406 THERMALLY RESPONSIVE NANOSPHERES WITH DUAL DRUG RELEASE PROFILES FOR COMBINED CRYOTHERAPY OF OSTEOARTHRITIS

Introduction: In this study, diclofenac (DCF) and kartogenin (KGN) were chosen as the combined osteoarthritic (OA) cryotherapy to induce anti-inflammatory activity and cartilage regeneration. Here, we designed a dual drug delivery system with thermoresponsiveness for combined therapy of OA which is composed of pluronic F127 (F127)-chitosan oligosaccharide (COS)-KGN conjugated nanoparticles (F127-COS-KGN NPs) encapsulating DCF. The aims of this study were to: (1) characterize the F127/COS/KGN, for controlled dual release by temperature change and F127-COS-KGN, and (2) evaluate the combined therapeutic effects of the F127-COS-KGN in vitro. Subjects and Methods: (1) Preparation of F127-COS-KGN NPs loading DCF. The F127/ COS/KGN were made by emulsification/solvent evaporation method. Conjugation of F127-COOH and KGN with COS was carried out by EDC/NHS catalysis during the NPs synthesis process. DCF was encapsulated inside the NPs by change of wall-permeability according to temperature control. (2) Combined dual release by temperature change. The amounts of KGN and DCF released from the NPs were determined by HPLC chromatography. (3) In vitro thermochromic differentiation. The hBMSC (2.5 × 10^5 cells, passage 3-5) were made by pellets. (4) In vitro anti-inflammatory activity. After induction of inflammation with lipopolysaccharide (LPS), the F127/COS/ KGN were used to treat the cells. (5) In vivo thermo-responsiveness & retention time of OA rat. OA was induced surgically using ACLT and DMM in rats. After IA injection of the fluorescence dye-label F127/COS/KGNDCF, cold temperature (5°C) were applied around the joint for 10 minutes with a cryotherapy device. Fluorescence spectrum was scanned using an IVIS-spectrum measurement system. (6) In vivo cyclooxygenase inhibition. Serum and synovium were collected in OA rats after IA injection of the F127/COS/KGNDCF. COX inhibition was evaluated by RT-qPCR and ELISA. (7) In vivo cartilage regeneration. The OA rats were treated with F127/COS/KGNDCF by IA injection at weeks 6 and 9 after OA induction. The distal femur in each group were dissected at 14 weeks after OA induction and evaluated by Safranin-O staining and QLS scoring. Immunohistochemistry of COL2 and ACAN was also carried out.

Results: (1) Preparation of F127-COS-KGN NPs loading DCF. The F127/COS/KGN are 300 nm at 37°C expand to ~650 nm when cooled to 4°C. (2) In vitro release study. While the encapsulated DCF showed burst release for 6 hours after cold shock, the encapsulated KGN showed sustained release for 144 days even though the temperature changed. (3) In vitro thermo-responsification. The gene expression of COL2A1 and ACAN increased in hBMSC pellets exposed to unconjugated KGN and both F127/COS/KGNDCF for 21 days compared with those of untreated hBMSCs. (4) In vitro anti-inflammatory activity. After cold shock treatment, the F127/COS/KGNDCF treated chondrocytes showed rapid decrease in IL-6 secretion. (5) In vivo thermo-responsification & retention time in OA joint. The fluorescence signals from F127/COS/KGNDCF were observed in the knee joint of OA rats up to 21 days. In particular, F127/COS/KGNDCF treated rats after cold temperature treatment showed significantly higher fluorescence intensity than those of untreated rats with cold temperature on days 2 (p < 0.01) and 5 (p < 0.05). (6) In vivo cyclooxygenase inhibition. After cold temperature treatment, the F127/COS/KGNDCF injected rats showed decrease of COX-2 activity. Discussion and Conclusion: Both KGN and DCF were released independently from the F127/COS/KGNDCF by temperature control. COX-2 inhibition by DCF released from the NPs after cold temperature treatment was confirmed. The F127/COS/KGNDCF can be effectively combined therapeutic for OA by thermally controlled dual drug delivery.

