Methods: We used the Col2.3-11β-HSD2 transgenic (Tg) mouse in which the GC-inactivating enzyme, 11β-hydroxysteroid-dehydrogenase type 2, is overexpressed in osteoblasts and osteocytes, resulting in disruption of intracellular GC signaling in these cells. 14-week old Tg mice and their wild type (WT) littermates were unloaded by tail suspension for the duration of 4 weeks.
Results: Unloading resulted in tibia loss of cortical bone in WT but not Tg mice when compared to non-unloaded controls. This was mainly due to a decrease in cortical area (Ct.Ar), cortical volume, mean total cross-sectional tissue area (Tt.Ar) and mean total cross-sectional tissue perimeter (Tt.Pm) in WT mice only. Trabecular bone in the tibia was similarly affected in WT and Tg mice by unloading. However, there are only significant effects on WT mice in the tibia cancellous bone of bone volume fraction (BV/TV), trabecular thickness (Tb.Th) and trabecular pattern factor (Tb.Pf). Moreover, the maximum force (F_{max}) and polar moment of inertia (pMOI) of tibia were significant reduced in WT mice after hind limb unloading, but no change in Tg mice. The ashing data in lumbar (L5) showed a significant decrease in wet weight, dry weight and ash weight of WT mice, but not in Tg mice. Meanwhile, the effect of hindlimb unloading on trabecular bone of the lumbar vertebrae was similar to the tibia in WT and Tg mice.
Conclusion: These results indicated that GC play a role in hindlimb unloading-induced bone loss of mice. Above all, the effect of GCs on cortical bone was more significant than that of cancellous bone in the bone loss induced by hindlimb unloading. The results above offered a new mechanism for the unloading-induced bone loss in mice.
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Session: Disease & Treatment — Osteoarthritis

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EFFECT OF COMBINATION SALVIANOLATE AND PREDNISONE THERAPY ON BONE HISTOMORPHOMETRY AND BIOMECHANICS IN COLLAGEN-INDUCED ARTHRITIS RATS

Jiaqi Lu [a], Xuemei Zhang [a], Xuna Wu [a], Jiaxin Bei [a], Liyi Zou [a], Liao Cui [a,b], Bilian Xu [a,b]
[a]*Department of Pharmacology, Guangdong Medical University, Zhanjiang, Guangdong, China*
[b]*Guangdong Key Laboratory for Research and Development of Natural Drugs, Guangdong Medical University, Zhanjiang, Guangdong, China*

Background: Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease with clinical manifestation of erosive and symmetric poly-arthritis. The combination therapy with prednisone and other drugs is now a research focus, but there's rare report about the effect of the combined application of salvianolate and prednisone on bone metabolism. We established the rat model of rheumatoid arthritis (RA) induced by type II collagen, and investigated the effect of combination therapy on bone metabolism in rats with collagen-induced arthritis (CIA).
Subjects and Methods: Forty-six 8-week-old female Lewis rats were randomly divided into 2 groups, control group with 6 rats and the remaining 40 rats were used to establish the CIA model. Four weeks after immunization, screening 27 CIA rats which successfully infected with arthritis (arthritis index≥4) were randomly divided into 4 groups. Rats in control group were given vehicle as well as in CIA group, while in the other groups were treated with prednisone at 4.5mg/kg/d or/and salvianolate at 20mg/kg/d. Drugs were administrated for 90 days. After sacrificed, the femur of rats was collected for bone biomechanical properties assay. The proximal tibial metaphysis (PTM) of rats was performed for histomorphometric analysis.
Results: Biomechanical properties (elastic load, maximum load, break load, stiffness coefficient) of femur in CIA group were significantly decreased compared with control group. Compared with CIA group, biomechanical properties (maximum load, break load and stiffness coefficient) were increased in CIA+PDN+Sal group, but there were no significant changes in CIA+PDN group and CIA+Sal group. Poor trabecular structure and less trabecular bone of PTM were seen in CIA rats. Compared with CIA group, percent trabecular area (%Tb.Ar) and trabecular number (Tb.N) in CIA+PDN group and CIA+PDN+Sal group were increased. Furthermore, %Tb.Ar and Tb.N in the treatment of salvianolate and prednisone group were lower than those in combination therapy group.
Discussion and Conclusion: Using prednisone to treat CIA ameliorated the cancellous bone loss of tibia, but did not improve biomechanical properties. Using combination prednisone and salvianolate therapy to treat CIA that was better to ameliorate the cancellous bone loss of tibia, and significantly improve biomechanical properties. The protective effect of combination salvianolate and prednisone therapy on bone loss in CIA rats was greater than that of prednisone and salvianolate alone.
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Session: Disease & Treatment — Pharmaceutical Interventions

