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OSTEOGENESIS IMPERFECTA: CURRENT AND FUTURE TREATMENTS

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SUMMARY

Osteogenesis imperfecta (OI) is a heritable disease of bone due to mutations involving type I collagen synthesis. Current concerns in both children and adults involve the effectiveness of intravenous and orally administered bisphosphonates, which are generally considered the standard of care. However, questions remain in terms of fracture prevention rates in children and adults, as well as questions related to dosing schedules and the duration of bisphosphonate treatment. This paper reviews collagen synthesis in OI as related to potential treatment strategies. Potential pharmacological treatments for OI involve: RANK ligand inhibitors, cathepsin K and sclerostin inhibitors. Teriparatide is currently in clinical trials. Molecular strategies include the use of hammerhead ribozymes and siRNA methodology to silence the mutant COL1 allele. Fetal intrauterine mesenchymal cell transplantation has shown promising results despite limited trials. In the situation of quantitative collagen defects, genetic treatment would involve the addition of a COL1 functional allele into the mesenchymal stem cells of a patient. In dominant/negative COL1 mutations the strategy would be to silence the mutant allele (gene targeting). Stem cell therapy involving induced pleuripotent stem cells as a means of repopulating the marrow with functional osteoblasts producing normal type I collagen may offer great therapeutic potential.

INTRODUCTION

The heritable bone disorder osteogenesis imperfecta (OI), or brittle bone disease, has a long and storied medical history dating to the description by Malebranche in 1674 of a man appearing "as broken on the wheel" and a 1,000-year-old Egyptian skeleton at the British Museum (1, 2). In his graduate thesis (1788), O.J Ekman first described OI, termed osteomalacia congenita, in three generations of one family in the Netherlands (3). Over the ensuing 222 years, various treatment regimens for OI were notable for their diversity, assorted vitamins, minerals and hormones, as well as for their lack of effectiveness in preventing fractures. Although progress had been slow, this changed with the introduction of bisphosphonates in the 1990s. However, despite the wide utilization of bisphosphonates, a diversity of treatment regimens remains when the use of individual bisphosphonates, the doses and treatment schedules for individuals with OI are taken into consideration.

OI is the most common of the inherited skeletal disorders, affecting approximately 25,000 individuals in the U.S. Patients frequently have blue sclera, multiple fractures, early-onset hearing loss and dentinogenesis imperfecta. The majority of them carry mutations altering the synthesis of type I collagen, which is the major structural protein in bone and tendon. Because type I collagen is present in all tissues including the eye, ear ossicles and blood vessels, OI is a systemic disorder affecting more than just bone. With the identification of several hundred mutations in the genetic information encoding type I collagen, advances in molecular biology allow a diagnosis based on DNA analysis in 98% of suspected cases (4). However, it has not been possible to consistently relate genotype to phenotype, although this was initially sought as a potential benefit of genetic testing. It is also appreciated that marked phenotypic variability exists, even with the same type I collagen mutation. Also, it has not as yet been possible to use DNA analysis to devise a uniformly effective pharmacological treatment for children or adults. This is in part because the majority of type I collagen mutations are unique to the individual and are widely distributed throughout the large collagen molecule. The objective of this report is to review the metabolic pathways involved in type I collagen synthesis in order to identify potential drug targets, to summarize therapies that are currently used to treat OI and to suggest future treatment possibilities.

TYPE I COLLAGEN SYNTHESIS

Type I collagen is a heteropolymer composed of two procollagen α-1(I) chains and one procollagen α-2(I) chain in a triple helical array (Fig. 1). The three α chains, each containing 1,000 amino acids, are constructed of a repeating triplet that can be represented as

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Gly-X-Y, with glycine in the first position and proline and hydroxyproline as the major X and Y components (5). Although eight first-position amino acid substitutions can occur in the triplet (Ala, Ser, Cys, Val, Arg, Asp, Glu), the most common are single-base substitutions replacing one glycine residue. A single glycine substitution destabilizes the triple helix by disrupting hydrogen bonding and causing a discontinuity in the register of the helix (6).

The bone-forming osteoblast synthesizes type I collagen and several other proteins and proteoglycans that provide the bone extracellular matrix with bulk, strength and flexibility, which is why OI is mainly a disease of the osteoblast (7). Post-translational procollagen α chain processing involves interaction with molecular chaperones (47 kDa heat shock protein [HSP47] and 65 kDa FK506-binding protein [FKBP65]), which regulate collagen folding and processing through the rough endoplasmic reticulum. Post-translational procollagen modifications include proline hydroxylation and α chain glycosylation, followed by secretion into the extracellular matrix. Cross-link formation and cleavage of *N*- and *C*-propeptide domains leads to chain assembly in the extracellular matrix, followed by the formation of the collagen fibril (Fig. 1).

