Biomaterials 67 (2015) 308–322

Contents lists available at ScienceDirect

Biomaterials

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

Synergy between IL-6 and soluble IL-6 receptor enhances bone morphogenetic protein-2/absorbable collagen sponge-induced bone regeneration via regulation of BMPRIA distribution and degradation

Ru-Lin Huang [a, 1], Gang Chen [a, 1], Wenjin Wang [a, 1], Tanja Herller [b], Yun Xie [a], Bin Gu [a], Qingfeng Li [a, *]

[a] Department of Plastic and Reconstructive Surgery, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, 639 Zhizaoju Road, Shanghai 200011, China
[b] Department of General, Trauma, Hand, and Plastic Surgery, University of Munich, Munich, Germany

ARTICLE INFO

Article history:
Received 11 April 2015
Received in revised form
22 July 2015
Accepted 23 July 2015
Available online 26 July 2015

Keywords:
BMP-2
IL-6
sIL-6R
BMPRIA
Bone regeneration
Cell surface translocation

ABSTRACT

Bone morphogenetic protein-2/absorbable collagen sponge (BMP-2/ACS) implants have been approved for clinical use to induce bone regeneration. We previously showed that exaggerated inflammation characterized by elevated level of inflammatory cytokines including TNF-α, IL-1β, and IL-6 has been shown to inhibit BMP-2/ACS-induced bone regeneration. Furthermore, unlike the negative effects of TNF-α and IL-1β, IL-6 seemed not to affect BMP-2-induced osteoblastic differentiation of bone marrow mesenchymal stem cells (BMSCs). We hypothesized that there may be a regulatory loop between IL-6 and BMP-2 singling to affect BMP-2/ACS-induced bone regeneration. Here, we established a BMP-2/ACS-induced ectopic bone formation model in rats and fund that IL-6 injection significantly increased BMP-2/ACS-induced bone mass. Consistent with this animal model, an in vitro study demonstrated that synergy between IL-6 and soluble IL-6 receptor (IL-6/sIL-6R) promotes BMP-2-induced osteoblastic differentiation of human BMSCs through amplification of BMP/Smad signaling. Strikingly, IL-6 injection did not activate osteoclast-mediated bone resorption in the ectopic bone formation model, and IL-6/sIL-6R treatment did not affect receptor activator of NF-κB ligand (RANKL)-induced osteoclastic differentiation of human peripheral blood mononuclear cells (PBMCs) in vitro. Furthermore, IL-6/sIL-6R treatment did not affect expression of BMP receptors, but enhanced the cell surface translocation of BMP receptor IA (BMPRIA) and inhibited the degradation of BMPRIA. Collectively, these findings indicate that synergy between IL-6 and sIL-6R promotes the cell surface translocation of BMPRIA and maintains the stability of BMPRIA expression, leading to enhanced BMP-2/ACS-induced bone regeneration.

1. Introduction

Bone morphogenetic protein-2 (BMP-2) plays widely recognized roles in bone formation during mammalian development [1,2] and has been approved by the U.S. Food and Drug Administration (FDA) for clinical use to enhance bone regeneration in specific clinical scenarios [3–5]. In current clinical practice, recombinant human BMP-2 (rhBMP-2) is delivered by impregnation of an absorbable collagen sponge (ACS); this sponge is used to retain rhBMP-2 at wound sites and to permit slow release into the extracellular milieu. Due to BMP-2's short half-life, a high concentration of BMP-2 dose (1.5 mg/mL) is needed in clinical setting (greatly exceeding native concentrations of 18.8–22 pg/mL) to