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ICARIIN AUGMENTS BONE FORMATION THROUGH ACTIVATION OF CANONICAL Wnt SIGNALING PATHWAY

Junming Huang, Jiaming Zhang, Fengjing Guo
Tongji Hospital, Tongji Medical College, China

Background: Traditional Chinese herbal medicine has been widely used for thousands of years for the treatment of bone diseases. Icariin belong to a class of flavonoid which is used to prevent bone loss and can be extract from several species of plants in the Epimedium family commonly known as Yin Yang Huo. In some reports, Icariin has been mostly reported to boost bone fracture healing and treat postmenopausal osteoporosis in ovariectomized animal model. Wnt signaling pathway with a foundational role during embryogenesis and normal cell development, which can regulates bone development and gene expression in whole process of bone metabolism. In this manuscript, we want to identify the role of icariin via regulating Wnt signaling pathway in bone formation.
Subjects and Methods: Alizarin red staining was used to estimate matrix calcification of mesenchymal stem cells in osteogenic differentiation basal medium compared to the same medium supplemented with icariin. Immunofluorescence test to detect expression of P-GSK in cytoplasm and localization of β-catenin in nuclear. Western blot were used to test expression of proteins of Wnt signaling pathway. Real-time PCR (RT-PCR) were used to test related osteogenic gene expression.
Results: Icariin added in osteogenic medium compared with normal osteogenic medium could promote the formation of calcium nodules in number and morphology by alizarin red staining. RT-PCR showed that Icariin could upregulate expression of osteogenic genes RUNX2, OCN, OSX. The result of western blot suggested that icariin could upregulate expression of P-GSK and active β-catenin. The result of immunofluorescence suggested that icariin could upregulate expression of P-GSK in cytoplasm and boost β-catenin expression in nuclear.
Discussion: Icariin, a flavonoid isolated from Epimedium family, has previously been identified to exert beneficial effects on preventing bone loss and promoting bone regeneration. Meanwhile in skeletal development, Wnt signaling, which play progression, is implicated in and commitment lineage MSC a crucial role in adipogenesis. In Wnt signaling, and chondrogenesis, osteogenesis, myogenesis non-phosphorylated GSK play an important role in degradation of β-catenin. In this study, these results have confirmed that icariin can promote the formation of calcium nodules in number and morphology, and upregulate phosphorylation of GSK so that inhibiting degradation of β-catenin in cytoplasm. With less degradation in cytoplasm, more β-catenin can into nuclear and play their bio-function.
Conclusion: In conclusion, icariin augments bone formation may via activate Wnt signaling pathway.
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Session: Biomaterials and Implants—New (Bio)materials

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A NOVEL CLAY-BASED NANOCOMPOSITE HYDROGEL WITH ATTRACTIVE MECHANICAL PROPERTIES AND ITS APPLICATION FOR BONE TISSUE REPAIR

Xinyun Zhai [a,b,c], Wenguang Liu [c], Changshun Ruan [a], Haobao Pan [a], William Weijia Lu [a,b]
[a]*Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, China*
[b]*The University of Hong Kong, Hong Kong*
[c]*Tianjin University, China*

