Injury, Int. J. Care Injured 47S2 (2016) S21–S26

Contents lists available at ScienceDirect

Injury

journal homepage: www. elsevier.com/locate/Injury

Fracture healing in osteoporotic bone

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KEYWORDS

Fracture healing
Osteoporotic bone
Aging
Metaphyseal fracture
Estrogen receptor

ABSTRACT

As the world population rises, osteoporotic fracture is an emerging global threat to the well-being of elderly patients. The process of fracture healing by intramembranous ossification or/and endochondral ossification involve many well-orchestrated events including the signaling, recruitment and differentiation of mesenchymal stem cells (MSCs) during the early phase; formation of a hard callus and extracellular matrix, angiogenesis and revascularization during the mid-phase; and finally callus remodeling at the late phase of fracture healing.

Through clinical and animal research, many of these factors are shown to be impaired in osteoporotic bone. Animal studies related to post-menopausal estrogen deficient osteoporosis (type I) have shown healing to be prolonged with decreased levels of MSCs and decreased levels of angiogenesis. Moreover, the expression of estrogen receptor (ER) was shown to be delayed in ovariectomy-induced osteoporotic fracture. This might be related to the observed difference in mechanical sensitivity between normal and osteoporotic bones, which requires further experiments to elucidate.

In mice fracture models related to senile osteoporosis (type II), it was observed that chondrocyte and osteoblast differentiation were impaired; and that transplantation of juvenile bone marrow would result in enhanced callus formation. Other factors related to angiogenesis and vasculogenesis have also been noted to be impaired in aged models, affecting the degradation of cartilaginous matrixes and vascular invasion; the result is changes in matrix composition and growth factors concentrations that ultimately impairs healing during age-related osteoporosis.

Most osteoporotic related fractures occur at metaphyseal sites clinically, and reports have indicated that differences exist between diaphyseal and metaphyseal fractures. An animal model that satisfies three main criteria (metaphyseal region, plate fixation, osteoporosis) is suggested for future research for more comprehensive understanding of the impairment in osteoporotic fractures. Therefore, a metaphyseal fracture or osteotomy that achieves complete discontinuity fixed with metal implants is suggested on ovariectomized aged rodent models.

Introduction

Bone tissues demonstrate a remarkable ability to regenerate following fracture injury, recovering from structural failure and lost physiological function [1]. The cascade of events following traumatic bone injury is well-documented in both stabilized and non-stabilized fractures. The former primarily heal via intramembranous ossification in which bone regenerates directly from mesenchymal cells, while the latter primarily heal via endochondral ossification in which bone regenerates through a cartilage intermediate [1–5]. Both events begin with the formation of a hematoma between the damaged bone ends and surrounding soft tissues. Inflammatory cells are recruited by local chemokines to debride the wound, which allows for the migration of mesenchymal stem cells. In stabilized fractures, these cells differentiate directly into osteoblasts and form trabecular bone [5]. In non-stabilized fractures, these cells alter their fate and differentiate into granulation and cartilage tissues [1]. A predominantly cartilaginous soft fracture callus develops and stabilizes the injury site. Then, a hard fracture callus develops through vascularization and mineralization of the extracellular matrix, which yield trabecular bone. Once trabecular bone is generated in both ossification processes, a series of bone depositions and resorptions by osteoblasts and osteoclasts, respectively, reform lamellar bone.

Despite the fine degree of orchestration during fracture healing, the process may be impaired. Currently, 10–15% of the approximately 15 million fractures that occur annually result in poor or unresolved

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healing [6]. As the aging population is expected to double by 2050 [7] and the occurrence of osteoporotic fractures rise in the near future, impairment in osteoporotic fracture healing is becoming an emerging public health concern. Moreover, it has previously been reported that the risk of non-union increases with age [8,9]; and that osteoporotic fracture is associated high morbidity, mortality rate [10,11] and increased healthcare costs.

As the pathophysiology of both post-menopausal estrogen deficiency (type I) and senile (type II) account for the major causes of osteoporosis and subsequently osteoporotic fractures, this paper is intended to review our current understanding on fracture healing in osteoporotic bone in both types and to discuss a number of key determining factors that are impaired during osteoporotic fracture healing. These factors include the recruitment, proliferation and differentiation of progenitor cells; the revascularization of callus; and also the role of mechanical sensitivity in the healing osteoporotic bone. These factors are of high potential as therapeutic targets in future research. Some experiences in animal studies on diaphyseal osteoporotic fracture are summarized in this paper; nonetheless, a general direction of future development in metaphyseal osteoporotic fracture model is suggested in order to improve our research work in terms of clinical relevance and translational applicability.

Mechanical sensitivity in estrogen deficiency-induced osteoporotic fracture (type I) and the role of estrogen receptors

A number of reports revealed the differences of mechano-biology between osteoporotic and normal bones [12] and osteoporotic fracture healing was impaired in both early [13] and late phases with decrease in callus cross-sectional area, bone mineral density (BMD) and mechanical properties [14]. The mechanism of impaired osteoporotic fracture healing is multi-factorial and some reports indicated that low sensitivity of osteoblasts to mechanical signals [15,16], reduced angiogenesis [17,18], and decreased mesenchymal stem cells [19] might be the causes. To enhance fracture healing, mechanical stimulation by means of weight bearing is the current commonest clinical approach. However, previous finding showed that osteoblasts from osteoporotic donors were less responsive to 1% cyclic strain stretching in terms of proliferation and TGFβ release, as compared with younger normal donors [15]. Therefore, this is generally believed that osteoporotic bone is less responsive to mechanical stimulation; however, there were some opposite reports, e.g. Leppänen et al showed that osteoporosis was not attributable to impaired mechano-responsiveness of aging skeleton [20]; also, male adult rats with lower estrogen level demonstrated better mechanical responses than females [21]. Hence, mechanical sensitivity of osteoporotic bone remains obscure.

