoral BMD decreased in the next 6 months and increased thereafter. (Proximal Radius: BMD mg/cm^2: before: from 397 to 541, during GnRHa: from 541 to 528, after: from 528 to 622; L2-L4: BMD mg/cm^2: before: from 519 to 729, during GnRHa: from 729 to 707, after: from 707 to 787; Femur (total) BMD mg/cm^2: before: from 416 to 596, during GnRHa: from 596 to 749, after: from 749 to 709 after 6 months and 824 after 12 months).

Conclusions. In our experience there was an evidence for negative effect of GnRHa treatment on BMD in OI patients and we do not recommend this treatment further; finally, the DXA BMD response to hormonal changes may be site- and time-dependent.

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BONE IN BONE IMAGE AFTER TREATMENT WITH INTRAVENOUS INFUSIONS OF DISODIUM PAMIDRONATE IN A CHILD SUFFERING FROM OSTEOGENESIS IMPERFECTA.

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A boy aged 12 suffering from osteogenesis imperfecta, complicated by several peripheral and thoracic vertebral fractures was treated by intravenous infusions of disodium pamidronate (1 mg/kg per infusion), 3 consecutive days every 3 months for 2 years. His BMD measured by DXA increased from 0.416 to 0.600 g/cm^2 at the (L1-L4) lumbar spine and from 0.590 to 0.804 g/cm^2 at the total hip after 4 years. In the same time, he grew 16 cm. The view of the postero-anterior DXA scan of the lumbar spine obtained 2 years after the last course of therapy demonstrated the presence of “arrest lines” close to the plateaus of the vertebral bodies. X-ray films confirmed that this aspect was attributable to a “bone in bone image” due to bisphosphonate therapy, a well-known radiological aspect in growing children treated by cyclical intermittent bisphosphonates (1). This DXA aspect corresponding to the radiological image should be recognized, because much of the bone gain can be explained by it.

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Notes

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CHILDREN WITH MILD OI HAVE A GREATER RESPONSE TO BISPHOSPHONATE THERAPY THAN THOSE WITH SEVERE OI

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Bisphosphonate therapy increases bone mineral density (BMD) and reduces fractures in OI children. Children 3-13 yrs of age were stratified by bone age, pubertal stage, and type of OI and then randomized to receive oral alendronate 1 mg/kg/day up to 20 mg as tablets or intravenous pamidronate, 3 mg/kg/4 months for 2 yrs in an open-label, prospective study. Nine received oral and 9 intravenous therapy. In both groups, 3 had severe OI (type III/IV) & 6 had mild OI (type I). Children with milder OI (Type I) were taller than those with more severe OI (Type III/IV) (height SDS -1.4±1.8 vs -5.3±1.3, $p<0.001$). With treatment, height Z-score increased 0.4 in the Type I children ($p <0.01$) whereas it changed only 0.2 in the Type III/IV group ($p=0.47$). Children with mild OI had greater mean total body (TB) area, BMD, and BMC and L2-L4 BMD and BMC than those with severe disease Increases in TB BMC, area, and BMD were all significantly greater in the type I group than in the type III/IV group. Mean TB BMD z-score in the type I group increased from -1.1 to 0.3 at 24 months ($p<0.001$) whereas in the type III/IV group it did not change significantly, -2.2 to -2.1 ($p=0.45$)). Increases in L2-L4 were only significantly different between groups for BMC at 12 months. In type I mean BMD z-score at L2-L4 increased from -2.9 to -0.5 at 24 months ($p=<0.001$) whereas the increase in type III/IV was less, -3.9 to -2.5($p=0.02$). There were few significant differences in response to treatment by severity in turnover markers or calcium-regulating hormones. At baseline, children with milder OI had lower serum phosphorus levels and lower urinary NTX levels (568±278 nMBCE/mM versus 921±199 nMBCE/mM, $p<0.02$); by 4 months these levels were comparable. Osteocalcin tended to be higher in the type I group at all time points, and significantly different from type III children at 12 months (56±39 vs. 22±7, $p<0.02$). In our long-term studies beyond two years, three mild patients increased BMD above +1.5 SD for age and were discontinued from treatment as dictated by our institutional protocol, whereas none of the severe cases have done so over the same period of time. This difference in BMD effect by severity implies that bisphosphonate treatment should be tailored by type of OI in children.

