THE EFFECTS OF β-MERCAPTOETANOL ON NEUROGENIC DIFFERENTIATION ABILITY OF ADULT HUMAN STEM CELLS: A VISUAL CONFIRMATION

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This preliminary study attempted in vitro protocols to isolate, culture, and induce human adipose tissue and teeth pulp derived mesenchymal stem cells (MSCs) into neuronal-like cells. MSCs were isolated from adipose tissue and teeth pulp, which were obtained with informed consent from UMMC and Dentistry clinical patients, respectively. Isolated MSCs were cultured and maintained in complete medium and incubated at 37°C. Media was replaced every three days and the cells were subcultured on reaching 70%-80% confluence. Passage 5 MSCs were initially treated for 24 hours with different low concentrations of treatment doses (0.6mM, 0.9mM and 1.2mM) of β-mercaptoethanol (β-ME) to induce neuronal differentiation. After 24 hours treatment, media was changed and replaced with fresh serum free media. MSCs were then treated with higher β-ME concentrations (6mM, 9mM and 12mM) for 3 hours. Adipose and teeth pulp derived MSCs showed spindle-shape or fibroblast-like morphology. Upon exposure to β-ME, these MSCs displayed neuronal-like phenotype as detected under phase contrast microscopy. Morphologically neuronal-like cells were observed; each having extended processes attached to the cell body ( soma). Thus, the findings propose the current protocol as suitable to differentiate human adipose tissue and teeth pulp MSCs into morphologically neuronal-like cells.

Keywords: neuronal human stem cells, β-mercaptoethanol, human adipose derived MSCs, human teeth pulp derived MSCs

INTRODUCTION

Stem cells are defined functionally as cells that have the capability to self-renew as well as the ability to generate differentiated cells [1]. The adult stem cells offer great opportunity for utilization of patients’ own cells from small samples of adult tissues for expansion and subsequent implantation without the ethical issues of embryonic stem cells [2].

Accumulated knowledge from bone marrow mesenchymal stem cells (MSCs) researches has enabled the isolation of stem cells populations from teeth pulp of human (TPMSCs), which exhibit similar properties to those of bone marrow MSCs including self-renewal capacity [3]. According to Anghiéri et al. (2008), adipose-derived mesenchymal stem cells (AMSCs) may also represent a valid alternative to bone marrow MSCs because of their ability to differentiate and quickly proliferate in vitro, in addition to being readily accessible [4]. Human MSCs including adipose (AMSCs) and teeth pulp derived mesenchymal stem cells (TPMSCs) may become important for stem-cell based regenerative therapies due to their multi-
lineage differentiation potential into various lineages of cells including neurons [5]. Pre-treatment of the stem cells by demethylating agents appears to be a good strategy to overcome deregulated differentiation of stem cells. Woodbury and his colleagues (2000) differentiated bone marrow MSCs into neuronal-like cells using β-mercaptoethanol (β-ME) as inducer [6]. In contrast to neuronal differentiation of bone marrow MSCs, only few studies have been conducted using β-ME for neuronal differentiation of human AMSCs and TMSCs [6, 7, 8]. Differentiation of adult stem cells into neuron and neural regeneration have the potential to benefit patients who have neurodegenerative health issues [6].

The current preliminary study attempted in vitro protocols to isolate, culture, and induce human AMSCs and TMSCs into neuronal-like cells.

**MATERIALS AND METHODS**

AMSCs and TPMSCs were separately isolated from abdominal region of cesarean surgeries and teeth pulp tissues of extracted teeth, respectively. Informed consents for the tissues were obtained from concerned specific patients of the University of Malaya Medical Centre (UMMC) and Dentistry clinic, respectively. The ethical committee has approved the harvesting of selected tissues. The isolated MSCs were then cultured and maintained in complete Dulbecco’s Modified Eagle Medium (DMEM) (Gibco, USA) and incubated at 37°C with 5% of CO2. The media was changed every three days to remove the non-adherent hematopoietic stem cells. MSCs were subcultured several times on reaching 70%-80% confluency. MSCs from passage 5 were initially treated 24 hours with different low concentrations of treatment doses (0.6mM, 0.9mM, and 1.2mM) of β-ME (Life Technologies, USA) in complete DMEM to induce neuronal differentiation. After 24 hours of treatment, the media was changed and replaced with fresh serum free media. MSCs were then treated with higher concentrations of β-ME (6mM, 9mM and 12mM) for 3 hours. Each culture work experiment was repeated three times in order to ensure reproducibility. The treated cells were observed under phase contrast microscope (Leica, Germany) for morphological analysis.