Introduction: In our study a novel nanocomposite hydrogel (NC gel) was successfully prepared by in situ free-radical photo-polymerization of the acrylic acid derivatives, macromolecular crosslinker (PEGDA) and nano-clay. The obtained hydrogel exhibits dramatic improvements in mechanical properties and the pre-in vivo test shows that this kind of novel hydrogel can accelerate bone formation [1—3].
Subjects and Methods: The NC gel was synthesized by in situ free-radical photo-polymerization of acrylic acid derivatives (monomer) and PEGDA (macromolecular crosslinker) in the presence of exfoliated clay. The clay content was varied from 1 to 10 wt % with respect to the monomer weight, and the solid content of the nanocomposite hydrogel varied from 20% to 30%. Characterization was carried out by tensile tests, compression tests, XRD, SEM and TEM. Pre-in vivo bone formation was studied using a rat bone defect model system.
Results: Mechanical tests show that the obtained novel clay-based nanocomposite hydrogel has the best tensile strength (about 800 kPa) and excellent stretch ability (higher than 5000%) when clay content and solid content are 5% and 20% respectively. The compression strength of the hydrogel is higher than 10 MPa

and can recover to its original shape when compression ratio is less than 80% which will be very attractive for bone tissue engineering. SEM analysis shows that the pores in the hydrogel was uniformly present and highly interconnected which is very important for nutrients transplantation. XRD image indicates that both polmers and clay were well distributed. Adding macromolecular crosslinker (PEGDA) into the system can not only increase the biocompatibility but also improve the bone formation compared with the nanocomposite hydrogel without PEGDA.

Discussion and Conclusion: A novel nanocomposite hydrogels composed of exfoliated clay showed attractive fracture strain up to 5000% and good compression strength. This kind of hydrogel was synthesized by in situ photo-polymerization of monomer in the presence of clay, acrylic acid derivatives and macromolecular crosslinker (PEGDA). More attractive part is that the obtained nanocomposite hydrogel has good biocompatibility and can accelerate bone formation. So that we will explore why this kind of hydrogel can accelerate bone formation and find out the best PEGDA molecular weight, clay content and solid content for bone formation.

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Session: Others

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MODULATION OF SUPERFICIAL ZONE PROTEIN/LUBRICIN/PRG4 BY KARTOGENIN AND TRANSFORMING GROWTH FACTOR-β1 IN SURFACE ZONE CHONDROCYTES IN BOVINE ARTICULAR CARTILAGE

Kazumasa Miyatake [a,b], Kenjiro Iwasa [b], Sean McNary [b], Gordon Peng [b], A. Hari Reddi [b]
[a]*Tokyo Medical and Dental University, Japan*
[b]*University of California, Davis, USA*

Introduction: Articular cartilage is an anisotropic structure with a zonal design and consists of surface or superficial, middle, and deep zones. Superficial zone protein (SZP)/lubricin/PRG4 functions as a boundary lubricant in articular cartilage to decrease friction and wear. (Reference 1, 2) As articular cartilage lubrication is critical for normal joint function, the accumulation of SZP at the surface of cartilage is important for joint homeostasis. Recently, a heterocyclic compound called kartogenin (KGN) was found to induce chondrogenic differentiation and enhance mRNA expression of lubricin. (Reference. 3) The objective of this study was to determine whether KGN can stimulate synthesis of SZP in superficial zone articular chondrocytes and synoviocytes.

Subjects and Methods: We investigated the effects of KGN and transforming growth factor-β1 (TGF-β1) on articular cartilage and synovium of the bovine knee joint by evaluating SZP secretion by enzyme-linked immunosorbent assay analysis. Monolayer, micromass, and explant cultures of articular cartilage, and monolayer culture of synoviocytes, were treated with KGN and TGF-β1. Explant was also treated with KGN and IL-1β to evaluate the anti-catabolic effect of KGN. SZP accumulation in the medium was evaluated and mRNA expression was measured through quantitative polymerase chain reaction.

Results: TGF-β1 stimulated SZP secretion by superficial zone chondrocytes in monolayer, explant, and micromass cultures as expected. In addition, SZP secretion was inhibited by IL-1β in explant cultures, and enhanced by TGF-β1 in synoviocyte monolayer cultures. Although KGN elicited a 1.2-fold increase in SZP mRNA expression in combination with TGF-β1, KGN neither stimulated any significant increases in SZP synthesis nor prevented catabolic decreases in SZP production from IL-1β.

Discussion and Conclusion: These data suggest that the chondrogenic effects of KGN depend on cellular phenotype and differentiation status, as KGN did not alter SZP synthesis in differentiated, superficial zone articular chondrocytes. However, these apparent differences between progenitor and differentiated cell types in response to KGN merit additional investigation since important new, mechanistic insights into the effects of KGN may be revealed.