Mutations affecting type I collagen synthesis destabilize the bone matrix by decreasing the total levels of collagen and the production of the large chondroitin sulfate proteoglycan osteonectin, biglycan and decorin (8). In mild type I OI, 50% of the osteoblasts are normal and 50% demonstrate a "null gene" with the mutated pro-α-1(I) chain degraded intracellularly so that only half of the normal amounts of type I collagen are produced (quantitative defect). In the more severe phenotypes in which the mutated chain is processed by the cell, each osteoblast deposits structurally defective procollagen into the extracellular matrix (qualitative defect). Because the mature type I collagen molecule is a heterotrimer composed of two pro-α-1(I) and one pro-α-2(I) chains, the incorporation of a defective pro-α chain into the heterotrimer interferes with normal α chain assembly, producing the so-called "dominant-negative" result. Delayed intracellular processing of procollagen chains leads to enhanced post-translational modification, mainly excess glycosylation of the peptides, recognized as altered chain migration in gel electrophoresis (9).

In 1956, David Sillence categorized OI into four types (I-IV) based on autosomal dominant inheritance, sclera color and clinical severity (Table I). Figures 2 and 3 illustrate two common OI phenotypes: Sillence type I (mild) and Sillence type III (severe progressive OI) (10).

Various mutations involving the type I collagen genes *COL1A1* and *COL1A2* were subsequently identified in these four phenotypes (for a current listing of mutations, see http://www.le.ac.uk/genetics/collagen/newdata.xml). Types I-IV account for approximately 90% of OI cases. Mutations have not been defined for OI types V and VI (Table I). Recently, two additional genotypes (types VII and VIII) associated with recessive inheritance and severe or lethal disease have been added to the Sillence classification (11). These account for approximately 3% of OI cases.

Types VII and VIII involve mutations affecting three additional genes: *CRTAP* (encoding cartilage-associated protein), *LEPRE1* (encoding leucine- and proline-enriched proteoglycan 1) and *PPIB* (encoding peptidyl-prolyl *cis-trans* isomerase B) (12, 13). These three proteins function as a complex to regulate 3-hydroxylation of proline-986 in the procollagen chain (14). Recently, two instances of mutations affecting endoplasmic reticulum chaperone proteins with recessive inheritance were reported in severely affected patients with OI. The first was detected in five Turkish families and involved *FKBP65*, the gene encoding the chaperone protein FKBP65, and the second was a missense mutation in *SERPINH1*, which encodes the collagen chaperone-like protein HSP47, reported in a severe autosomal recessive OI phenotype (15-17).

It is instructive to review how drugs used to treat osteoporosis alter net bone synthesis. Bisphosphonates (alendronate sodium, risedronate sodium, ibandronate acid monosodium salt monohydrate, pamidronate sodium, olpandronate and zoledronic acid) decrease osteoclast function by inhibiting the enzyme farnesyl pyrophosphate synthase in the mevalonate pathway, increasing the accumulation of unprenylated proteins, thus blocking protein transport (18). Less well appreciated is the fact that bisphosphonates also affect osteoblast protein synthesis. Alendronate treatment will increase type I collagen and osteocalcin synthesis, as well as alkaline phosphatase activity and the expression of the *BMP2* gene (encoding bone morphogenetic protein 2) by MG-63 osteoblastic cells (19). Bisphosphonates also increase osteoclast apoptosis and inhibit osteoclast differentiation by mechanisms that are independent of effects on protein prenylation (20, 21). Denosumab (AMG-162, Prolia®; Amgen) which is not currently approved by the FDA for the treatment of osteoporosis, is a human monoclonal antibody that blocks receptor activator of NF-κB (RANK) ligand (RANKL), thus inhibiting osteoclast differentiation and activity (22). Teriparatide (rhPTH-[1-34], Forsteo®, Forteo®; Lilly) has been shown to upregulate the transcriptional cell growth factors zinc finger protein osterix and runt-related transcription factor in marrow-derived mesenchymal stem cells, leading to accelerated osteoblast maturation and net bone synthesis (23). Furthermore, an increased level of canonical Wnt signaling has been observed in PTH-treated bones (24). However, because of the multiple mutations responsible for altered intracellular collagen synthesis in the OI osteoblast, it is unlikely that pharmacological methods could be used to promote the synthesis of more normal type I collagen or to stabilize the molecule in the extracellular matrix.

OSTEOGENESIS IMPERFECTA TREATMENT: PAST AND CURRENT

It is important when considering drug development or other potential treatments to differentiate the pathophysiology of age-related osteoporosis from that of OI. In the former, bone loss occurs because of an imbalance between bone formation and resorption; the aging osteoblast cannot compensate for an increase in osteoclastic bone resorption. In older individuals with osteoporosis, antiresorptive treatment with bisphosphonates suppresses bone resorption, permitting the osteoblast to complete, be it incompletely, unfilled resorption cavities (the so-called "bone transients"), leading to an increase in net bone mass. Similarly, in older individuals with osteoporosis, the osteoblast responds effectively to stimulation with teriparatide by increasing osteoblastic bone synthesis. However, in OI, osteoblasts have limited bone-forming capacity from the start. Because of mutations affecting the synthesis of multiple proteins