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411 ESTABLISHMENT OF OSTEOPOOROSIS MODEL IN C57/B6 MICE BY OVARIECTOMY

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Background: To investigate the optimum timing and how long to build the osteoporosis model in C57/B6 mice by ovariectomy (OVX). Methods: Fifty-week-old female C57/B6 mice were divided into ten groups (A-J). Group A and F underwent BMD measurement by DEXA on cranium at eight-week-old and twelve-weeks-old, respectively. The BMD analysis of group B-E was performed at 8 weeks, 10 weeks, 12weeks, and 14 weeks after the mice underwent OVX at eight weeks old. The BMD analysis of group G-J was performed at 6 weeks, 8 weeks, 10 weeks, and 12 weeks after the mice underwent OVX at twelve weeks old. Results: The mean BMD on the cranium of twelve-week-old mice (0.131±0.030g/cm^2) was significantly higher than the BMD of eight-week-old mice (0.113±0.042g/cm^2) (P<0.05). There was no significant difference between groups A-E. The mean BMD on the cranium of group F (0.131±0.030g/cm^2) was significantly higher than the BMD of group H (0.113±0.014g/cm^2) (P<0.05). The BMD decreased smoothly from H-J (P<0.05). Discussions and Conclusions: The optimum age to build up the osteoporosis model in C57/B6 mice is twelve weeks old and we should wait at least 8 weeks before the model is established.

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407 CORRELATION ANALYSIS OF DXA AND COMBINED USE OF QUS AND OSTA

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Background: At present, the population aging situation in China has become more and more serious. Osteoporosis is one of the deadliest diseases that affect the health of the elderly. Early detection and early prevention of osteoporosis can help to avoid serious complications of osteoporosis, such as limb brittle fractures or vertebral compression fractures. The extended life expectancy and increasing number of elderly in the population means the arrival of an aging society. Quantitative ultrasound (QUS) is a non-invasive method for evaluating bone mass density developed in the 90s. It not only reflects the bone density, but also contributes to show the bone strength and bone structure characteristics; therefore, it has the value of diagnosing osteoporosis and predicting potential fracture risks as well. At the same time it is convenient to carry and easy to operate. Asian osteoporosis self-assessment tool (OSTA) is an easy and effective way to evaluate Asian people’s osteoporosis. Neither OSTA nor QUS can solely achieve the desired sensitivity and specificity when screening for osteoporosis, but it is a feasible way to combine both methods. This study aims to explore the use of combining QUS and OSTA to evaluate the risk of osteoporosis in a community of postmenopausal women.

Methods: From September 2014 to December 2014, bone mineral density of 118 postmenopausal women was measured in Guangzhou communities by quantitative ultrasound measurement and relative information such as their ages and BMI were collected through questionnaires. Patients also went through lumbar and dual-energy X-ray scans. DXA test results were taken as the gold standard of osteoporosis diagnosis, by drawing an ROC curve, this research evaluates the feasibility of the joint use of QUS and OSTA score in osteoporosis screening and determine the appropriate diagnosis point.

Results: When combined use of OSTA and for screening, the regression curve was fitted as Y = -1.188*QUS+0.186*OSTA–3.973. Y was considered to be a predicted value. Meanwhile, the AUC of ROC drawn by predicted value and DXA screening result is 0.847, SE = 0.041.

Discussions and Conclusions: Quantitative Ultrasound (QUS) and OSTA score is a simple and economic method of predicting the incidence of osteoporosis in the elderly. By setting the QUS and OSTA threshold, it can effectively screen osteoporosis in patients at high risk.

