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Targeted delivery of mesenchymal stem cells to the bone

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ABSTRACT

Osteoporosis is a disease of excess skeletal fragility that results from estrogen loss and aging. Age related bone loss has been attributed to both elevated bone resorption and insufficient bone formation. We developed a hybrid compound, LLP2A–Ale in which LLP2A has high affinity for the α4β1 integrin on mesenchymal stem cells (MSCs) and alendronate has high affinity for bone. When LLP2A–Ale was injected into mice, the compound directed MSCs to both trabecular and cortical bone surfaces and increased bone mass and bone strength. Additional studies are underway to further characterize this hybrid compound, LLP2A–Ale, and how it can be utilized for the treatment of bone loss resulting from hormone deficiency, aging, and inflammation and to augment bone fracture healing. **This article is part of a Special Issue entitled "Stem Cells and Bone".**

Introduction

Osteoporosis is a syndrome of excessive skeletal fragility that results from a combination of a reduction in bone mass and bone strength. The two most significant determinants of osteoporosis are estrogen deficiency and aging. Estrogen loss leads to a reduction in trabecular bone mass and an irreversible alteration of the trabecular bone structure. The decline of trabecular bone structure secondary to estrogen deficiency is suppressed by treatment with anti-resorptive agents (estrogen, bisphosphonates, calcitonin and selective estrogen receptor modulators) [1,2]. These agents are hypothesized to work by reducing the activation of new bone multicellular units (BMUs) while still allowing normal bone formation to continue in already activated BMUs. This results in a more complete secondary mineralization of basic structural units due to reduced turnover and an increased degree of bone mineralization (DMB). These agents have been associated with preservation of trabecular microarchitecture. However, an important limitation of this class of drugs is that they do not restore the lost bone structure. There is currently an anabolic agent, rhPTH (1–34), that can stimulate new bone formation on existing trabeculae, increase trabecular bone mass, and reduce the risk of incidental vertebral fractures [3]. However the requirement of daily injections of rhPTH (1–34) for two years and the lack of data on hip fracture risk reduction have limited the use of this medication in clinical practice.

Age related bone loss has been attributed to an increase in osteoclast driven bone resorption, with an insufficient increase in osteoblast number to drive bone formation. Over time this can then lead to an uncoupling of bone turnover and bone loss. However, a more detailed review of the bone microenvironment in preclinical and clinical studies of aging has provided additional insights. Aging is associated with a reduction in the number of mesenchymal stem cells (MSCs) that can differentiate into osteoblasts. This leads to a reduction in osteogenesis and bone formation [4–6]. However, it is not clear if age related reduction in bone formation results from a reduction in MSCs in the bone marrow due to cell death, if MSCs are directed to differentiate into adipocytes, or if MSCs are unable to migrate to the bone surface due to changes in the bone microenvironment. A number of these factors may be present in the aging bone marrow that results in reduced bone formation.

Over the past few years, the idea that increasing the ability of MSCs to differentiate into osteoblasts in aged or estrogen deficient animal models to increase osteogenesis and facilitate new bone formation has been investigated. In the majority of the experiments, MSCs from a number of sources including whole bone marrow, fat, MSC enriched peripheral blood, or purified and cultured MSCs have been injected intravenously (IV) into the peripheral circulation in both animal and a few human studies and have generally failed to engraft within the bone marrow. Also, more than 90% of the intravenously transplanted MSCs became trapped in the lung microvasculature and while a small number of MSCs did engraft in the bone marrow, the residence time within the marrow was limited [7–9]. Also intravenous administration of MSCs *in vivo* has failed to promote an osteogenic response in bone due to the inability of MSCs to home to the bone surface unless they

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were genetically modified [10–14] or following bone trauma [8,15] or fracture [8,16]. The successful application of MSCs to bone has been limited to the repair of injuries in which the MSCs are presented by local subcutaneous implantation or intramedullary injection or combined with scaffolds within the bone[17–20].

However, administration of systemic MSCs in *in vivo* models does not find MSCs migrating to the bone surface or forming new bone. Generally the infused MSCs are found in the upper metaphysis, epiphysis, bone marrow sinusoids or Haversian systems and are usually removed from the bone marrow within a few weeks [7–9,21]. One solution to this problem of insufficient numbers of MSCs in the bone marrow of older individuals that can differentiate into osteoblasts would be to inject MSCs into the systemic circulation and allow the MSCs to move to the bone surface. However, the movement of MSCs from the bone marrow to the bone surface is complex. MSCs undergo osteogenic differentiation in the bone marrow and mobilization of the osteoblast progenitors to the bone surface is a crucial step for osteoblast maturation and the formation of mineralized tissue[22–24]. Bone cells at all maturation stages are dependent on cell–matrix and cell–cell interactions [25–28]. Once the osteoblast progenitors are "directed" to the bone surface, they synthesize a range of proteins including osteocalcin, osteopontin, bone sialoprotein, osteonectin, collagen-I and fibronectin that further enhance the adhesion and maturation of osteoblasts [29–31]. These interactions are largely mediated by transmembrane integrin receptors that primarily utilize an arginine–glycine–aspartate (RGD) sequence to identify and bind to specific ligands. MSCs express integrins α1, 2, 3, 4, 6, 11, CD51 (integrin αV), and CD29 (integrin β1) [32]. Integrins α1β1, α2β1, αvβ1, αvβ5, α5β1and α4β1 are expressed in the osteoblastic cells [26,30,31,33]. Integrin α5 is required for MSC osteogenic differentiation [34] and overexpression of α4 integrin on MSCs has been reported to increase homing of the MSCs to bone [25]. These studies suggest that a therapeutic strategy for bone regeneration could be directed toward the integrins on the surface of the MSCs and could bring the MSCs to the bone surface. In addition to their initial development of cell therapies for tissue regeneration and wound healing, MSC paracrine and functions have been increasingly recognized as important factors that contribute to their efficacy[35–37].