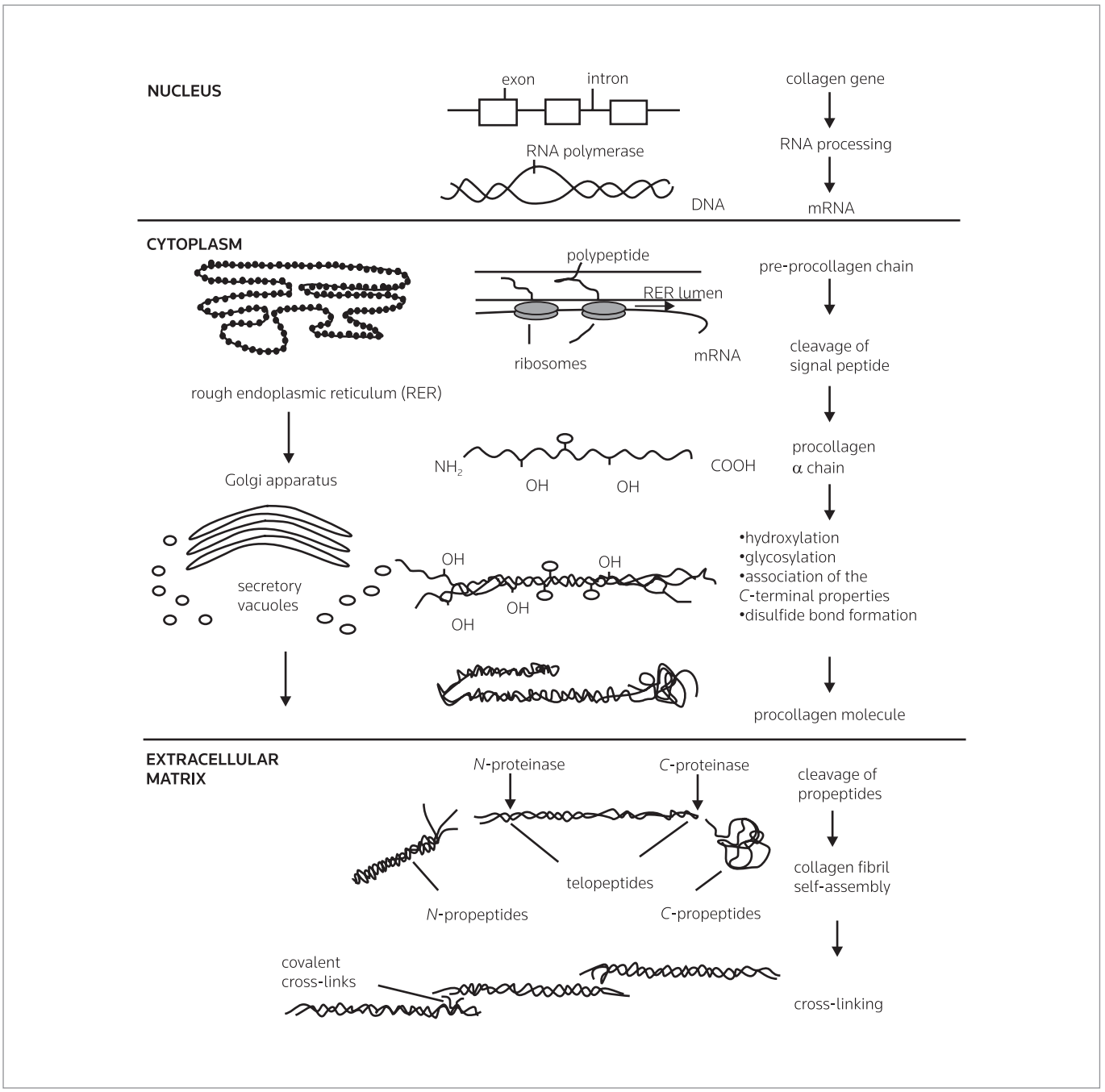

Figure 1. Intra- and extracellular type I collagen processing. Reproduced with permission from Ahtikoski, A. *Synthesis and degradation of muscle collagen during immobilization, glucocorticoid treatment and in neuromuscular diseases*, Acta Universitatis Ouluensis. Medica, D772, 2003.

and proteoglycans, bone formation is compromised throughout the life span (25). Thus, the therapeutic strategies for the two disorders are quite different. As discussed below, antiresorptive drugs may have limited effectiveness in OI and therapies promoting new bone formation may be similarly limited because of decreased osteoblast reserve. The solution may rest in finding methods to effectively alter gene function, rather than attempting to pharmacologically stimulate osteoblast bone formation. This requires suppression of the mutant protein at the molecular level to ultimately affect the composition of the extracellular matrix.

Pharmacological treatment

Ineffective treatments for OI include anabolic steroids, thymus extract, vitamin D and vitamin C in large amounts, sodium fluoride (NaF), magnesium oxide (MgO), flavonoids and calcitonin (26, 27).