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HEIGHT AND WEIGHT DEVELOPMENT DURING BISPHOSPHONATE THERAPY IN CHILDREN WITH PRIMARY OSTEOPOROTIC DISEASES

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Children with osteoporotic diseases are frequently treated with bisphosphonates. The impact of bisphosphonates on body height remains unclear whereas some authors claim an increase in body weight, although the cause for the latter is unknown so far.

We analyzed data of 40 children (34 diagnosed with osteogenesis imperfecta; 6 with idiopathic juvenile osteoporosis) treated intravenously with pamidronate (1 mg/kg on 3 consecutive days every 2-4 months[1]). The mean (SD) treatment period was 2.0 (0.9) years. At the start of therapy, the patients age was 6.7 (SD 4.7) years and the body mass index (BMI) was 17.9 (4.2). The standard deviation score (SDS) of body height was -3.1 (3.1). At the end of the observation period, the BMI was 19.0 (5.1) and height SDS was -2.9 (3.0). During treatment, body height improved by 0.25 SDS. While growth was reduced >1 SDS in 6 children (15%), it increased >1 SDS in 8 children (20%) and remained constant (± 1 SDS) in 26 (65%). The mean increase in body mass index (BMI) was 1.7 (4.9). BMI increased >1 in 17 children (40%) and in 3 children >5 (7.5%), it decreased >1 in 6 and remained constant (± 1) in 17 patients.

In conclusion, we found an increase of body height in children with primary osteoporosis treated with pamidronate i.v. indicating a small catch-up growth. This was accompanied with a slight increase in BMI in most patients but 3 developed obesity during treatment.
Further investigation have to show whether this is due to bisphosphonate treatment or related to changes in live style (physical activity, nutrition).

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Notes

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POSTPARTUM OSTEOPOROSIS IN TYPE I A OSTEOGENESIS IMPERFECTA: RESPONSE TO BISPHOSPHONATES

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Transient osteoporosis is an uncommon, self-limited disorder involving the hip, femur and pelvic bones. It most often affects healthy women during the third trimester of pregnancy but may occur in middle-aged men and in OI. Pain at rest, which may be severe, prevents weight bearing, restricts motion at the hips, and radiates to the groin and lower back. X-ray shows diffuse osteoporosis. ^{99m} Technetium scanning shows increased uptake involving the head and shaft of the femur or ankle, which may precede x-ray changes. Magnetic resonance imaging (MRI) T1 images show decreased signal intensity of bone marrow. T2 weighted images reveal increased signal intensity characteristic of bone marrow edema that is uni- or bilateral. Stress fractures may occur. Conventional treatment involves limited weightbearing, joint protection and analgesics. Symptoms may last 8-12 months. Bone scans normalize after 12-15 months. A 26 year old woman with type I A OI, gravida 1, 3 weeks post-partum, presented with severe bilateral hip pain starting 2 weeks prior to caesarean section at 36 weeks. Her son had a fractured clavicle. 3 weeks post partum she was confined to bed and wheelchair due to bilateral hip and groin pain which prevented weightbearing. Hip x-rays were normal. MRI T1 images showed a small right hip effusion. T2 weighted images demonstrated increased signal consistent with bone marrow edema in the right and left femoral heads, the right anterior pubis and superior sacrum not consistent with acute vascular necrosis or fracture. Sedimentation rate (ESR) was 105 mm/hr, alkaline phosphatase 152 IU/L (30-120), 25 (OH) vitamin D 38 ng/ml, NTX excretion 133 nMBCE/nMcreatinine (14-87). Pamidronate (60 mg) was administered IV at 3 week intervals, but due to vein irritation following the first two infusions, treatment was changed to oral alendronate, 40 mg/d. She experienced a rapid and substantial decrease in hip pain by 4 weeks of treatment and was weightbearing by 5 weeks. ESR decreased to 32 mm/hr. Weitzel (J. Orthopedics, 23: ‘03) reported post partum osteoporosis of the hip in a type I OI woman treated with restricted weightbearing and analgesics which resolved after 6 months, but recurred following a second pregnancy. Here, administration of bisphosphonates significantly diminished hip pain and permitted weightbearing after 5 weeks of treatment. Along with clinical improvement, major resolution of MRI abnormalities in sacrum and femur occurred after only 3 months of bisphosphonate treatment.