Abbreviations: BMP-2, bone morphogenetic protein-2; BMPRIA, bone morphogenetic protein receptor IA; BMPRIB, bone morphogenetic protein receptor IB; BMPRII, bone morphogenetic protein receptor II; IL-6, interleukin-6; sIL-6R, soluble interleukin-6 receptor; mIL-6R, membrane-bound interleukin-6 receptor; BMSCs, bone marrow mesenchymal stem cells; TNF-α, tumor necrosis factor-α; Runx2, runt-related transcription factor 2; ALP, alkaline phosphatase; ACS, absorbable collagen sponge; CTX-1, C-terminal fragment of collagen 1; TRAcP-5b, tartrate-resistant acid phosphatase 5b; OCN, osteocalcin; COL1A1, collagen type 1 alpha 1; RANKL, receptor activator of NF-κB ligand; M-CSF, macrophage colony-stimulating factor; TRAP, tartrate-resistant acid phosphatase; NFATC1, nuclear factor of activated T cells, cytoplasmic 1; PBMCs, peripheral blood mononuclear cells; ELISA, enzyme-linked immunosorbent assay.

* Corresponding author.
E-mail address: dr.liqingfeng@shsmu.edu.cn (Q. Li).
[1] These authors contributed equally to this manuscript.

http://dx.doi.org/10.1016/j.biomaterials.2015.07.047
0142-9612/

exert its osteoinductive capability. Although BMP-2/ACS implants have been demonstrated to be safe and effective in promoting bone regeneration, a review of publicly available data suggests that the risk of adverse events, such as inflammatory response [6,7], seroma [8,9], and bone resorption [10], is 10–50 times [11,12] higher than reported in trial publications, which raise concerns about the safety and effectiveness of rhBMP-2 [13,14].

Following BMP-2/ACS implantation, the inflammatory response is characterized by the recruitment of both inflammatory cells and stem cells to the implantation site and by the presence of inflammatory cytokines in the sera, such as TNF-α, IL-1β, and IL-6, in clinical patients as well as experimental animals [8,15,16]. Among these inflammatory cytokines, TNF-α and IL-1β have been reported to play an inhibitory role on BMP-2-induced osteogenic differentiation of BMSCs [17–21]. However, in contrast with TNF-α or IL-1β, IL-6 seemed to be an exception, as no significant effect of IL-6 on BMP-2-induced osteoblastic differentiation was observed in our previous work [20,22]. Because the in vivo inflammatory environment is more complex than in vitro inflammation models mimicked by single or multiple inflammatory cytokine treatment, further investigation on the precise roles of IL-6 is required and may be helpful for developing novel therapies to improve the clinical osteoinductive efficiency of BMP-2.

IL-6 is a pleiotropic cytokine that exerts its action by binding to the soluble IL-6 receptor (sIL-6R), which is present in the serum in vivo and acts as an agonist to facilitate ubiquitous IL-6 signaling. IL-6 is also known to be a potent stimulator of osteoclast-mediated bone resorption. However, in IL-6-knockout mice, the absence of IL-6 leads to low bone mass, reduced osteoblast numbers, and retarded fracture healing [23]. These mice have been reported to experience more alveolar bone loss, but they also experience less bone loss in an experimental model of periodontitis [24,25]. Furthermore, IL-6 promotes the differentiation of pre-osteoblasts into mature osteoblasts and has no effect on MLO-Y4 osteocyte-stimulated osteoclastogenesis [26,27]. By contrast, IL-6 and its soluble receptor, sIL-6R, were shown to negatively regulate osteoblastic differentiation in MC3T3-E1 cells and primary murine calvarial osteoblasts [28]. In other words, these reports indicate that IL-6 also affects bone cells and that the effects are conflicting and may occur in opposite directions [29–31].

The BMP-2 signaling cascade is initiated via activation of three related transmembrane serine/threonine kinase receptors, termed BMP receptor IA (BMPRIA), BMPRIB, and BMPRII. Upon binding of BMP-2 to heteromeric complexes of BMPRI and BMPRII at the cell surface, BMPRII kinase phosphorylates and activates BMPRI kinase, which in turn activates the downstream BMP/Smad pathway. The response of target cells to BMP-2 at least depends partly on BMP receptor expression, which can be modulated by certain drugs [32] and cytokines [33]. We hypothesized that the existence of a functional loop between IL-6 and the BMP receptors could provide new insights into the role of the inflammatory environment in the osteoinductive efficiency of BMP-2/ACS implants. Therefore, in this study, we investigated the effect of IL-6 on BMP-2/ACS-induced ectopic bone formation in a rat model and on BMP-2-induced osteoblastic differentiation in human BMSCs (hBMSCs). We identified a complex regulatory relationship between IL-6 and the BMP receptors that controls BMP-2-induced osteoblastic differentiation in inflammatory environments. Our results reveal that this regulatory pathway represents a promising approach for enhancing the osteoinductivity of BMP-2/ACS implants in clinical applications.