**RESULTS AND DISCUSSION**

AMSCs and TPMSCs were successfully isolated and sub-cultured up to passage 5 after reaching 70%-80% confluency. MSCs from adipose tissue and teeth pulp were analyzed and observed under phase contrast microscopy at different stage after and before treatment. Cultured MSCs were morphologically homogeneous, having spindle-shape and fibroblast-like appearance with the characteristic property of attaching to plastic culture dishes [Fig. 1].

![Fig. 1](image_url)

**Fig. 1:** Phase contrast morphology of primary culture of (A) human adipose (AMSCs), and (B) teeth pulp mesenchymal
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...stem cells (TPMSCs) after passage 5. AMSCs and TPMSCs were cultured in DMEM and sub-cultured every 18-20 days. They exhibited fibroblast-like morphology with the characteristic property of attaching to plastic culture dishes.

...contrast microscopy with (A) 0.6mM, (B) 0.9μL, and (C) 1.2mM of β-ME for 24 hours. AMSCs were morphologically changed; somas were larger in size and many with pyramidal shapes. Unconfirmed connections appeared to be made by the processes.

In contrast, no obvious morphological changes of TPMSCs into neuronal-like cells were observed after the first two initial low dose treatments. However, under 1.2mM dose the cells slightly changed morphologically from thicker fibroblast-like cells to thinner immature neuronal-like cells with small “dendritic spines” seen [Fig. 3]. Subsequently, AMSCs and TPMSCs exhibited obvious neuronal-like structures when treated with higher concentrations of β-ME in serum free medium after 3 hours [Fig. 4 and Fig. 5]. Unconfirmed connections seemed to be made by these extended processes, which resembled dendrites and axons.

**Fig. 2:** Morphological changes of treated adipose derived mesenchymal stem cells (AMSCs) after first treatment under phase contrast microscopy.
Fig. 3: Morphological changes of treated tooth pulp mesenchymal stem cells (TPMSCs) after first treatment with (A) 0.6 mM, (B) 0.9 mM, and (C) 1.2 mM of β-ME after 24 hours. Some adherent cells died, and the surviving cells proliferated, differentiated and started to have processes. No obvious differences, especially for those under the first two low doses.

Fig. 4: Morphology of AMSCs after second treatment with β-ME. AMSCs were treated for the second time with (A) 9 mM, and (B) 12 mM β-ME in free serum DMEM for 3 hours. AMSCs showed better differentiation into neuronal-like cells displaying close proximities of extended processes, which were attached to the cell bodies.

Fig. 5: Morphology of TPMSCs after second treatment with β-ME. TPMSCs exhibited neuronal-like cell with extended processes after second treatment with (A) 9 mM, and (B) 12 mM of β-ME for 3 hours in free serum DMEM, although less compared to AMSCs.

Numerous studies have been conducted using different inducers such as retinoic acid (RA), ascorbic acid (AA), and 5-azacytidine to evaluate the neuronal differentiation...
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potential of bone marrow MSCs [7, 8, 9]. Woodbury and co-workers (2000) have used β-ME (1-10mM) to differentiate human and rat bone marrow MSCs into neurons [6]. However, only few studies investigated the differentiation of human AMSCs and TPMSCs into neurons using β-ME [7]. In this study, we followed Woodbury protocol with some modifications in β-ME concentration, to differentiate human AMSCs and TPMSCs into neuronal lineage. The separate treatments by the selected inducer resulted in each type of MSCs having extended processes structures, which were similar to neuronal phenotype. However, further study is needed to distinguish the functions of these displayed extended processes; either having axonal or dendritic-like functions. Less neuronal-like cells were formed from TPMSCs as compared to AMSCs when treated with the same β-ME concentration. Among the different concentrations of β-ME, 9mM and 12mM had the best differentiation effect for both AMSCs and TPMSCs.

CONCLUSIONS

Visual confirmation in this study proved that human AMSCs and TPMSCs could be successfully isolated and differentiated into neuronal-like cells after treatment with β-ME in an in vitro protocols using complete DMEM with serum for the first treatment and incomplete DMEM without serum for second treatment.

REFERENCES