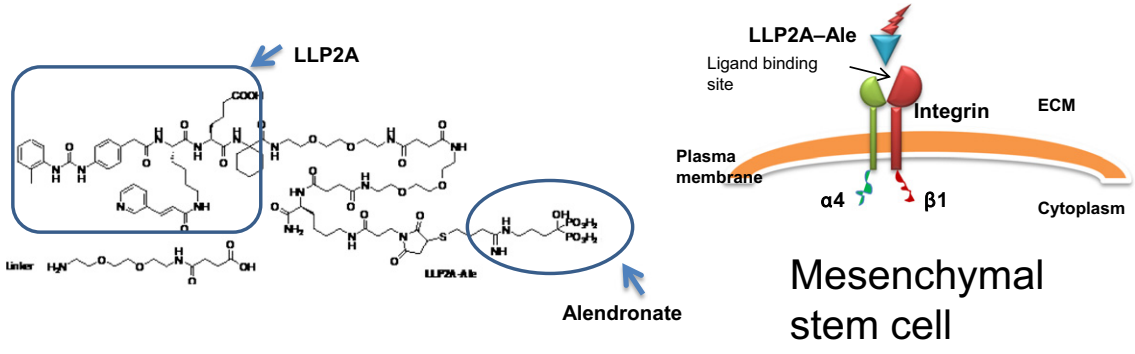
Our research team wanted to try to improve the engraftment of MSCs in the bone marrow to form new bone. To accomplish the goal of delivering the MSCs to the bone surface, we collaborated with chemists and developed a compound, LLP2A–Ale that binds to both MSCs and to bone. The scientists screened a combinatorial library for peptidomimetric ligands that were able to bind to the α4β1 integrin on the MSC surface, and LLP2A was identified as a potential ligand to bind to the integrin. Next, the chemists worked on conjugating LLP2A to a bisphosphonate so that the LLP2A would carry the bound MSCs to the bone surface. A number of bisphosphonates were attempted to bind to LLP2A, and alendronate (Ale) was the only one that could bind to LLP2A through a chemical linker and then link LLP2A with alendronate, LLP2A–Ale (Fig. 1).

General *in vitro* and *in vivo* effects

Our research group performed a number of studies in young mice with a wide dose range of LLP2A–Ale,assessed the weight, kidney function, liver function and calcium metabolism, and determined that they were not affected by the treatment. Also, no extraskeletal calcifications were observed in the mice treated with LLP2A–Ale.

It was critical to determine if the synthetic peptide against α4β1 integrin, LLP2A, had affinity for MSCs that were undergoing osteoblast differentiation. We determined that the α4β1 integrin was highly expressed in the osteoprogenitor cells and had a high affinity for LLP2A[38]. *In vitro* studies with MSCs showed that LLP2A–Ale increased both the number of MSCs that differentiated into osteoblasts as well as the migration of the MSCs to hydroxyapatite crystals [39]. The effects of LLP2A–Ale on MSC migration appeared to be mainly chemotactic as increased chemokine levels were observed, including monocyte chemotactic protein-1 and macrophage-inflammatory protein-1α. Also, as MSCs can differentiate into osteoblasts, chondrocytes or adipocytes, it was observed that treatment of MSCs with LLP2A–Ale did not increase either the chondrogenic or adipogenic phenotypes of the MSCs in the culture media.

Next, to determine if LLP2A–Ale could direct transplanted MSCs to the bone surface two *in vivo* proof-of-concept studies were performed. NOD/SCID/mucopolysaccharidosis type VII (NOD/SCID/MPSVII) immune deficient mice were treated with human bone marrow (huMSCs) or with LLP2A–Ale. This mouse strain lacks the β-glucuronidase (GUSB) enzyme, which facilitates human cell detection by a simple enzymatic substrate reaction as described [40,41]. The donor cells were detected using biochemical detection of β-glucuronidase [9,42]. Twenty-four hours after the injections, LLP2A–Ale increased the number of huMSCs on the bone surface as compared to all the other control groups (PBS, LLP2A–Ale or huMSCs). Three weeks after a single injection of human MSCs and LLP2A–Ale, the transplanted huMSCs were observed adjacent to the bone surface. Moreover, the transplanted huMSCs were embedded within the bone matrix in the MSC + LLP2A–Ale treated group,



" MSC specific ligand", LLP2A, that targets the surface integrins (α4β1) on the MSCs
"Bone seeking agent" , Alendronate, that deliveries/guides the MSCs to bone surface

Fig. 1. LLP2A–Ale is a hybrid compound. It is composed of the bisphosphonate alendronate that has high affinity for the bone tissue. Alendronate is bound to a chemical linker that also is attached to LLP2A, a synthetic protein that has high affinity for alpha 4, beta 1 integrin that is on the surface of mesenchymal stem cells and hematopoietic stem cells.