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OSTEOGENESIS IMPERFECTA IN CHILDHOOD; HABILITATION STRATEGIES REGARDING FAMILY, FUNCTION AND FITNESS

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Habilitation strategies in Osteogenesis Imperfecta in childhood, what is new since Annecy (Fr) 2002 ?. Based on current literature, the contradictory effects of bisphosphonates on motor development and functional ability will be highlighted. Sakkers et al reported, based on a randomized placebo-controlled clinical trial, no beneficial effects of oral medication on ambulation, functional ability and muscle strength [1]. A controlled trial in children with types III and IV OI confirms vertebral gains but not short-term functional improvement. [2] Others reported increase in isometric grip as well as functional ability, mobility and aspects of quality of life. [3-5] In young children bisphosphonates have been reported to be safe, increase BMD and reduces fracture frequency. [6] We recently found that intravenous bisphosphonates in young children with severe OI enhance the development of particularly dynamic motor milestones, increase body height, weight, bone mineral density and decrease fracture incidence. [7] Recently, attention is focussed on intervention in fitness in OI children. In patients with OI type I, no pulmonary or cardiac abnormalities at rest were found. The exercise tolerance and muscle strength were significantly reduced in patients with OI, which might account for their increased levels of fatigue during activities of daily living. [8] We currently study the effect of a fitness program on functional ability, muscle strength and exercise tolerance in children with OI. In future, research should focus on family dynamics in OI and how interventions should be beneficial in functional ability, family dynamics and fitness. Collaboration between experienced OI centers remains to be essential.

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Notes

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TECHNOLOGY IN THE CLINIC: UNDERSTANDING FUNCTION IN CHILDREN WITH OSTEOGENESIS IMPERFECTA.

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Introduction:

Children with Osteogenesis Imperfecta have significant impairments, some of which limit their ability to achieve being upright. We describe findings, determined using newer technologies, likely to influence upright posture and activity.

Methods:

Prospective clinical data were collected from patients coming to the National Institute of Child Health and Human Development to participate in studies of Osteogenesis Imperfecta. These included: anthropometrics, video based gait analyses including kinetics and kinematics, radiographics of thorax and spine and a performance measure of gross motor function using the Brief Assessment of Motor Function (BAMF). Correlations were performed among these datasets using Spearman rank order and Kruskal-Wallace test to evaluate relationships.

Results:

Static and dynamic measures of leg lengths were obtained in 71 children which showed an association between leg length discrepancy (LLD) and pelvic height difference, ($p=0.031$). There were statistically significant correlations between single limb stance (SLS) and LLD ($r=0.437$, $p=0.0134$). When subjects who used walkers were eliminated from the analysis, the correlation between SLS and LLD was ($r=0.673$, $p=0.0043$). Chest deformities were not static as children aged. Ratio of chest AP to body length measures correlated with BAMF score in girls ($r=-.83$) and boys ($r=-.64$); chest circumference ratio to body length for boys ($r=.4738$). All p values $\leq .0001$. 31 children (76%) had chest deformity 8 (20%) changed from no deformity to greater or different deformity during 14 mo interval. The presence of pectus carinatum was significantly associated with below normal joint range of motion and lower BAMF gross motor scores ($p=.015$). Pectus excavatum was significantly associated with higher motor performance BAMF scores and joint hypermobility ($p=.02$) BAMF scores were significantly correlated with: speed: ($r=.68$, $p=.0001$); stride length: ($r=.71$, $p=.0001$); the number of weak muscles: ($r=-.74$, $p=.0001$) specifically, hip extensors, flexors, abductors; and quadriceps ($r>.52$, $p<.003$ for any one muscle).

Conclusions:

LLD, pectus carinatum, lower extremity proximal muscle weakness, all significantly correlate with motor performance. Interventions aimed at their correction and improvement are likely to result in improved upright activity.

Notes

HEARING LOSS IN OI – A POPULATION STUDY IN FINLAND

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In a nationwide study during the years 1995-2003, 299 Finnish OI patients were ascertained (5.74/100 000). Of the 183 patients with audiometry, 46 were children and 137 were adults. Results of middle ear surgery, vestibular problems and search for molecular defects with genotype-phenotype correlation analyses were studied in adults.

Hearing loss may already be present in childhood. It was found in 58% of adults. Almost 20% of adult patients misjudged their hearing ability. Hearing loss affects patients with all types of OI, but tends to be more common in OI types I and III than in OI type IV. Sensorineural hearing loss, especially at an early age, may be more common in OI type I. Because the early detection and treatment of hearing loss is important to avoid aggravation of physical handicap, audiometry should be performed in all OI patients at the age of 10 years and repeated every third year thereafter.