Table I. *Clinical classification of osteogenesis imperfecta (OI) types.*

OI type	Clinical phenotype	Inheritance	Biochemistry
I (Sillence)	Normal stature, mild skeletal deformity, blue sclera, hearing loss	AD: new mutations are common	50% reduction in type I collagen synthesis (null allele)
II (Sillence)	Lethal, beaded ribs, broad or narrow femurs, pulmonary insufficiency	AD: new mutations, parental mosaicism; AR?	Structural alteration in type I collagen, CRTAP, LEPRE1
III (Sillence)	Progressively deforming, short stature, scoliosis, dentinogenesis imperfecta	AD: structural alteration in type I collagen chains; AR (South Africa)	Structural alteration in type I collagen, CRTAP, LEPRE1
IV (Sillence)	Moderate severity, scoliosis, light blue or white sclera, dentinogenesis imperfecta, assisted ambulation common	AD	Type I structural mutations
V	Variable severity, radial head dislocation, calcified interosseous membranes, hyperplastic callus, white sclera	AD	No mutation
VI	Moderate severity, early fractures before 18 months, osteomalacic bone biopsy	Not ascertained	Normal collagen
VII	First Nations, Quebec, moderate to severe bone changes, rhizomelic	AR with full penetrance	CRTAP (short arm chromosome 3)
VIII	South African blacks, severe, lethal and recessive	AR	LEPRE1, CRTAP, PPIB

AD, autosomal dominant; AR, autosomal recessive; CRTAP, cartilage-associated protein; LEPRE1, leucine- and proline-enriched proteoglycan 1; PPIB, peptidyl-prolyl *cis-trans* isomerase B.

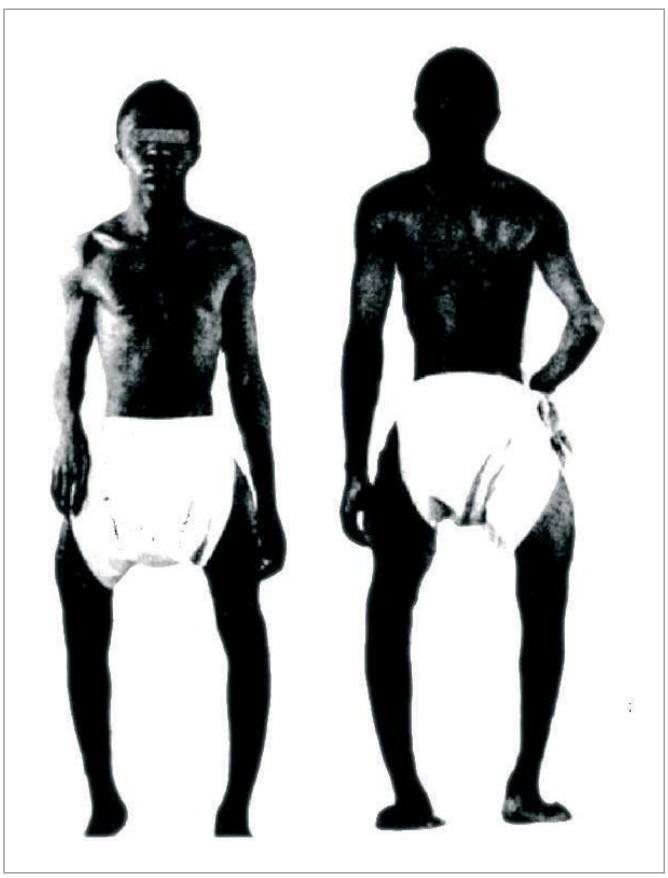

Figure 2. A 21-year-old male with osteogenesis imperfecta type I with 13 fractures, no decrease at puberty, deep blue sclera, bowing deformities of the extremities and molding of the skull.

Figure 3. Osteogenesis imperfecta type III in a 34-year-old woman with marked scoliosis and lower limb deformities, which have prevented ambulation.

Growth hormone

Growth hormone (GH) has been used in OI patients with short stature, although these persons are not classically GH deficient (28). Most individuals with OI, whether mild or severe, have short stature with normal GH function and normal baseline insulin-like growth factor I (IGF-I) levels: OI patients, whether children or adults, are not GH deficient. When GH is administered to individuals with sufficient GH, it can stimulate net bone turnover.

Studies with GH in children with OI have shown mixed results. Antoniazzi et al. studied growth rate, bone density and bone metabolism in 14 patients aged 4.8-10.8 years (29). Seven participants were treated for 12 months with human GH at a weekly dose of 0.6 IU/kg and 7 were followed as controls. After 12 months, linear growth velocity in treated subjects increased significantly in comparison with that observed during the pretreatment period (from 3.57 ± 0.55 to 6.04 ± 0.69 cm/year; $P < 0.05$) and in the untreated group ($P < 0.05$). Bone age did not advance faster than chronological age. Before therapy, individuals with OI had significantly lower lumbar bone density than normal. After treatment with GH, bone density increased significantly at all sites measured. However, because of the low fracture incidence, an effect of fracture rate could not be evaluated in this study.

