drive MEFs to an intermediate stage. Then chondrogenic growth factors were used to complete the induction by 2D/3D culture. Cells of each stage were characterized by immunofluorenscence and single cell sequencing. A col2-pd2EGFP reporter system played a role in the following high-throughput screening for efficiency optimization.
Results: Col2+ chondrocyte-like clusters could be visualized after chemical induction by 2D/3D culture. Consequently, 3D culture (micromass and suspension culture) with TFGβ3 promoted the chondrogenesis of chemical-induced MEFs compared to monolayer culture, resulting in a cartilage-like particle. For the whole chemical lineage conversion, the first stage was essential, during which fibroblasts were driven into an intermediate stage with pluripotent colonies and heterogenous cell subpopulation. With the help of col2-pd2EGFP screening system, we discovered that adding other 2 small molecule compounds during the first stage significantly increased the fibroblast-to-chondrocyte conversion efficiency.
Conclusion: This study demonstrates the possibility of chemical conversion from mouse fibroblasts to cartilage-like tissue without genetic manipulation. This proof of concept study lays a foundation for high-throughput screening of chondrogenic inducers and *in vivo* chemical conversion in clinical cartilage repair.
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DIFFERENT EFFECTS OF PREDNISONE TREATMENT FOR 30 DAYS AND 90 DAYS ON BONE METABOLISM IN COLLAGEN-INDUCED ARTHRITIS (CIA) RATS

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Background: Glucocorticoids (GCs) are often prescribed to treat RA for a long time, but there is still controversy in the administration of GCs, mainly because of the adverse reactions, such as osteoporosis. How to control the dosage and duration of GCs treatment is the problem we need to solve. The aim of this study was to investigate the different effects of prednisone treatment for 30 days and 90 days on bone metabolism in collagen-induced arthritis (CIA) rats and hope to provide a reasonably clinical application of glucocorticoids for the treatment of RA.
Subjects and Methods: Fifty 8-week-old female Lewis rats were randomly divided into 2 groups, normal group with 12 rats and the remaining 38 rats were used to establish the CIA model. Four weeks after immunization, 24 rats which were successfully infected with arthritis (arthritis index $\geq$4) were randomly divided into CIA treated with vehicle group and CIA treated with prednisone group. 6 rats were killed in each group on the 30d and 90d respectively. Bone histomorphometry, bone mineral density (BMD), micro-computer tomography (micro-CT), biomechanical test and enzyme-linked immunosorbent assay were performed.
Results: Compared with normal rats, in fourth lumbar vertebrae (LV4), percent trabecular area (%Tb.Ar) and trabecular number (Tb.N) were significantly decreased, while trabecular separation (Tb.Sp), percent osteoclast surface perimeter (%Oc.S.Pm), percent labeled perimeter (%L.Pm) and bone turnover rate (BFR/BV) were significantly increased in CIA rats. Poor trabecular structure and less trabecular bone of femur were seen in CIA rats by micro-CT scanning. BMD and biomechanical properties of femur were significantly decreased in CIA rats. Serum level of PINP, sRANKL and CTX-I were increased in CIA rats treated with vehicle for 90 days. Compared with CIA rats, %Tb.Ar and Tb.N of LV4 in CIA rats treated with prednisone for 30 days were increased, but there was no significant change in CIA rats treated with prednisone for 90 days. Prednisone treatment for 30 days increased bone volume and improved microarchitecture of distal femur. Prednisone treatment for 90 days increased bone volume, but there was no significant improvement in microarchitecture of femur. In additional, serum levels of OPG and PINP were decreased in CIA rats treated with vehicle for 90 days. There was no significant change in biomechanical properties of femur between CIA rats treated with vehicle and prednisone.
Discussion and Conclusion: High bone turnover osteoporosis could be shown in CIA rats, manifested by decrease of trabecular bone mass, structural degradation. Reduction of bone mineral density and biomechanical weak-ness. Prednisone treatment for 30 days decelerated the degeneration of trabecular bone in CIA rats, but did not improve bone mineral density and bone biomechanics. In additional, the protective effect of prednisone treatment for 30 days on bone in CIA rats was better than that of 90 days. Decrease of bone mass was not directly seen in CIA rats treated with prednisone for 90 days, but there was a negative effect on bone metabolism.
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PROMOTING EFFECT OF STRONTIUM ON THE DIVISION OF STEM CELLS IN BONE FORMATION

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Introduction: Strontium ranelate has been shown to reduce the risk of vertebral fracture in postmenopausal women [1]. The beneficial effects of strontium on promoting bone formation are closely related to its capability to increase the osteogenic differentiation of mesenchymal stem cells [2,3]. However, the molecular mechanisms are still not fully understood. The aim of this study is to investigate the mechanism underlying the promoted effect of strontium in inducing osteogenic differentiation of mesenchymal stem cells in early stage.
Subjects and Methods: Eight-week-old male C57BL/6 mice were randomly divided into experiment group and control group. Sr was daily oral administration in experimental group and vehicle (0.9% saline) in control group respectively for 4 weeks. The effect of Sr on trabecular bone microstructure was analyzed by micro-CT on proximal tibia. Human fetal BMSC (hfBMSC) which characterized by multi-differentiation were cultured in control group (control), osteogenic group (Osteo) and Sr promoted osteogenic group (Osteo+Sr). The osteogenic and asymmetric differentiation related genes were analyzed by quantitative PCR. The protein level of asymmetric differentiation related gene was detected by western blot. The effect of Sr on trabecular bone microstructure was analyzed by micro-CT on proximal tibia.
Results: The microstructure analysis of trabecular bone in proximal tibia showed that Sr significantly increased trabecular number and connectivity density. In the first week of hfBMSC culture, the related osteoblastic genes Osterix, osteopontin and BSP were significantly enhanced in osteogenic induced group from day3. With the addition of Sr, these genes were expressed at even higher levels. The gene Numb associated with asymmetric differentiation was promoted in Osteo+Sr groups in day3. At the same time in the immunofluorescence images, the osteogenic differentiation could be observed in Osteo and Osteo+Sr group. Oster+Sr showed higher proportion of the osterix positive cells. It should be noted that the divided cells showed one osterix positive cell and one oct-4 positive cell. In western blot, Sr stimulated the expression of Numb in the early stage of osteogenic differentiation.
Discussion and Conclusion: During the osteogenic differentiation of hfBMSC, Sr is related to the enhanced effect in asymmetric division of stem cell in the early stage.
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Session: Disease & Treatment — Osteoporosis

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DISRUPTION OF GLUCOCORTICOID SIGNAL IN OSTEOBLASTS AND OSTEOCYTES ATTENUATES HINDLIMB UNLOADING-INDUCED BONE LOSS IN MICE

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Objective: Bone loss induced by weightlessness has been a hot issue in space physiology, but the accurate mechanism is still not clear. At present, the lack of mechanical stimulation is considered to be an important factor for bone loss under weightlessness environment. However, besides mechanical unloading, it is inevitable that the stress in weightlessness environment induced increase of endogenous glucocorticoid (GC) levels. High levels of GCs have been established as risk factors for low bone mineral density and an increase in fracture risk. A recent progress showed that osteoblast-targeted disruption of intracellular GC signaling can prevent transgenic mice in chronic stress from bone loss, which indicated GC play an important role in stress-induced bone loss and a possible role in weightlessness induced bone loss. Therefore it is the aim of the present study to explore the role of GC in unloading-induced bone loss of mice.