Vestibular dysfunction is common in OI. Inner ear damage appears to be the main reason for vertigo. Occasionally, it is caused by basilar impression (BI), but some OI patients without BI or hearing loss also suffer from vertigo.

The surgical anatomy in OI differs from otosclerosis especially by thick and vascular mucosa with excessive bleeding tendency, and elastic, fractured or atrophic stapes crurae. These anatomical peculiarities cause technical problems. A correct preoperative differential diagnosis of OI-related hearing loss and otosclerosis is a prerequisite for successful surgery. Furthermore, the hearing gain appears to be better after surgery centralized in units with a larger annual number of operations and more experienced surgeons.

Mutations in COL1A1 or COL1A2 were found in 49 unrelated patients, representing the molecular genetic background of 41.1 % of the Finnish OI population. Mutation type is associated with OI type, while null allele mutations most often produce OI type I, and single base substitutions resulting in glycine substitutions in proα2(I) tend to produce OI type I and IV. Neither the mutated gene nor the type of mutation correlated with the presence, type or severity of hearing loss. The hearing loss in OI apparently is a result of multifactorial, yet unknown genetic and environmental effects.

Notes

COCHLEAR IMPLANTATION IN OSTEOGENESIS IMPERFECTA

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Although the most widely recognized manifestation of osteogenesis imperfecta is the tendency toward multiple spontaneous fractures, hearing loss is also a common feature. Hearing loss occurs in 42 to 58% of patients with deafness occurring in 25 to 60%. An association between osteogenesis imperfecta (OI) and otosclerosis has been demonstrated and many patients with OI present with a conductive hearing loss similar to otosclerosis. In cases of stapes fixation, stapedectomy can be performed. Ossiculoplasty can be performed for other forms of conductive hearing loss.

When sensorineural hearing progresses to bilateral severe to profound levels and conventional hearing aids are no longer effective, cochlear implantation is the only therapeutic option. Technical challenges may occur during implantation because the mucosa overlying the promontory may be thickened and the promontory overlying the cochlea may consist of vascular, spongiotic bone. In addition, intracochlear fibrosis and ossification may occur. We report a case in which all of the above were encountered, in addition, a wound infection occurred which required explantation and reimplantation. Inspite of these obstacles, cochlear implantation was successfully accomplished. Postoperative sound awareness improved to 25dB and Hint sentence recognition improved from 0% to 61% at 6 months postimplantation.

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SKULL BASE ABNORMALITIES IN OSTEOGENESIS IMPERFECTA - CEPHALOMETRIC EVALUATION OF 54 ADULT PATIENTS AND 108 ADULT CONTROLS

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In Osteogenesis imperfecta (OI) head is often abnormally shaped, and changes in skull base anatomy in the forms of basilar impression and basilar invagination have been reported. Although the basilar region can be best visualized by CT or MRI, lateral skull radiographs provide a low-cost and simple method for epidemiological studies and screening purposes. We analyzed the skull base anatomy from standardized lateral cephalograms of 54 adult OI patients with types I, III, and IV OI, as well as of 108 controls. In several previous studies on OI patients, basilar abnormality has been diagnosed when the tip of the odontoid process lies more than 5.0 mm above Chamberlain's line or more than 7.0 mm above McGregor's line. Of our unaffected Finnish controls, 7.4% and 6.5% exceeded these diagnostic limits, respectively, and hence there was an obvious need for re-evaluation. We calculated control population mean values for McGregor's, Chamberlain's and a novel reference line. These three mean values were exceeded by more than 2SD in 28.3-35.2%, and by more than 3SD in 13.2-16.6% of the OI patients. Moreover, the odontoid process protruded into the foramen magnum or touched the foramen magnum line in 22.2% of the OI patients, whereas none of the controls showed this feature. We therefore suggest that basilar abnormality can be reliably diagnosed in adults when the tip of the odontoid process does not entirely lie below the foramen magnum line (and call this condition basilar impression), or exceeds one of the following measures: 11.9 mm for McGregor, 10.5 mm for Chamberlain, or 9.4 mm for the vertical distance from odontoid tip to the lowest occipital point (and call this basilar invagination). Platybasia (anterior cranial base angle > 146 ^{o}) was present in 11.1% of the patients but not in any of the controls. Platybasia, basilar impression and basilar invagination were often co-expressed, but each was also found to be present as an isolated abnormality. These three abnormalities as well as wormian bones were predominantly found in types III and IV OI, and in patients exhibiting dentinal abnormality.

