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Research Report

Induction of adipose-derived stem cell into motoneuron-like cells using selegiline as preinducer

*Alireza Abdanipour, Taki Tiraihi**

Department of Anatomical Sciences, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran
Shefa Neurosciences Research Center, Khatam Al-Anbia Hospital, Tehran, Iran

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ABSTRACT

Cell therapy is one of the approaches taken to treatment of spinal cord disorders. In this study, adipose-derived stem cells (ADSCs) were induced to form motoneuron-like cells (MNLCs) using selegiline as preinducer, as well as Shh and all trans-retinoic acid (RA) as inducers. Selegiline was reported to induce the embryonic stem cells and bone marrow stromal cells into neuronal phenotype. ADSCs were evaluated using CD90, CD44, CD 49d, CD106, CD31, CD45, lipogenesis and osteogenesis. Dose response and time course studies were used in selecting the optimal concentration for selegiline using the percentage of viable cells (PVC) and percentages of immunoreactive cells (PIC) to nestin and neurofilament 68. Accordingly, such studies were used in selecting the optimal dose for RA using PVC and PIC to islet-1 and oligo-2. The expression of islet-1, oligo-2 and HLXB9 was evaluated using RT-PCR and immunocytochemistry. Real-time PCR was utilized in order to quantify the expression of islet-1, oligo-2 and HLXB9. ADSCs were immunoreactive to CD90, CD44 and CD 49d with consistent differentiation osteogenic and lipogenic cells. The optimal concentrations of selegiline and RA were 10^{-9} mM and 2×10^{-8} M, respectively. After two days, MNLCs showed high oligo-2 expression. MNLCs innervated myotubes; also, the release rate of synaptic vesicles using FM1-43 followed exponential decay model, and this rate in the induced MNLCs was approximately three times of that in the preinduced cells.

1. Introduction

Cell therapy is one of the options for ameliorating the locomotive deficits in spinal cord trauma and neurodegenerative diseases (Hedlund et al., 2007; Lindvall and Kokaia, 2010). Several types of cells have been so far considered for transplantation, such as embryonic stem cells (ESCs), fetal derived cells, umbilical vein derived stem cells and adult stem cells (Boncoraglio et al., 2010). Motoneuron transplantation was suggested in the treatment of several diseases such as amyotrophic lateral sclerosis and spinal cord injury (Clowry et al., 1991; López-González et al., 2009; Nayak et al., 2006; Nógrádi and Szabó, 2008; Thonhoff et al., 2009). Motoneurons were generated from ESCs and inducible pleuripotent stem cells (iPSCs) (Nizzardo et al., 2010; Ronaghi et al., 2010), several authors reported the potential tumorogenesis in these transplants (Asahina et al., 2006; Fong

* *Corresponding author at:* Department of Anatomical Sciences, School of Medical Sciences, Tarbiat Modares University, P.O. Box 14155-4838, Tehran, Iran. Fax: +98 21 801 6544.
E-mail addresses: takialtr@modares.ac.ir, ttiraihi@gmail.com (T. Tiraihi).

Abbreviations: ADSCs, adipose-derived stem cells; MNLCs, motoneuron-like cells; PVC, percentage of viable cells; PIC, percentages of immunoreactive cells; NSCs, neural stem cells

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et al., 2010; Kooreman and Wu, 2010). Also, motoneurons reported to be derived from fetal spinal cord (Guo et al., 2010) and forebrain of the embryo (Currle et al., 2007), however, there is certain limitation in the use of these cells (Piacibello et al., 1998; Tung et al., 2010). Neural stem cells derived from iPSCs and ESCs were used to generate motoneurons (Robertson et al., 2008; Schwartz et al., 2008), but these sources had ethical limitation and transplant rejection (Kabos et al., 2002). Adult stem cells are another source for motoneurons, for example adipose derived stem cells were induced to transdifferentiate into motoneurons using isobutylmethylxanthine and ß-mercaptoethanol (BME) as preinducers (Liqing et al., 2011). While BME was reported to be associated with sister chromatin exchange (Speit et al., 1980), selegeline was documented as a safe and, efficient and potent inducer for the bone marrow stromal cells (Ghorbanian et al., 2010). Moreover, Zavan et al. (2010) confirmed that the adipose tissue was an enriched source for stem cells with neural differentiation potential. In this study, we tried to induce the adipose tissue derived stem cells into motoneuron-like cells using selegiline as preinducer.