Marini administered recombinant human growth hormone (rhGH) to 26 children with type III and IV OI aged 4.5-12 years (30). Children were treated with rhGH at 0.1-0.2 IU/kg/day for 6 days/week for at least 1 year. Approximately 50% of the treated children sustained a ≥ 50% increase in linear growth over their baseline growth rate. Most responders (10 of 14) had moderate type IV OI; other types showed less response. Responders, largely with OI type IV, were distinguished from nonresponders by higher baseline carboxy-terminal propeptide of type I procollagen (PICP) values ($P < 0.05$), suggesting they had an intrinsically higher capacity for collagen production. There is an apparent decrease in fracture rate for responders during the treatment year when compared with the average fractures/year preceding treatment.

GH has not been accepted as a primary treatment for children with OI because the gains in height are limited to certain phenotypes and the effect on fracture rate is not well established. There are issues of potential side effects; a GH-induced increase in muscle strength could adversely affect weak bone, as well as the annual cost of GH treatment.

Bisphosphonates

It was following the introduction of bisphosphonates for the treatment of age-related osteoporosis in 1973 that these agents were first employed to decrease fracture risk in children and adults with OI (31, 32). However, now 20 years after the first reports of bisphosphonate treatment of OI, some uncertainty remains about their effectiveness in children and adults. Intravenous pamidronate is most frequently used to treat children based on the initial reports of Glorieux and others (33, 34).

In limited trials, oral alendronate and risedronate were reported by DiMeglio and Bishop to improve bone density and decrease fracture rate in children with OI (35, 36). However, in the DiMeglio study, an effect on fracture rate must be qualified in that where alendronate was compared with pamidronate in 18 children (9 receiving alendronate and 9 pamidronate) "fracture coincidence showed a trend to decrease in both groups, with a significant decrease in fracture rates when the oral and intravenous groups were pooled" (i.e., neither drug independently decreased fracture rate in this small study). This suggests an effect other than that ascribed to the drug treatment. In the Bishop study, "Poisson regression with age and prior fracture as covariates showed that there was no difference in incident nonvertebral fracture between groups. Fracture rate diminished in each group during the trial compared with the previous 2 years ($P = 0.005$)." The results of both trials suggest that inclusion in a drug trial will diminish fracture incidence and raises questions about the efficacy of the bisphosphonate in decreasing fracture risk.

Oral olpandronate has also been reported to decrease fracture rate in children (37). In a group of 34 children, 16 receiving olpandronate, "treatment was associated with a 31% reduction in relative risk of fracture of long bones (hazard ratio: 0.69 [95% confidence interval (CI): 0.52-0.91], $P = 0.01$)." In contrast, Rauch reported that oral risedronate treatment (15-30 mg/week) in 26 children and adolescents increased lumbar spine bone mineral density (BMD) but did not result in any change in iliac crest bone histomorphometry or fracture rate (38). Intravenous zoledronic acid (Reclast®; Novartis), which is effective for up to 12 months in adults with osteoporosis, has been administered to children with OI and compared to pamidronate in a multicenter trial, but the results of this study have not been published. Thus, current concerns in both children and adults involve the effectiveness of bisphosphonates in fracture prevention, as well as questions related to the dosing schedules and duration of treatment (39). Nevertheless, bisphosphonates are generally acknowledged as the standard of care in children with OI.

Two literature-wide surveys have compiled an evidence-based analysis of bisphosphonate treatment in pediatric patients with OI. A Cochrane review of 8 randomized controlled trials including 403 participants reported a significant reduction in the number of fractures after treatment with bisphosphonates in only 1 of these studies; no differences in fracture rate were reported in 3 others (40). Spine Z score or BMD was reported to increase in two trials. Reviewing studies of intravenous bisphosphonate versus placebo, two trials showed differences in the number of participants experiencing at least one fracture and another showed no difference in fracture incidence. Therefore, it was not clear that either oral or intravenous bisphosphonates significantly decreased fracture rate. Castillo et al. also identified eight studies that confirmed improvement in bone density and found a 30-60% reduction in fracture risk in three of four small randomized, controlled trials (41).

Data on bisphosphonate effects on fracture rate in adult OI patients are similarly limited. Chevrel et al. studied the effects of oral alendronate on BMD in 64 adult patients in a 3-year randomized, placebo-controlled trial (42). Mean increases in the lumbar spine BMD were 10.1 ± 9.8% and total hip BMD increased by 3.3 ± 0.5%. However, they concluded that the sample size was not sufficient to determine an effect of alendronate on fracture rate. Adami et al. treated 46 adults with OI for 2 years at 3-month intervals with intravenous neridronate, compared with a control group of 23 individuals (43). Spine and hip BMD rose by 3.0 ± 4.6% (SD) and by 4.3 ± 3.9%, respectively, within the first year of treatment, and an additional 3.91

and 1.49%, respectively, during the second year. Following treatment, the relative risk in fracture rate was 0.14 (95% CI: 0.02-1.09). The authors' data suggest that the effect of bisphosphonate agents in adult OI patients is to produce a modest increase in bone density over 2-3 years, with only a marginal effect on fracture rate in subjects with type III OI but no effect on fractures in individuals with type I OI.