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PULMONARY FUNCTION ABNORMALITIES IN CHILDREN WITH OSTEOGENESIS IMPERFECTA CORRELATE WITH OI TYPE AND LOCATION OF COLLAGEN MUTATION.

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Osteogenesis Imperfecta (OI) is characterized by osteoporosis, bone fragility and other connective tissue abnormalities. OI patients with severe chest wall deformities and scoliosis develop respiratory insufficiency. Cor pulmonale is a major cause of morbidity and mortality in adults with severe OI. To determine whether early pulmonary abnormalities are detectable in children with types III and IV OI and to learn whether these abnormalities correlated with OI type, severity of scoliosis or location of type I collagen mutations, we designed a retrospective cross-sectional observational study. Forty-seven children with types III and IV OI(25 type III, 22 type IV), age 4.9 - 23 years underwent 131 pulmonary function evaluations (PFT) over 7 years. The type I collagen mutations were identified in 39 of the children; there were 24 mutations in the α1chain and 15 mutations in the α2 chain. Multiple regression analysis of Forced Vital Capacity, Total Lung Capacity, Vital Capacity (% of predicted), age, mutation, OI type and degree of scoliosis were performed. Our results reveal a PFT decline during childhood in both types of OI, (TLC: $r=-0.41$, $p<0.0001$; VC: $r=-0.48$, $p<0.0001$; FVC: $r=-0.45$, $p<0.0001$). Children with progressive type III OI had greater loss of FVC than moderate type IV OI. PFT abnormalities correlated with severity of scoliosis. However a significant decline also occurred in children with minimal scoliosis. (TLC: $r=-0.42$, $p=0.01$; VC: $r=-0.31$, $p=0.0005$; FVC: $r=-0.43$, $p= 0.008$). The decline of PFT with age was significantly greater in children with α2(I) than in α1(I) mutations. We conclude that significant pulmonary abnormalities are detectable in children with type III and IV OI, most of whom are asymptomatic. PFT's decline with age in both moderate and severe OI, although the decline is greater in type III OI. PFT abnormalities correlate with severity of scoliosis but also decline in those with minimal spine curvature, suggesting a direct effect of the abnormal collagen in lung tissue. This data provides the basis for careful early monitoring and therapeutic intervention in OI children to prevent cor pulmonare in adulthood.

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HEARING LOSS IN CHILDREN WITH TYPES III AND IV OI: CORRELATION WITH SP1 GENOTYPE AND OI CLINICAL FEATURES

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Previously reported data (Letocha et al., 2002; Kuurila et al, 2000 & 2004) demonstrate a higher occurrence of hearing loss in children with OI than in unaffected children, and a similar prevalence of hearing loss in children with COL1A1 and COL1A2 mutations. Previous data were expanded to investigate the associations between hearing loss and clinical and molecular parameters. The NIH pediatric OI population receives regular audiology exams, L1-L4 DEXA and surveillance for BI. The collagen mutations in most of our study population are known and genotypes for the polymorphic Sp1 site in COL1A1 intron 1 were determined. Associations between hearing loss, mutation location, SP1 genotype, DEXA z-score, and presence of BI were analyzed. There are 31 patients with COL1A1 mutations, 15 of whom have hearing loss, and 18 patients with COL1A2 mutations, 7 of whom have hearing loss. Frequency of hearing loss does not differ for mutations in α1(I) vs α2(I) ($p = 0.56$), nor does age at which hearing loss began (8.91 ± 5.33 yrs for patients with COL1A1 mutations; 10.18 ± 2.91 yrs for COL1A2 mutations, $p = 0.27$). Age at hearing loss was not correlated with DEXA z-score (low = -2 to -4, moderate = -4 to -6, severe = < –6; $p = 0.33$). Forty-two patients have glycine substitutions; the remaining 7 have exon skipping, alternative splicing, or other amino acid substitutions. Hearing loss was not associated with mutation type ($p = 0.22$). Frequency of hearing loss did not differ significantly for SS vs. Ss genotypes of the Sp1 polymorphism ($p = 1$), with heterozygotes evenly distributed between patients with and without hearing loss. Frequency of hearing loss also did not differ between types III and IV OI patients ($p=0.13$). Hearing loss was not significantly associated with BI ($p = 0.57$), BI with platybasia ($p = 0.33$), or BI with platybasia and syrinx ($p = 0.24$). Neither type of hearing loss (conductive, sensorineural, or mixed) nor its severity differed significantly for the 2 alpha chains ($p = 1$ and 0.81), severity of osteoporosis as measured by DEXA z-scores ($p = 0.74$ and 0.47), or presence of BI ($p = 1$ for both). In the NICHD OI study population of children with types III and IV OI, the presence, type and severity of hearing loss were not statistically related to molecular or clinical parameters. This data confirms and extends similar results on OI patients in Finland.

