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Honey Bee Venom Will Differentiate Mesenchymal Stem Cells in to the Osteocyte

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Abstract

Umbilical cord (UC) is an important source of multipotential mesenchymal stem cells (MSCs). It has observed that bee venom (BV) is effective in survival and differentiation of the cells. We hypotheses that BV can cause differentiation of MSCs to osteocytes. The cells obtained from mouse UC tissues were digested and suspended in DMEM medium. After 24 hours, to induce osteogenic differentiation, cells were cultured for 14 and 21days respectively in DMEM medium contain different concentrations of BV (1,2,3,4,5,6 μgr/ml). Following the treatment, calcium's level in the cells was determined by Alizarin red staining. Cytotoxic effects of BV on MSCs were tested by MTT assay which are shown that BV inhibits MSCs growth. Furthermore, by Alizarin red test, we found that BV increases calcium level in MSCs on dose and time dependent manner. In conclusion we suggest that the MSCs from UC have differentiation potential to osteocyte under BV treatment and it may be useful in cell therapy.

Keywords: mesenchymal stem cells, Bee venom, osteogenic differentiation.

1. Introduction

Umbilical Cord (UC) is a rich source of multipotential mesenchymal stem cells (MSCs). MSC's are a type of multipotent adult stem cell that was originally described as early as the 1960's in animal experiments [1]. Many studies have demonstrated that MSCs have an enormous therapeutic potential for cell therapy [2]. These cells are also considered to have regenerating potential for certain degenerative conditions. MSCs can be differentiate into bone, adipose, cartilage, muscle, and endothelium if these cells are cultured under specific conditions MSC's is obtainable from placental blood, bone marrow, fatty tissue and amniotic fluid. It is also easily obtainable from the tissue of the cord itself [3,4]. In this study MSC's present in the UC were selected. UC is a low cost source of MSCs with multiple differentiation capacities and so it is a much better source than other sources, such as bone marrow or fatty tissue [5,6]. Bee venom (BV) has been used for the treatment of chronic inflammatory diseases such as rheumatoid arthritis and relief of pain in oriental medicine. More recently, studies indicate that BV has potential role in cancer therapy [7,8]. BV contains a variety of biologically active components like melittin and phospholipase A2

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(PLA2) [9]. Previous observations have shown that bee venom or its components are effective in proliferation, survival and differentiation of the cells [10]. In this view the aim of this study was the examination of differentiating potential of BV on MSCs. Our hypothesis is that BV could cause differentiation of MSCs to osteocytes.

2. Materials and methods

UC from 1-2 pregnant mice were obtained from animal lab unit in Kharazmi university which were killed by ether and the harvested pieces of tissue were washed several times in sterile phosphate-buffered saline (PBS) and then mechanically minced and enzymatically digested with 0.25% trypsin-EDTA (Gibco-Invitrogen) for approximately 10 min at 37 ˚C. After centrifugation (1500rpm for 10 min), cells were collected and cultured in Dulbecco's modified Eagle's medium (Gibco-Invitrogen) supplemented by 10% fetal bovine serum (FBS), 100 U/ml penicillin-streptomycin (Gibco-Invitrogen). Cell cultures were maintained at 37 ˚C with a water-saturated atmosphere and 5% CO_2. Medium was replaced one to two times every week. Three to 5 days after initiating incubation, to calculate the proper doses of BV that cause differentiation of MSCs and also have low cytotoxicity, the cells were cultured with different concentrations of BV (1–12 µgr/ml) for 24h. Cell viability then measured by MTT assay. In continue, to induce osteogenic differentiation, the cells were treated by different concentration of BV (4,5,6 µgr/ml) respectively for 14 and 21 days. After these times, osteogenic differentiation was analyzed by alizarin red staining.

3. Results

Based on MTT assay, results the IC_{50} values of BV for MSCs were 7.5µgr/ml after 24h (fig.1). Also the results showed that BV induces cell death in MSCs in high doses while, at low doses it could inhibit cell growth and induce differentiation. Maximum BV-induced cytotoxicity was evident after 24 h exposure to 15µgr/ml concentration. Our results from alizarin red staining illustrated that undifferentiated MSCs (control group) showed almost no specific staining. Osteocyte-like cells could be seen after 14 days of osteogenic induction (Fig.2). But after 21 days of cultivation the overall color intensity of the differentiated MSCs was markedly enhanced (Fig.3).

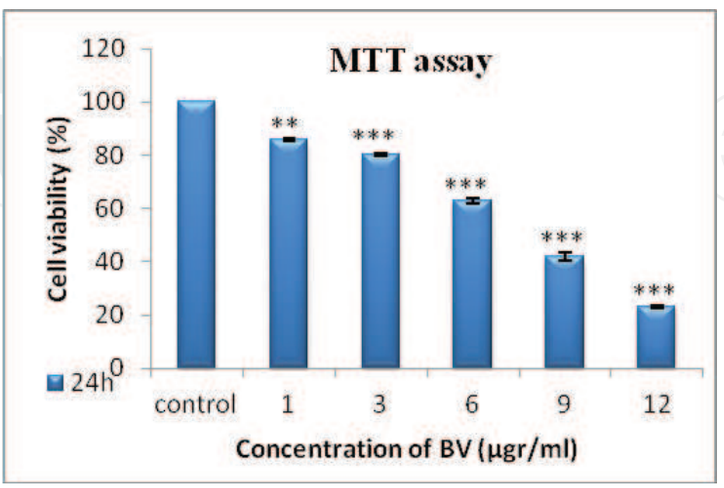

Figure 1. Effect of BV on cell viability. MSCs were treated with different concentration of BV for 24h.The cell viability was then determined by MTT assay, as described under Section 2. Data shown are the mean of three independent experiments ± SEM and are statistically significant in comparison to the control by one-way ANOVA.