Teriparatide

Teriparatide, which stimulates osteoblastic bone formation, is an effective drug for the treatment of age-related osteoporosis (44). However, no data are currently available from a three-center study in adults with OI that is currently in progress. Because of the defects in osteoblast protein synthesis noted above, the ability of rhPTH to stimulate osteoblast bone formation in adult OI patients remains to be determined. FDA regulations indicate that Forteo® cannot be administered to children.

Potential new drug treatments

Several new drugs are under study for the treatment of age-related osteoporosis. These are more significant because they are agents that affect not only osteoblast and osteoclast function but also target steps in bone matrix synthesis not previously explored. These include odanacatib, targeting the collagen-degrading enzyme cathepsin K (45-48).

The generally positive experience with antiresorptive bisphosphonates in children with OI suggests that RANKL inhibitors might prove equally effective. In recent studies in the *oim/oim* mouse (which lacks synthesis of pro-α-2[I] chain), the inhibitor RANK-Fc increased femur bone density and cortical thickness but did not decrease fractures (49). It is important to note that osteoblast function in OI differs from that in age-related osteoporosis. Thus, the effectiveness of antiresorptive agents requiring an osteoblast response, including RANKL inhibitors, in children or adults with OI remains to be confirmed.

Molecular treatment strategies

Inactivating one allele in the case of dominant-negative mutations related to severe OI would have the benefit of converting severe type III or IV OI to mild type I OI. Hammerhead ribozymes and short interfering RNAs (siRNAs) offer opportunities for this type of cell engineering. It is envisioned that these short RNAs would be synthesized ex vivo and administered systemically to affect resident bone-forming cells.

Hammerhead ribozymes are highly specific ribonucleases designed to attack specific cleavage sites in the mRNA encoding a target protein (50). Allele-specific ribozymes can inactivate mutant mRNAs and thus induce mRNA suppression in dominant-negative genetic disorders. In OI, this can be useful because of its specificity in targeting the mutant type I collagen allele without affecting expression of the functional allele. There are two requirements for this process: a specific cleavage site and a binding site. The binding site is of utmost importance because it provides specificity for targeting the point mutation, which cannot otherwise be targeted using other technologies, such as linear antisense oligonucleotides (antisense approach). In OI, approximately 25% of type I collagen point mutations generate a ribozyme cleavage site, which can be used as a target for mutant RNA suppression (51). Dawson demonstrated that active ribozymes transfected in cultured OI fibroblasts selectively decreased (approximately 50%) the level of mutant α-1(I) collagen mRNA. Normal α-1(I) mRNA levels were not significantly reduced when active ribozyme was introduced (52). The ribozyme approach accomplished a higher level of allele specificity than the antisense oligonucleotides. However, it is not known whether this level of RNA suppression is sufficient to alter the phenotype in type I OI. Smicun and colleagues tested the therapeutic efficiency of the hammerhead ribozyme Col1a1Rz547, which selectively cleaved a mutant *Col1a1* gene transcript in a murine calvarial osteoblast cell line (53). Using a vaccine-based system, these investigators enhanced the intracellular level of the Col1a1Rz547 ribozyme and the resulting cleavage of the mutant *Col1a1* gene transcript. The results suggested that the enhanced ribozyme cleaved mutant transcripts in OI at a more efficient rate. However, the clinical applicability of this procedure is limited.

Sequence-specific gene silencing can be accomplished using siRNA methodology (54). Double-stranded exogenous antisense RNA causes degradation of cellular RNA with sequence complementary to one of the strands. In OI, 21-nucleotide-long siRNA has been used to downregulate *COL1A2* T/C polymorphisms in heterozygous human bone cells (55). A specific siRNA decreased *COL1A2* abundance by 71%, of which 75% was due to suppression of the targeted T allele. However, some studies have shown that siRNA may not be specific to the level of a single nucleotide. If sequence specificity is attained, this would allow for two generations of siRNAs to target each of the polymorphic variants of *COL1A1*, which hypothetically could be used to treat those 40% of OI patients who test positive for dominant-negative *COL1A1* mutations, or those who are heterozygous for a polymorphism. *COL1A1*-targeting siRNAs have been tested in mesenchymal stem cells for their ability to differentiate between two *COL1A1* targets that differ by a single nucleotide. Millington-Ward et al. concluded that none of the siRNAs in their study were completely allele-specific (56).

Cell therapy

The more severe OI types involve a qualitative defect with mutant procollagen chains secreted into the extracellular matrix. Cell therapy is designed to deliver normal closely matched allogeneic osteoprogenitor cells, making the patient chimeric for exogenous normal and endogenous mutant cells. Because the mutant cells are at a disadvantage to normal cells regarding both growth and collagen, their deleterious effect on bone quality would gradually diminish as the normal turnover process replaces mutant cells with normal ones.