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CYCLIC INTRAVENOUS PAMIDRONATE IN OSTEOGENESIS IMPERFECTA TYPE I

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OI type I is characterized by bone fragility and distinctly blue sclerae. A cross-sectional and longitudinal study of bone density in 35 children and adolescents (average of 4 scans per subject) with OI type I showed a progressive decline with age.

Study Design

A cohort of children and adolescents with mild OI type I was studied. The subjects had either: symptomatic osteoporosis with fractures, evidence of progressive vertebral crush fractures, areal bone mineral density (BMD) z-scores $\leq$ -2.0, or a progressive decrease in serial BMD z-scores. Patients were treated with cyclic intravenous Pamidronate. Postpubertal subjects received 30mg/M² monthly. Prepubertal subjects received 30mg/M² monthly for 12 months and second monthly for 12 months.

All subjects were clinically assessed and had biochemistry, urinary deoxypyridinoline and BMD (by dual-energy x-ray absorptiometry, DXA, Lunar DPX) measurements performed every 6 months.

Results

Cohort consisted of 18 (9F) prepubertal children (mean age = 6.17 years, range 2.6-13.2) and 12 (4F) postpubertal children (mean age = 13.91 years, range 12.8-15).

Prepubertal subjects showed significant increases in Total Body and L2-L4 BMD z-scores, after both 12 months and 24 months of treatment. Post pubertal subjects showed significant increases after 12 months of treatment in L2-L4 BMD, and after 24 months, in both the Total Body and L2-L4 BMD. The greatest increases for both groups were seen in L2-L4 BMD. Prepubertal subjects had significantly greater responses than the postpubertal group at 12 months and 24 months ($p < 0.05$).

Positive increments in height z scores were observed in both prepubertal and post pubertal children. Treatment was characterized by a self-reported decrease in bone pain. Therapy was ceased in all children achieving a BMD in the normal range (z-score approaching 0 on 2 occasions).

The findings have significant implications for management of OI type I.

Notes

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ORAL AND DENTAL MANIFESTATIONS IN ADULTS WITH OSTEOGENESIS IMPERFECTA

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Introduction. The study is part of a project describing a population of adults with osteogenesis imperfecta (OI). The ongoing study includes individuals from 25 to 100 years and finally it will comprise about 90 persons. Until now 60 persons have been examined (22 males and 38 females) with a mean age of 46.8 years (range 27-83 years). The aim of our study is to describe oral manifestations of OI in an adult population.
Methods. The study material comprises a questionnaire, dental intra- and extraoral examination, photographs, orthopantomograms and study models of teeth and jaws. Tooth discolouration, missing teeth, filled teeth and malocclusion were recorded. Objective and subjective TMJ dysfunction was registered and tooth obliteration and wear were assessed.
Results. Dentinogenesis imperfecta (DI): In the study group 15 % showed clinical signs of DI. The affected teeth were abnormally translucent and discoloured, exhibiting a yellowish, greyish or brown-bluish hue. Missing and filled teeth: 25 % of the participants were missing 5 permanent teeth or more. The mean number of missing and filled teeth in the study group was not statistically different from the general population. Malocclusion: 13 % of the participants had mandibular overjet and mesial molar occlusion. Mandibular opening capacity: 50 % of the individuals had reduced mouth opening capacity, measured between front teeth. In the study group 85 % of the individuals described minor temporomandibular problems while 3 % had severe problems. A majority of the OI-population visited the dentist or dental hygienist regularly, at least once yearly. 73 % received full coverage for dental treatment from the national health insurance system.
Conclusions. Mandibular overjet, reduced mouth opening capacity and TMJ – problems are much higher than in comparable groups. The study group showed good dental health compared with the general population.