To compare the responses of normal and osteoporotic fractured bones to mechanical signals, fracture healing of nine-month-old normal (Sham) and ovariectomy (OVX)-induced osteoporotic SD rats in response to cyclic vibration (35 Hz, 0.3 g where *g*=gravitational acceleration; 20 min/day and 5 days/week) were assessed using radiography, microCT, histomorphometry and four-point bending mechanical test at 2, 4, and 8 weeks post-treatment. Results showed that fracture healing in OVX animals responded to cyclic vibration very well, as reflected in all the assessment outcomes, particularly in the early phases of healing [22]. Callus formation, mineralization and remodeling were enhanced by 25–30%, while energy to failure was increased by 70% as compared to corresponding OVX control. The outcomes were comparable to those of age-matched normal fracture healing in Sham group. These findings also revealed that both intramembranous and endochondral ossification were enhanced well in osteoporotic fracture healing augmented by cyclic vibration. In the meantime, these osteogenesis findings were further substantiated by the angiogenesis data performed in another study using the same experimental design and cyclic vibration treatment [17]. Significantly increased blood flow velocity (+10–19%) and vascular volume (+25–57%) than corresponding OVX control were demonstrated at the fracture sites of OVX-induced osteoporotic rats at week 2 and 4 post-treatment, whereas its non-OVX counterpart showed +2.2–13.2% increase of vascular volume (Sham treatment vs. Sham control) at week 2–4 only. Also, similar findings were found when the mechanical loading was changed to low intensity pulsed ultrasound (1.0 kHz, 30.0 mW/cm^2 spatial-averaged temporal-averaged intensity; 20 min/day and 5 days/week) with the same study design [23], which again showed comparable responses (similar increase of energy-to-failure of OVX treatment over OVX control vs. Sham treatment over Sham control at week 8) to acoustic loading between osteoporotic fractured bone and age-matched normal one. Rubinacci et al. also verified that OVX non-fractured rats treated with vibration treatment (30 Hz, 3 g) showed significant increase in cortical and medullary areas, periosteal and endosteal perimeters but not in Sham animals, illustrating that OVX might sensitize cortical bone to mechanical stimulation [24]. All these evidences confirm that osteoporotic bones respond effectively to mechanical loading (regardless of physical or acoustic form), which was not worse than normal ones.

As the immediate effects of estrogen depletion is sensed and relayed by estrogen receptors (ERs), as well as ERs was known to function as mechanical signal transduction through its ligand-independent function [25], this is not surprising to postulate the quantity of ERs may play a role in determining bone formation during fracture healing. Furthermore, ERs have been reported to localize in fracture callus [26] that indicates the potential roles of ERs in fracture healing. When comparing the gene expression of ERs at fracture callus between 9-month-old Sham and OVX closed fractured rats, it was found that ERs expressions were significantly higher in Sham group at week 2 but later significantly lower at week 8 than OVX group, while the OVX group demonstrated an opposite trend [27]. Meanwhile, moderate correlations were found between ER-α and BMP-2 ($r = 0.545$, $p = 0.003$), between ER-α:ER-β ratio and BMP-2 ($r = 0.601$, $p = 0.001$), between BMP-2 and callus width/callus area ($r = 0.709$, $p = 0.000$/$r = 0.588$, $p = 0.001$). These gene expression data were also validated by immunohistochemistry at protein level. These findings depict that impaired healing of OVX-induced osteoporotic fracture may be associated with delayed expression of ERs.

As delayed expression of ERs may be the cause of impaired osteoporotic fracture healing, this is interesting to look into the changes of ERs expression in osteoporotic fracture healing augmented by mechanical stimulation. In the study, the fractured rats were randomly assigned to 4 groups – Sham control (SHAM), OVX-induced osteoporotic control (OVX), OVX vibration treated at 35 Hz, 0.3 g for 20 min/day and 5 days/week (OVX-VT) and OVX vibration supplemented by daily 1.5 mg/kg/day ICI182,780 (Fulvestrant, a complete ER antagonist) (OVX-VT-ICI). The results demonstrated that ER-α expression level was higher in SHAM and OVX-VT groups at week 2 and gradually decreased at week 4 and week 8, while that of OVX group showed lower expression at week 2 and later surged at week 8 [28]. Also, ER-α gene expression levels were similar between SHAM and OVX-VT groups with no significant difference between two groups. This indicated that cyclic vibration could induce the increase of ER-α level in osteoporotic fractured bone close to SHAM normal level. Interestingly, in OVX-VT-ICI group, the ER-α expression was suppressed to a significantly lower level. Similarly, the osteogenesis gene expressions (Col-1 and BMP-2) and callus morphometry parameters (callus width, callus area) echoed the ER-α data with the highest levels in SHAM and OVX-VT groups from week 2–4, while the group of OVX-VT-ICI was the lowest. This further substantiates the fractured bone's ability to transmit mechanical strain to stimulate callus formation. Both gene expression data and fracture outcomes suggested that the presence of ER-α was essential for mechanical transduction and responsible for the enhancement effects induced by cyclic loading. The induced increase of ER-α level at fracture callus may be sourced