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mineralisation ability was conducted to identify whether STIM1 is involved in osteogenesis. We next investigated the effects of 17β-estradiol on STIM1. MC3T3-E1 were cultured in the presence of 17β-estradiol and 17β-estradiol inhibitor (ICI) while osteogenic induction was conducted. RT-PCR and Western Blot was used to detect the expression and mRNA transcript levels. To elucidate the underlying mechanism of oestrogen on STIM1, we added an inhibitor of PI3K and mTOR to determine whether this signalling pathway was involved. To investigate the role of STIM1 in osteoblast differentiation and osteogenesis regulated by 17β-estradiol, we compared the osteogenic gene markers, ALP activity, and mineralization ability between STIM1 knockdown and control group in the presence of 17β-estradiol.

Results: The function of the CRAC channel and osteogenic differentiation of BMASCs is decreased in postmenopausal osteoporosis patients. Knockdown of STIM1 weakened the osteogenic differentiation and mineralisation of osteoblasts derived from MC3T3-E1 dramatically. We found that 17β-E2 can promote the expression and transcription of STIM1 via the PI3K-mTOR signalling pathway. STIM1 also plays an essential role in osteogenic differentiation of osteoblasts regulated by oestrogen.

Discussion and Conclusion: In summary, we found that the expression of STIM1 is decreased due to low activity of PI3K-mTOR signalling pathway, because of the low level of oestrogen after menopause. The decreased expression of STIM1 affects the differentiation and bone formation. Our study preliminarily clarified the underlying mechanism of the role of STIM1 on osteogenesis and bone formation, further studies are needed to clarify potential mechanisms and provide new strategies for treatment.

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CHEMOKINES: A POTENTIAL MOLECULAR LINK BETWEEN CHONDROCYTE APOPTOSIS AND OSTEOCLAST MIGRATION AND FORMATION FOLLOWING DEXAMETHASONE THERAPY

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Introduction: Glucocorticoid therapy is known to cause growth plate chondrocyte damage, bone growth arrest, bone resorption, and bone loss, for which the mechanisms remain unclear. The current study aims to identify whether apoptotic chondrocytes secrete factors that may stimulate osteoclast differentiation. It was hypothesised that dexamethasone (Dex) will induce chondrocyte apoptosis, causing increased secretion of chemokines with the capacity to promote osteoclast recruitment and formation.

Subjects and Methods: The current study utilized an in vitro Dex treatment model in ATDC5 chondrogenic cells and examined treatment effects on molecular marker expression, induction of apoptosis, and expression of chemokines and receptors in differentiated Dex-treated chondrocytes. In addition, ability of promoting osteoclast differentiation in RAW264.7 osteoclast precursor cells was assessed for conditioned media taken from Dex-treated or -untreated chondrocytes.

Results: In a time course of chondrogenic culture, the increased type II and X collagen expression suggests the dynamic chondrocyte differentiation. The significantly enhanced increased density of apoptotic cells as observed by Hoechst dye staining as well as markedly increased expression level of Fas-L as examined by real-time quantitative RT-PCR suggests increased chondrocyte apoptosis following Dex treatment. Meanwhile, chemokine PCR array analysis of Dex-treated or -untreated chondrocytes identified up-regulation of various chemokines in treated chondrocytes. Chemokine SDF-1 was demonstrated to be the chemokine with the highest induction following treatment, and its induction was also supported by conformational RT-PCR. Furthermore, the SDF-1 protein level was also increased as assayed by ELISA in the conditioned medium of Dex-treated chondrocytes. Moreover, it was found that conditioned medium from Dex-treated chondrocytes was able to stimulate migration of RAW264.7 cells as well as enhance formation of TRAP-positively stained RAW264.7 cells. In particular, inhibition of these promoting effects of the conditioned medium by a neutralising antibody for SDF-1 was also observed.

Discussion and Conclusion: These findings suggest that Dex treatment can cause apoptosis of chondrocytes and elevated expression of chemokines by apoptotic chondrocytes, which may be responsible for enhanced osteoclast migration and formation, serving as a potential molecular link between chondrocyte apoptosis and osteoclast differentiation.

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