Notes

Prospects for Gene and Cell Therapy

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TARGETED DISRUPTION OF THE TYPE I COLLAGEN GENES IN MESENCHYMAL STEM CELLS RESTORES PROPERTIES OF NORMAL COLLAGEN

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Therapeutic prospects for osteogenesis imperfecta present a challenge due to the dominant nature of the mutant collagen produced from osteoblasts. Therefore, a feasible approach for development of a treatment includes elimination or prevention of mutant collagen expression. OI results from mutations in the type I collagen genes *COL1A1* and *COL1A2*. Transplantation of autologous osteoblast precursors, or mesenchymal stem cells (MSCs), with one normal and one inactivated mutant *COL1A1* or *COL1A2* allele could convert severe OI to mild disease *in vivo*.

Our approach for development of a treatment for OI combines methods of both cell and gene therapy. We previously showed that adeno-associated virus (AAV) vectors can efficiently introduce specific genetic changes into homologous genomic sequences of normal human cells. Here we present experiments in which AAV vectors were used to inactivate either *COL1A1* or *COL1A2* in MSCs. We have designed AAV vectors to insert a selectable marker at both *COL1A1* and *COL1A2* to eliminate gene expression of the mutant allele. The current targeting vectors consist of regions of homology to either *COL1A1* or *COL1A2* flanking an internal ribosome entry site (IRES), *neo* gene, and polyA. AAV vector infection of MSC lines established from individuals with OI results in selection of 0.03-0.4% of the initial cell population. Based on Southern blot analysis 31-90% of the cells were targeted at either at exon 1 of *COL1A1* or exon 4 of *COL1A2* depending on gene sequence included in a targeting vector. Mutant and wild type alleles were targeted at roughly equal rates based on SDS-PAGE analysis of collagen proteins. OI MSCs that were targeted differentiated into osteoblasts and formed bone following subcutaneous implantation of a seeded matrix into NOD/SCID mice. Gene-targeted OI MSCs were analyzed for their ability to synthesize collagen with normal biochemical properties. We showed that collagen biosynthesis and structural resistance to enzymatic digestion return to normal following gene targeting of mutant alleles. Establishment of polyclonal populations with up to 90% of cells that had undergone gene targeting suggests a simpler strategy more suitable for clinical applications. Our findings establish the potential of AAV-mediated gene targeting in combination with stem cell therapy for the treatment of OI and other dominant genetic diseases.

Notes

RNA INTERFERENCE FOR NEUROGENETIC DISEASE THERAPY

Beverly L. DAVIDSON, Ph.D.
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Director, Gene Transfer Vector Core
Associate Director, Gene Therapy Center for Cystic Fibrosis and Other Genetic Diseases

Notes

ENDOGENOUSLY EXPRESSED MULTIMERIC SELF-CLEAVING HAMMERHEAD RIBOZYMES ABLATE MUTANT COLLAGEN

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Hammerhead ribozymes are small catalytic RNA molecules that can be targeted to any RNA molecule containing a putative cleavage site, and can selectively eliminate mutant gene products producing dominant negative effects, such as those found in severe variants of osteogenesis imperfecta. We previously reported the development of a self-cleaving multimeric hammerhead ribozyme (Smicun *et al*, 2003). A vector was developed (pCOLZ) that used the *COL1A1* promoter to drive expression of the multimeric ribozyme (M8Rz547) and its monomeric counterpart (Rz547). The multimeric and monomeric ribozymes in this vector were stably co-expressed in MC3T3-E1 osteoblasts expressing the truncated *COL1A1* target transcript and protein derived from the plasmid pMG155. The multimeric ribozyme exhibited self-cleavage to derivative fragments, including monomers. Increased expression of ribozymes was found in cells expressing the multimeric ribozyme compared to the cells expressing the monomeric ribozyme. A modest reduction of truncated target transcript and protein was seen in cells expressing the ribozyme monomer, while nearly complete ablation of target transcript and protein was produced in cells expressing the ribozyme multimer. Both monomeric and multimeric ribozymes expressed from the pCOLZ vector were found in the nucleus and cytoplasm, and target transcript reduction appeared to be more active in the nucleus than the cytoplasm. A reversion to a more normal collagen phenotype, measured as an increase in fibril diameter and restored fibrillar architecture, and in a decreased rate of collagen turnover, was produced in cells expressing the ribozyme multimer. M8Rz547 is highly expressed in vivo, and mice doubly transgenic for the truncated collagen gene and M8Rz547 show behavioral evidence of reversion to normal phenotype. Self-cleaving multimeric ribozymes expressed from an endogenous promoter are more effective than monomers at eliminating target gene products.

