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ORIGINAL ARTICLE

Effect of intra-articular injection of mesenchymal stem cells in cartilage repair in experimental animals

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Abstract Osteoarthritis (OA) is characterized by degeneration of the articular cartilage and, ultimately, joint destruction. Human umbilical cord blood (hUCB) may prove to be a new source of mesenchymal stem cells (MSCs) for cellular therapeutics used for cartilage repair.

Aim of the work: This study was carried out over a nine-month period of time, to study the effect of intra-articular injection of hUCB MSCs in cartilage repair by histopathological and ultra structural assessment.

Materials and methods: We conducted our study on 20 adult rats, which were subjected to the induction of cartilaginous defect in both knee joints. This was followed by injection of MSCs suspended in Hyaluronic acid solution in the right knee of each rat while the left knee served as a control. Histopathological and electron microscopic studies were performed.

Results: The present study revealed: In the injected knees; In 73% of the cases, the tissue was typical of fibrohyaline cartilage and appeared more cellular than fibrous. In 27% of the cases the repaired tissue appeared more fibrous than hyaline. In the control knees; the newly formed tissue was an undifferentiated connective tissue and the cells were covered with a thin layer of fibrous tissue. The electron microscopic pictures of the injected knees showed mitotic chondrocyte activity. The pictures indicated a repaired fibrohyaline cartilage.

Conclusion: We can conclude that the intra-articular injection of hUCB MSCs is an effective method for cartilage repair in rats. This makes it a very promising tool for the treatment of patients with OA.

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1. Introduction

Osteoarthritis (OA) is a degenerative joint disease that is characterized by erosion of the articular cartilage, growth of bone at the margins i.e., osteophytes, subchondral sclerosis and a range of biochemical and morphologic alterations of the synovial membrane and joint capsule [1]. The primary tissue affected is the thin rim of hyaline articular cartilage interposed between the two articulating bones [2].

In early OA, the articular cartilage surface becomes irregular, and superficial clefts within the tissue become apparent. As the condition worsens, the clefts deepen, surface irregularities increase and the articular cartilage eventually ulcerates, exposing the underlying bone [3]. Chondrocytes in areas surrounding an injured zone are unable to migrate, proliferate, repopulate, regenerate or repair tissue with similar structure, function, and biomechanical properties of normal hyaline cartilage [4].

The burden of OA is exacerbated by the inadequacies of current therapies. Nonpharmacologic and pharmacologic treatments are used for early and moderately early cases of OA [5].

Mesenchymal stem cells (MSCs), which have the ability to differentiate into cells of the chondrogenic lineage, are very promising candidates to develop new cell-based articular cartilage repair strategies; this strategy entails the use of MSCs as trophic producers of bioactive factors to initiate endogenous regenerative activities in the OA joint [6]. Autologous bone marrow (BM) represents the main source of MSCs for both experimental and clinical studies; however as the number of MSCs and their differentiation capacity decline with age, their therapeutic potential might be diminished as well [7]. As several ethical and practical issues arise from the use of BM and fetal stem cells, umbilical cord blood (UCB) has turned out to be an excellent alternative source of MSCs for clinical-scale allogeneic transplantation [8].

It was postulated that hyaluronic acid might facilitate the migration and adherence of MSCs to the defect, which might explain the occurrence of partial healing at 6 weeks in animals that were treated with hyaluronic acid alone. The repaired tissue in animals treated with hyaluronic acid alone was of inferior quality and was shown to deteriorate further after 12 weeks, so the combination between MSCs and hyaluronic acid will result in synergistic effect [9].

Aim of the Work: This study was carried out over a nine-month period of time, to study the effect of intra-articular injection of human umbilical cord blood (hUCB) mesenchymal stem cells (MSCs) in cartilage repair by histopathological and ultra structural assessment.

2. Materials and methods

The study was carried out on twenty albino rats of Wistar strain (adult males) during a nine-month period of time. They were brought from the medical research center, faculty of medicine, animal house of Ain Shams University. This study was approved by the local ethical committee.

The animals were maintained under conditions of controlled humidity, they were fed with commercial rat pellets and water. The animal house staff detected the age and weight of the animals and supervised their feeding. All animals were healthy and had no joint problems.

2.1. All animals were subjected to the following

Induction of cartilaginous defect in both knee joints by scratching the cartilage using a sterile needle [10,11]. In maximal flexion, longitudinal and diagonal grooves were made on the weight-bearing parts of femoral condyles without damaging the subchondral bone. The latter was checked by histology in 2 of the rats which were sacrificed; one of them after 1 week and the other after 4 weeks from the scratching which was done in the beginning of the experiment in order to ensure development of osteoarthritis (OA).

2.2. Preparation of MSCs from human umbilical cord blood (UCB)

2.2.1. Collection of UCB

Umbilical cord blood was obtained from the labor room of Obstetrics and Gynecology Department, Faculty of Medicine, Ain Shams University after written consent from the mothers.

4 UCB samples from full-term deliveries were collected from the unborn placenta using complete aseptic technique in sterile 15 ml Falcon tubes (*Nunclon*, *Germany*) containing 2 ml of acid citrate dextrose (ACD) anticoagulant (*Lonza*, *Switzerland*). The samples were stored at 22 ± 4 °C before processing [12]. Isolation and culture of MSCs were carried out in the medical research center, Faculty of Medicine, Ain Shams University as follows.

2.2.2. Isolation of mononuclear cells (MNCs)

Under complete asepsis each UCB sample was diluted 1:1 with phosphate-buffered saline (PBS) (lonza) and was carefully loaded onto Ficoll–Hypaque solution 2:1 ratio. After density gradient centrifugation at 2000 rpm for 30 min at room temperature, MNCs were removed from the interphase (the Buffy coat) and were washed three times with PBS; each time were centrifuged at 1500 rpm for 5 min, a clear cell pallet was formed in the bottom of the tube [13].

2.2.3. Culture of mesenchymal stem cells

The cells were cultured in complete culture medium; Dulbecco's modified Eagle's medium (DMEM) (*Lonza*, *Switzerland*) containing 12% fetal calf serum, 1% antibiotics – antimycotic; 100 units/ml of penicillin, 100 μg/ml of streptomycin and 250 μg/ml Amphotericin B. Cells were placed in 25 ml Falkon flasks (*Nunclon*, *Germany*). The flasks were incubated at 37 °C in 5% CO_2 (*NuAire*, *USA*).

On the fifth day of culture, non-adherent cells were discarded and adherent cells were examined microscopically for morphological evaluation of spindle shaped cells. The complete medium was changed and the flasks were reincubated. Flasks were examined every other day for MSCs (spindle shaped, fibroblast like cells).

After 2 weeks of culture, the adherent cells were almost confluent. Adherent cells were harvested using 0.25% trypsin for 5 min at 37 °C, 5 ml of medium was added to deactivate it. Cells were then counted with a haemocytometer. Cells were then collected in a 15 ml Falcon tube and centrifuged at