USE OF MESENCHYMAL STEM CELLS TO FACILITATE BONE REGENERATION IN NORMAL AND CHEMOTHERAPY-TREATED RATS

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INTRODUCTION: Cisplatin and doxorubicin, the most commonly used chemotherapy drugs for malignant bone tumors, have been demonstrated to have detrimental effects on fracture healing and on mechanical strength of the skeleton. The toxicity of cisplatin and doxorubicin on proliferation and the osteogenic differentiation of mesenchymal stem cells (MSCs) has also been shown. Therefore it is important to apply tissue engineering strategies to facilitate bone regeneration in patients undergoing chemotherapy. The purpose of this study is to investigate the feasibility of using MSCs from isogenic rats to enhance bone regeneration in normal and chemotherapy-treated rats.

MATERIALS AND METHODS: A license for the use of rats in this experiment was obtained from the Home Office of the United Kingdom. Thirty-six adult male wistar rats, at the average weight of 600 grams, were used in the study. 18 rats (chemotherapy group) received three intraperitoneal injections of combined cisplatin (1mg/Kg body weight) and doxorubicin (1.2 mg/Kg body weight), the neoadjuvant regime suggested by the European Osteosarcoma Intergroup. The interval between the injections was two weeks. Normal saline was injected in the other 18 rats (control group) intraperitoneally. Seven days after the last injection, the rats were operated. A 1.5 mm bone gap osteotomy was created on the left femoral shaft and external skeletal fixators were used to stabilize the bone ends, with two pins at each side and the pins were connected by a plate (Figure 1). Eighteen rats in each group were divided into three subgroups; there was a subgroup in which the osteotomy remained unfilled, a subgroup with fibrin glue filling in the defect only, and a subgroup using MSCs suspended in fibrin glue to fill in the defect. The study groups were numbered as followed: Group 1, normal rats with nothing in the osteotomy gaps; Group 2, normal rats with fibrin glue only in the gaps; Group 3, normal rats with MSCs loaded in fibrin glue in the gaps; Group 4, chemo rats with nothing in the gaps; Group 5, chemo rats with fibrin glue only in the gap; Group 6, chemo rats with MSCs loaded in fibrin glue in the gaps.

MSCs were harvested from the femora of skeletally immature male wistar rats. Under sterile condition, the ends of the femur were removed and the marrow content was flushed out with culture media containing Dulbecco’s Modified Essential Medium (DMEM) with 10% fetal calf serum (FCS) and 1% penicillin/streptomycin. The flushed marrow content in the media was collected by centrifugation. The cell pellet was re-suspended, washed, and cultured in 175 cm² culture flasks. Media was changed twice a week. The cells which adhered to the plastic reached confluence at 10-14 days after initial seeding and were passaged using trypsin. Passage 2 and passage 3 cells (Figure 2) were transplanted to the bone defects. 10⁶ cells were then suspended in 0.25ml of thrombin component of the fibrin glue (Tissucel®, Baxter Hyland Immuno, UK) immediately before mixing with 0.25ml of fibrin component. 0.5 ml of fibrin glue was injected to the gap. Rats were kept for 5 weeks after the operation. Evaluation of bone formation included radiography, histology and bone mineral density measurement at the osteotomy gap using dual energy X-ray absorptiometry (DEXA) scanning. The results of DEXA scanning were analyzed with one-way analysis of variance (ANOVA) test at the level of significance of p<0.05. For ANOVAs with significant F tests, a Tukey’s post-hoc procedure was performed to determine which treatment groups were significantly different from each other.

RESULTS: There was no significant difference in body weight of the rats among the groups at the time of sacrifice. One X-ray from each group is shown in Figures 3 to 8. It was noted that there was less bone formation in chemotherapy-treated rats than in normal rats when there was nothing or only fibrin glue added to the fracture gap. However, bone formation was quite obvious in both normal and chemotherapy treated rats in which MSCs were delivered in the fibrin glue. For DEXA scan results (Figures 9 and 10), statistical significance was shown. Fibrin glue alone did not enhance bone regeneration in either normal or chemotherapy-treated rats. However, when the bone defects were filled with fibrin glue and MSCs, significantly greater bone regeneration was noted. Bone mineral density at the osteotomy site was not different between normal and chemotherapy-treated rats if the gaps were filled with MSCs and fibrin glue. Histology was shown in Figures 11-16, bone regeneration was evident in Group 3 (Fig. 13) and 6 (Fig 16), in which groups MSCs were delivered to the bone defects.

DISCUSSION: It has been demonstrated in this study that MSCs were effective in enhancing bone regeneration in normal and chemotherapy-treated rats. Also, fibrin glue, which is injectable, can be used to deliver MSCs. Impaired bone regeneration rate in chemotherapy-treated rats can be accelerated and comparable to normal rats with the help of MSCs.