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Notes

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BONE MARROW DERIVED OSTEOPROGENITORS TRANSPLANTED INTO NEONATAL OSTEOGENESIS IMPERFECTA MICE (OIM) CONTRIBUTE TO THE BONE FORMATION IN VIVO

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To explore the potential of mesenchymal stem cells to contribute to skeletal tissue cell phenotype, osteoprogenitors generated by repeated injection of the eGFP marked cells into neonatal mice were transplanted into 2-day old neonatal osteogenesis imperfecta mice (oim). Histological sections made from the femurs and tibia of the recipient mice at 14 days after cell transplantation demonstrated a larger number of GFP positive (GFP+) cells in the bone marrow as well as on the bone surfaces of the recipient mice. By 28 days after cell transplantation, gross examination of whole femurs, tibias and forelimbs harvested from the recipient mice under fluorescent microscope for GFP detection, demonstrated presence of a large number of GFP+ cells that appeared to be associated with the periosteum on all the bone surfaces. Tissue sections made from the femurs and tibia of the recipient mice demonstrated a large number of GFP+ cells on the surfaces of bone spicules in the spongiosa as well as in the endosteum and in the bone marrow cavity of the femurs and tibias of the developing OI mice. Some GFP positive cells were also present in the growth plate. Gene expression analysis of GFP+ cells recovered from the recipient mice at 28 days, demonstrated that the cells from bone were osteoblasts while the GFP+ cells from the bone marrow were osteoprogenitors. Injection of the cells locally into the femurs of the adult OI mice demonstrated that the cells were distributed on the bone surface as well as in bone marrow 2 weeks after cell injection. Cells recovered from the bones of the recipient mice again showed that the cells, which engrafted in the bones were osteoblasts and those recovered from the bone marrow were osteoprogenitors. Collagen extraction by pepsin digestion from the femurs that received the osteoprogenitor cells showed that the cells synthesized type I collagen comprising of a1(I)2a2(I) heterotrimers that is normally not synthesized by the OIM mice.These data demonstrate that the osteoprogenitors systemically transplanted into OI neonatal mice engraft in the most of the bones of the developing mice, differentiate into osteoblasts and contribute to the bone formation in vivo. These data encourage further studies in the investigation of the MSCs for the treatment of skeletal diseases like osteogenesis imperfecta.

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CELL AND GENE THERAPIES DESIGNED TO MINIMIZE THE CONSEQUENCE OF SEVERE OSTEOGENESIS IMPERFECTA (OI)

Philipp MAYER-KUCKUK[1, 2, 3], Terence GADE[2], Ian BUCHANAN[2], Ronald BLASBERG[2], Jason KOUTCHER[2], Debabrata BANERJEE[3] and Adele BOSKEY[1] [1]Hospital for Special Surgery, New York, [2]Sloan-Kettering Institute, New York and [3]University of Medicine and Dentistry, New Jersey

Cell and gene therapies designed to minimize the consequence of severe osteogenesis imperfecta (OI) are under active investigation. Development and clinical implementation of such treatments may substantially benefit from the ability to image delivery and activity of the therapeutic in OI individuals. We hypothesize that molecular imaging is a suitable tool to monitor cell and gene therapies *in vivo.* We will first discuss the principles behind two distinct yet complementary cell labeling strategies which can be used to analyze cells *in vivo*. Cells passively labeled with iron-oxide can be identified inside organs by magnetic resonance imaging (MRI), while cells engineered to express Firefly luciferase (FLuc) can be detected via time and cost-effective optical bioluminescence imaging (BLI). In a proof-of-principle study of combined MRI-BLI, we found that this combination strategy is feasible in mice and can be used (a) to identify cells within the anatomical structures of the mouse long bones and (b) to assess in real time transgene expression of the transplanted cells. As such, this approach is useful for the study of transplantation biology and it can be potentially applied to mouse models of OI cell therapy. Taken together, molecular imaging is likely to become a valuable tool for the non-invasive *in vivo* assessment of experimental OI therapies.

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