Initial attempts at bone marrow transplantation (BMT) in a group of severely affected infants should be classified as ineffective treatment (57). Five children with type III OI received whole marrow allogeneic BMT and two were followed as controls. Three infants with documented donor osteoblast engraftment had a median increase in body length of 7.5 cm (range: 6.5-8.0 cm) 6 months after transplantation, compared with 1.25 cm (range: 1.0-1.5 cm) for age-matched controls. These subjects reportedly gained 45-77% of their baseline values. Transplanted cell engraftment at 3 months after treatment was 1.2%. With extended follow-up, the patients' growth rates either

slowed or reached a plateau phase. Although fracture rate decreased after BMT, it is not uncommon for the fracture rate to decrease in the usual course of early growth in severely affected infants with OI. The study was not extended. Pochampally et al. used bone marrow mesenchymal stem cells from an individual with type III OI who was heterozygous for a mutation in *COL1A1* to overexpress wild-type hybrid genomic *COL1A1* cDNA in bone-derived mesenchymal cells in an attempt to partially correct the dominant-negative protein defect (58). Transfected mesenchymal stem cells showed increased expression of the wild-type mRNA and protein. In vitro assays demonstrated that the transfected cells more efficiently differentiated into mineralizing cells. The results suggested that it is possible to partially correct the type III OI dominant-negative protein defect by overexpressing *COL1A1* cDNA in mesenchymal stem cells.

Niyibizi used the *oim/oim* mouse model to explore the feasibility of gene therapy for OI (Table II) (59). This was done by inserting pro-α-2(I) cDNA into an adenovirus vector, which was then injected into bone marrow mesenchymal cells of *oim* mouse femurs. The results demonstrated that collagen was effectively secreted and the cells had osteogenic potential, as inferred from the alkaline phosphatase activity. Injection of the virus carrying the murine pro-α-2(I) cDNA into skin fibroblasts of *oim* mice showed synthesis of type I collagen made up of both α-1 and α-2 chains. These results suggested that collagen genes have the potential to be transferred into mesenchymal stem cells and fibroblasts in vivo.

Genetic treatment

This approach is designed to avoid the immune rejection problems associated with allogeneic transplantation by genetic engineering techniques that reduce the effect of the mutation on bone matrix formation. In the case of a quantitative defect, as occurs in mild type I OI (null allele), the most direct therapeutic strategy would involve the addition of a functional *COL1A1* allele in a patient's mesenchymal stem cells, which would then be delivered to bone. In contrast, the dominant-negative mutation associated with severe OI has to be silenced so as not to compromise the function of the remaining normal allele. As noted above, inactivation of a mutant allele in a patient's mesenchymal stem cells, which would then be implanted to bone, represents a significant potential therapeutic strategy for OI. Mutant gene inactivation (gene targeting) has been successfully attempted using adeno-associated virus (AAV) vectors to inactivate mutant *COL1A1* genes in OI mesenchymal stem cells expressing a dominant-negative mutation. These cells were then shown to produce normal collagen and to form bone in vivo. In a subsequent study, improved AAV vectors were used to target point mutations in *COL1A2* alleles in different OI mesenchymal cell lines (60). Gene targeting at exon 2 of the mutant *COL1A2* allele completely eliminated synthesis of the abnormal pro-α-2(I) chains in the mutant stem cells. The targeted mesenchymal stem cells produced normal type I collagen and formed bone in vivo. However, the most direct approach for either form of the mutation is the removal of the mutant segment of the gene using techniques of homologous recombination (see below), an approach that is probably not feasible for adult stem cells.

The more severe OI types involve a qualitative defect with mutant procollagen chains secreted into the extracellular matrix. A therapeutic strategy would involve replacing those cells with competent osteoblasts from a new patient-derived stem cell population. Recent studies by Guillot et al. involving intrauterine transplantation of first-trimester human mesenchymal stem cells to fetuses in mouse models of OI have increased bone strength, thickness and length, and have markedly reduced fracture rate (61).

Additional studies by Panaroni et al. evaluated in utero transplantation of adult bone marrow into heterozygous Brtl IV mice (62). Brtl is a classic knock-in mouse with a glycine substitution in type I collagen (α-1-[I] Gly-349Cys) with a phenotype comparable to moderate to severe OI. The transplantation effectively corrected perinatal lethality of the heterozygous Brtl IV mice. The femora of the treated Brtl IV mice (2 months) attained significant improvement in geometric and mechanical parameters compared with their untreated counterparts. The results supported the conclusion that engrafted cells effectively synthesize bone at a higher rate than normal endogenous cells. Thus, in utero transplantation may serve as a potential therapeutic approach for OI (62).