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Session: Disease & Treatment — Tumors

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DIFFERENTIATION THERAPY OF THE NEOPLASTIC STROMAL CELLS IN GIANT CELL TUMOR OF BONE USING THE U.S. FOOD AND DRUG ADMINISTRATION (FDA)-APPROVED DRUGS, RAPAMYCIN AND SIMVASTATIN

Carol P. Y. Lau [a,b], Stephen K. W. Tsui [b], Shekhar M. Kumta [a]
[a]*Department of Orthopaedics and Traumatology, Faculty of Medicine, The Chinese University of Hong Kong, China*
[b]*School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, China*

Background: Giant cell tumor of bone (GCT) is a common neoplasm in Chinese patients, constituting 20% of all benign bone tumors. The stromal cells of GCT are widely accepted to be the primary neoplastic cells, and originate from mesenchymal stem cells (MSC). Since GCT stromal cells could be induced to be mature osteoblasts, our research team has proposed the differentiation therapy of GCT stromal cells. It is noteworthy that rapamycin and simvastatin have been shown to induce osteogenic differentiation in a number of tumor types, and they are FDA-approved drugs and have been safely used in patients for decades. Therefore, we reasoned that they may be developed as adjuvant therapy agents for GCT.

Subjects and Methods: We investigated the effects of rapamycin and simvastatin on cell viability, proliferation and osteoblastic differentiation in GCT stromal cells in vitro. Cell viability was assessed using MTT assay, whereas, cell proliferation by BrdU assay. The markers for assessing the re-differentiation of GCT stromal cells are ALP, RUNX2, and OCN, and the mRNA level of those markers were detected by real-time PCR.

Results: Rapamycin decreased the cell viability and proliferation of GCT stromal cells significantly at 0.05 μM but no further inhibition was observed when increasing the dose of the drug up to 5 μM. Whereas, simvastatin showed a dose-dependent inhibition on cell viability and proliferation in GCT stromal cells. Moreover, the important osteoblastic markers RUNX2 and osteocalcin were up-regulated by both drugs.

Discussion and Conclusion: Rapamycin and simvastatin inhibited cell viability, and proliferation of GCT stromal cells. They also stimulated RUNX2 and osteocalcin gene expression, and may induce differentiation of the tumor cells. Previous studies have shown that simvastatin induces osteoblast differentiation by monitoring Smad signaling and Ras/Rho-mitogen-activated protein kinase pathway (Yamashita et al. 2008), whereas, rapamycin stimulates the osteoblastic differentiation of human embryonic stem cells by blocking the mammalian target of rapamycin (mTOR) pathway and activating the BMP/Smad pathway (Lee et al. 2010). The possibility of using rapamycin and simvastatin to promote the differentiation of GCT stromal cells into mature osteoblasts is appealing. Such a strategy has the dual advantage of preventing further bone destruction as a result of the osteolytic tumor while simultaneously promoting bone formation as the neoplastic cells are differentiated into mature osteoblasts. The mature osteoblasts will eventually undergo apoptosis. It will stop the tumor growth and thus reduce bone resorption.
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Session: Disease & Treatment — Tumors

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EXPRESSION OF GLYPICAN-3 AND PERIOSTIN IN MUSCULOSKELETAL TUMOR PATIENTS

Thamonwan Woraruthai, Sittisak Honsawek
Chulalongkorn University, Thailand

Background: Glypican-3 (GPC-3) is an extracellular matrix that functions in cell adhesion, migration and invasion during cell proliferation. It can induce abnormal cells to apoptosis. Additionally, periostin (POSTN) is also extracellular matrix that involve in tissue development and regeneration. This gene can bind to integrin for supporting adhesion and migration of epithelial cells. However, GPC-3 and POSTN expressions in soft tissue sarcoma remain unclear. The purpose of this study was to examine expression of GPC3 and POSTN in neoplastic and non-neoplastic adjacent tissues of musculoskeletal tumor patients.

Subjects and Methods: This research was conducted a cross-sectional study. Twenty musculoskeletal tumor patients who had liposacroma, osteosarcoma, lipoma, giant cell tumor and chondroma were enrolled in this study. The GPC-3