2. Results

2.1. *Preparations of adipose derived stem cells (ADSCs)*

The primary culture of the isolated ADSCs is presented in Fig. 1-A. They were cultured and the adherent cells became homogenous after 4 passages (see Fig. 1-B). The cells were maintained up to 15 passages. The cells isolated from the 4th passage were induced to differentiate into osteogenic and lipogenic phenotypes (Figs. 1-C and D, respectively). They were immunostained with anti-CD90, CD44 (both markers of mesenchymal stem cells) and CD49d (specific marker for fat cells) (see Figs. 1: E–G, respectively). Fig. 1 (I–H) presents the cells negatively immunostained with CD106 (a marker of mesenchymal stem cells derived from bone marrow stromal cells), CD31 (an endothelial cell marker) and CD45 (a hematopoietic cell marker). The percentage of immunoreactive cells to MSCs markers was 95%.

2.2. *Preinduction*

A combined histogram was drawn for the results of the dose response of selegiline (10^{-6}, 10^{-9}, 10^{-12} and 10^{-15}) using the time course of 3, 6, 12, 24 and 48 h in order to find the optimal concentration and time for preinduction, and the criterion for the selection was the viability of transdifferentiation of ADSCs into neuronal phenotype (Fig. 2-A). The highest percentage of viable cells (98.52%) was at concentration of 10^{-15} mM, which was incubated for 3 h; it is significantly higher than those of the other time points used in the study. The highest percentage of the immunoreactive cells to nestin in the preinduced ADSCs (65.4%) was at concentration of 10^{-9} mM incubated for 24 h, which was significantly higher than 10^{-6}, 10^{-12} and 10^{-15} mM at all other time points used in the study (Figs. 3 A). Accordingly, the highest percentage of the immunoreactive cells to neurofilament 68 (72.4%) was at the concentration of 10^{-9} mM which was incubated for 24 h; and the highest percentage of neural-like cells (63.8%) was at concentration 10^{-9} mM which was incubated for 24 h (Figs. 3B and C, respectively).

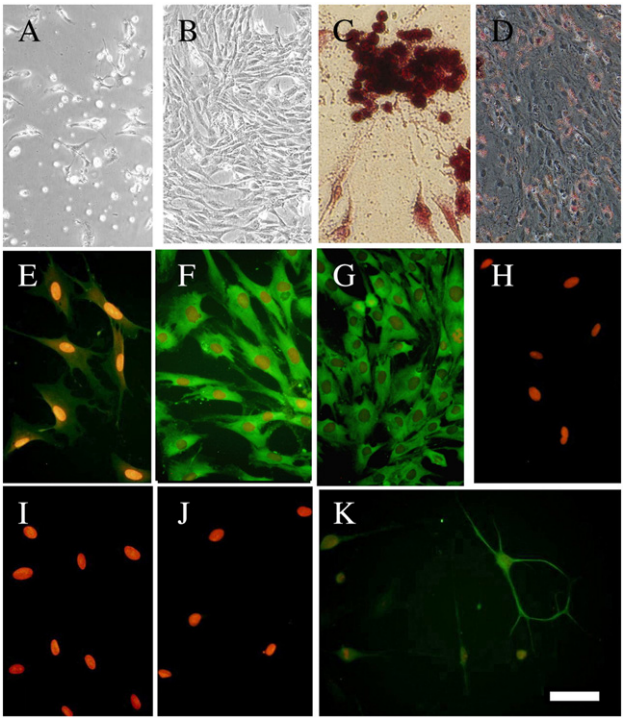

Fig. 1 – Demonstrates a phase contrast image from the adipose derived stem cells' (ADSCs) isolation, differentiation and immunostaining for different markers with the purpose of characterizing these cells. A: represents the primary culture (of 24 h) of the perinephric fat digested with collagenase I, which shows many fat globules in the culture medium. B: illustrates a phase contrast image of the 4th passage (15 days) of primary culture of ADSCs, which shows adherent spindle shape cells. C: represents the osteogenic differentiation of the cultured ADSCs stained with Alizarin red stain. D: represents the lipogenic differentiation of the cultured ADSCs stained with Oil red stain (scale bar, A, B and D: 120 and C: 75 μM). E, F and G represent the immunostaining of CD90, 44 and 49d (primary antibodies), respectively; they are markers for fat-derived mesenchymal stem cells (CD90 and 44) and fat cell specific marker (CD 49d). H, I and J represent immunostaining of CD106, 31 and 45 (primary antibodies), respectively; they are markers for mesenchymal stem cells derived from bone marrow stromal cells (CD106), endothelial cells (CD31) and hematopoietic stem cells (CD45). K presents the immunostaining of the induced ADSCs with anti-acetyl choline transferase antibody (primary antibody). The cells were immunolabeled with primary antibody, incubated with FITC conjugated secondary antibody, and counter-stained with ethidium bromide (E–K) (scale bar: 68 μm, all).

2.3. *Induction*

The induction was achieved with Shh at concentration of 1 μg/ml with RA, time course of (two days, one week and two weeks) at doses of (2×10^{-5}–2×10^{-9} M), in order to select the