The feasibility of allogeneic mesenchymal stem cell engraftment in human OI was tested by LeBlanc et al. in a female OI fetus with multiple intrauterine fractures (63). Using HLA-mismatched male fetal mesenchymal stem cells transplanted at week 32 of gestation, the investigators showed 0.3% XY-positive cells in bone biopsy specimens at 9 months of age, while whole Y genome staining showed 7.4% Y-positive cells. The utility of in vitro stem cell transplantation will depend on stem cell engineering to enhance engraftment and

Table II. *Mouse models of osteogenesis imperfecta.*

Model	Strain	Collagen gene	Mutation	Human	References
Mov13	NA	*Col1a1*	Moloney leukemia virus integrated into the *Col1a1* gene*	Type I	73
Brtl	NA	*Col1a1*	Gly-349 → Cys substitution in exon 23 of the α-1(I) chain	Type II	74
oim/oim	B6C3Fe-a/a (C57BL/6JLe x C3HeB/FeJLe-a/a) hybrid	*Col1a2*	G deletion at pro α-2(I) nucleotide 3983	Type III/IV	75
Brtl IV	Chimera X C57Bl/6J	*Col1a1*	α-1(I) Gly-349 → Cys	Type IV	76

NA, not applicable; *transgenic mouse model of the mild dominant form of osteogenesis imperfecta.

cell longevity. The method holds promise where identification of an affected fetus occurs in an already affected family.

Handschel et al. have reported that somatic stem cells from cord blood and embryonic stem cells can be differentiated into osteogenic cells, seeded on insoluble collagenous bone matrix, implanted in immunodeficient rats. Computerized tomography scans were performed to detect any calcification. The radiological examination showed a steep increase in the mineralized bone-like tissue in the groups (64).

Embryonic stem cells and induced pluripotent stem cells

Human embryonic stem (hES) cells have been widely anticipated as the ultimate source of progenitor cells for any tissue because they are totipotential, with the capacity to generate an entire animal. However, even if it were possible to efficiently direct these cells into tissue-appropriate progenitor cells, hES cells still have the significant drawback of tissue compatibility with their intended host. Therefore, the ability to develop patient-specific hES-like cells by viral transduction with a set of transcription factors (octamer-binding protein 4 [Oct-4], SOX-2, Krueppel-like factor 4 and Myc proto-oncogene protein), called induced pluripotent stem (iPS) cells, opens the possibility that this source of cells might solve both the immune tolerance and genetic engineering constraints (discussed next) that have limited the possibility for somatic gene therapy for OI. It is recognized that the autologous transplantation of these cells would involve patient preparation, as required for other transplantation procedures. However, there are currently a number of obstacles, including virus integration and the genetic alteration of iPS cells and potential tumor formation, which will need to be overcome before these cells may be considered safe for clinical application (65, 67, 68).

Genetic manipulation by homologous recombination (i.e., gene targeting to specifically correct disease-causing mutations) is the most promising technique for somatic gene therapy of a genetic disease. It involves the identification, isolation and expansion of a rare molecular event, designed to replace a segment of host DNA containing the disease-causing mutation (69). This process requires that the target cell population be expanded to provide the number of cells necessary to identify the rare event followed by multiple rounds of selection and expansion to purify the corrected cell population. Because no source of adult-derived progenitor cells will maintain its progenitor potential through these multiple rounds of selection and expansion, hES/iPS cells are a particularly attractive source of target cells because they can undergo unlimited numbers of replications without affecting their differentiation potential if properly maintained in their undifferentiated state (70).

Homologous recombination in hES cells has proven to be more technically demanding than the same technology in murine cells. Numerous laboratories are examining modification to the procedure that ultimately should overcome this problem. Currently, most promising is the use of zinc finger nucleases, which can be custom designed to cut host DNA at a specific site, and through this mechanism, direct the insertion of the foreign DNA fragment (71). In this manner, a mutation could be excised and replaced with a normal segment of DNA, rendering this endogenous gene capable of producing a normal gene product.

For OI, these developments point to a therapeutic path that ultimately could correct the underlying disease. The dominant-negative forms of the disease would be responsive to site-directed recombination that removes the mutant segment of the type I collagen gene. While those situations with low collagen production would also respond to this approach, they would also benefit from the insertion of an additional copy of the gene. Insertion of an entire collagen gene with its associated regulatory units will require directing a bacterial artificial chromosome-sized fragment into the host cell (72). For this reason, the use of zinc finger nucleases to direct this insertional event into a known and safe segment of host DNA will be an essential step to obviate the potential of oncogenic transformation due to insertional activation/disruption of a host growth factor or tumor suppressor gene that was observed with virally delivered gene therapy (73).

CONCLUSIONS

While these emerging technologies seem to be many years away from a practical application for the individual with OI, there is enormous interest and effort in the hES cell field because once developed for one genetic disease, they will be useful for all diseases. Progress has been made in generating acceptable forms of iPS cells and in the ability to safely manipulate the genome for insertion and/or replacement of a genetic element into these cells. Ultimately, the most significant challenge for somatic gene therapy for OI may be the efficient delivery of the corrected progenitor cells back to the affected bone, where they would be expected to replace the existing bone with a corrected extracellular matrix.

DISCLOSURES

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