2. Results

2.1. IL-6 injection increases BMP-2/ACS-induced bone mass by enhancing bone regeneration and does not activate osteoclast-mediated bone resorption

To investigate the role of IL-6 in BMP-2/ACS-induced bone formation, we designed a rat study using both subcutaneous BMP-2/ACS implantation to induce ectopic bone formation and IL-6 injection to artificially increase the serum level of IL-6 (Fig. 1A). During the first three weeks, the serum levels of IL-1β, IL-6, and TNF-α were measured regularly to confirm the elevation of the levels of these cytokines after IL-6 injection. As shown in Fig. 1B, compared with the sham-operated rats, the serum levels of IL-6 in the saline- and IL-6-injected rats significantly increased after day 1. More importantly, the concentration of IL-6 in the IL-6-injected rats was 2.7-fold higher than that in the saline-injected rats. However, although upward tendencies were also observed in the TNF-α and IL-1β levels in the IL-6-injected rats, the tendencies were much lower than the upward tendency observed for IL-6 (Supplemental Fig. 1A–B). These data indicate that IL-6 injection artificially triggers a significant elevation of the serum level of IL-6, but have a minimal impact on TNF-α and IL-1β secretion.

Following BMP-2/ACS implantation and IL-6 injection, the rats were sacrificed at 2 or 8 weeks, and the ectopically formed bony nodules were harvested to investigate the bone mass. Based on the representative gross views and three-dimensional (3D) reconstructed images, the bony nodules from IL-6-injected rats were obviously larger than those from the saline-injected rats (Fig. 1C–D). Histologically, newly formed bone matrix was observed at the periphery of the implants in the IL-6-injected rats at 2 weeks; however, no obvious sign of bone formation was observed in the implants in the saline-injected rats at the same time point. Eight weeks after implantation, the new bone or bone matrix was immature and lined with cells that were expected to become osteoblasts. Such newly induced woven bone was observed in implants from both the IL-6-injected rats and the saline-injected rats (Fig. 1F). However, the trabecular number (TbN), trabecular thickness (TbTh), and trabecular separation (TbSp) showed differences between the groups based on hematoxylin and eosin (H&E) staining (Fig. 1F). To quantitatively evaluate these differences, we analyzed the data from micro-computed tomography (μCT) scanning. The values of these indicators are presented in Fig. 1E. The results revealed that IL-6 injection enhanced BMP-2/ACS-induced bone mass in comparison with saline injection. To confirm this finding, immunohistochemical staining of a representative marker of bone mineralization, namely, osteocalcin (OCN), was performed. The area of positive expression of OCN in the IL-6-injected group was much larger than that in the saline-injected group (Fig. 1G). Furthermore, data from an enzyme-linked immunosorbent assay (ELISA) suggested that the serum OCN level in the IL-6-injected rats was much higher than that in the saline-injected rats (Supplemental Fig. 2A), indicating that IL-6 injection induces osteoblast-related bone regeneration in this model.

Due to the positive role of IL-6 in osteoclast-mediated bone resorption in several published reports [34–36], we stained the osteoclasts in the regenerated bony nodules by tartrate-resistant acid phosphatase (TRAP) staining and counted the TRAP-positive cells under a microscope. The data showed that the number of TRAP-positive cells in bony tissue sections was not significantly different between the IL-6-injected rats and the saline-injected rats (Fig. 1H). Similarly, the serum levels of the osteoclast marker tartrate-resistant acid phosphatase 5b (TRAcP-5b) and the bone