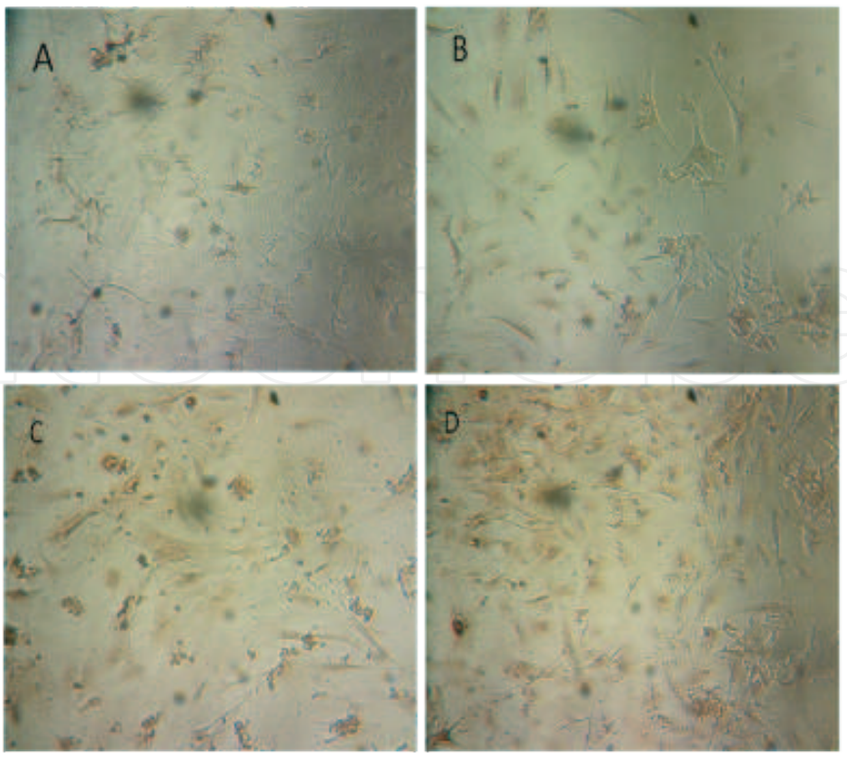

Figure 2. Light microscope micrograph of the alizarin red staining of MSCs following exposure to different concentrations of BV for 14 days. A: control group, B: 4µgr/ml BV treated cells, C: 5µgr/ml BV treated cells. D: 6µgr/ml BV treated cells (200x magnification)

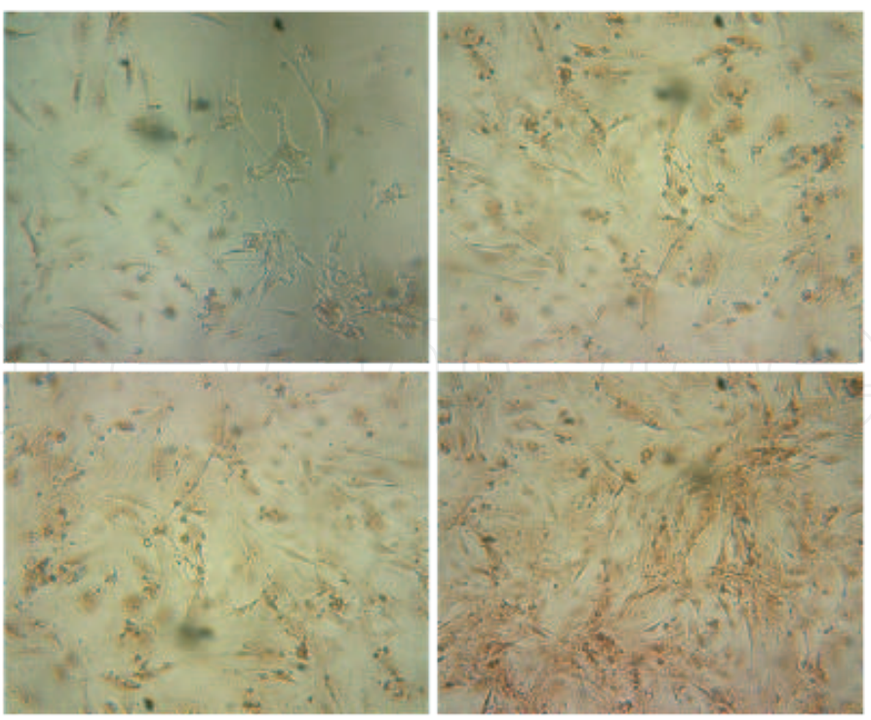

Figure 3. Light microscope micrograph of the alizarin red staining of MSCs following exposure to different concentrations of BV for 21 days. A: control group, B: 4µgr/ml BV treated cells, C: 5µgr/ml BV treated cells. D: 6µgr/ml BV treated cells (200x magnification)

4. Summary and conclusion

UC cells have many advantages because of the immaturity of newborn cells compared with other sources of stem cells. The UC appeared to be a source of fetal cells that could be easily used as multipotent stem cells [3]. Umblical cord derived mesenchymal stem cells can replicate stably in culture, possess the capability to differentiate into a wide variety of tissues after culture [5]. In this study we reported the osteogenic differentiation of MSCs, as characterised by alizarin red staining. By use of key markers of osteogenic differentiation (calcium deposition), we demonstrated that presence of BV can differentiate to osteocyte cells in the absence of osteogenic media. Osteogenic differentiation was greater when cultured-MSCs were treated with BV for 21 days rather than 14 days treatment. In conclusion, we suggest that BV can cause differentiation in the MSCs from UC and it may be useful in cell therapy.

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