Original Article

Effect of sertraline on proliferation and neurogenic differentiation of human adipose-derived stem cells

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Abstract

Background: Antidepressant drugs are commonly employed for anxiety and mood disorders. Sertraline is extensively used as antidepressant in clinic. In addition, adipose tissue represents an abundant and accessible source of adult stem cells with the ability to differentiate in to multiple lineages. Therefore, human adipose-derived stem cells (hADSCs) may be useful for autologous transplantation.

Materials and Methods: In the present study, we assessed the effect of antidepressant drug Sertraline on the proliferation and neurogenic differentiation of hADSCs using MTT assay and immunofluorescence technique respectively.

Results: MTT assay analysis showed that 0.5 μM Sertraline significantly increased the proliferation rate of hADSCs induced cells ($P < 0.05$), while immunofluorescent staining indicated that Sertraline treatment during neurogenic differentiation could be decreased the percentage of *glial fibrillary acidic protein* and Nestin-positive cells, but did not significantly effect on the percentage of MAP2 positive cells.

Conclusion: Overall, our data show that Sertraline can be promoting proliferation rate during neurogenic differentiation of hADSCs after 6 days post-induction, while Sertraline inhibits gliogenesis of induced hADSCs.

Key Words: Adipose-derived stem cells, Antidepressant drug, Neurogenic differentiation, proliferation, Sertraline

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INTRODUCTION

Depression is the most prevalent mood disorder. It suffers about 21% of the world population[1] and these disorders treat with antidepressants drugs.[2]

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Several studies have shown that antidepressants increase hippocampal neurogenesis in both animals and humans[3-5] and increase neuronal cell proliferation *in vitro*.[1,6]

Sertraline is an antidepressant drug from selective inhibitor of neuronal serotonin reuptake group.[7-10] Sertraline in the treatment of major depressive disorders[11] such as obsessive compulsive disorder[12] panic disorder[13] and post-traumatic stress disorder[14] is effective. This drug acted by elevating the concentration of monoamines,[15] some dopamine reuptake inhibitors[16] and inhibition of neuronal serotonin reuptake in the synaptic cleft of the central nervous system. Some reports showed that

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elevation of serotonin concentration in synaptic cleft cause to maintain mental balance.[17] Furthermore, rapid increase of monoamines in extracellular levels requires several weeks or months.[18,19]

Decrease of monoamine resulting a decline in hippocampal neurogenesis while elevation of serotonin and/or norepinephrine lead to increase in hippocampal neurogenesis, so this mechanism lead to consider Sertraline as an antidepressant in clinic.[2,20]

Mesenchymal stem cells (MSCs) have been isolated from different sources including bone marrow, muscle and adipose tissue, these cells can also differentiated to chondrocytes, adipocytes, myoblasts and osteoblasts *in vitro*.[21-23] and use for autologous transplantation.[24,25]

Several factors limit the use of MSCs-derived bone marrow, including: Morbidity, painful procedure and gain a few number cells. HADSCs can be isolated more easily than the other type of MSCs have a significant number of cells from fat tissue and less invasive method; thus, adipose stem cells have important criteria in compare with other source.[26]

It has been shown that human adipose-derived stem cells (hADSCs) can differentiate into several linage including: Chondrocyte, endothelial, adipocyte, cardiomyocyte[26-28] and also neurogenic cells that show neuron-like morphology and express neural markers.[28-31]

On the basis of these findings, it was interest to determine the influence of antidepressant drug sertraline on the proliferation rate and differentiation of hADSCs into neural lineage.

MATERIALS AND METHODS

Tissue collection and isolation of hADSCs

Samples of adipose tissue were obtained from three patients during abdominoplasty surgery, after receiving informed consent. HADSCs were isolated as previously described.[32]

Briefly, adipose tissues were washed several time with sterile phosphate-buffered saline (PBS) to remove contaminating debris and red blood cells. Then adipose tissue was enzymatically dissociated with 0.075% collagenase in PBS for 30 min at 37°C. The collagenase was neutralized with an equal volume of Dulbecco's modified Eagle's medium: F12 (DMEM: F12)/10% fetal bovine serum (FBS) (Gibco, BRL, Paisley, UK) and centrifuged for 10 min at 1200 g. The cellular pellet was resuspended in growth medium (DMEM: F12/10%FBS and 1% penicillin/streptomycin solution). Cell cultures were maintained in T25 flasks for 4-5 days in a 37°C incubator with 5% CO_2 until they confluent. Then, ADSCs were passaged at the ratio of 1:3.[32]

All chemicals, except where specified otherwise, were purchased from Sigma-Aldrich (St. Louis, MO).

In this study, after isolated ADSC from human abdominal fat, these cells differentiated to neuron-like cells and simultaneously treated with Sertraline as an antidepressant drug. MTT assay and immunofluorescent staining were used for assessment of proliferation rate and evaluation of neural markers status using neurogenic differentiation of hADSCs.

Neurogenic differentiation of hADSCs

For neural induction, hADSCs passage 3-6 was used. HADSCs were plated in low attachment plastic tissue culture dishes in culture medium that contained: DMEM: F12, 2% B27, supplemented with 20 ng/ml basic fibroblast growth factor and 20 ng/ml epidermal growth factor. This media renewed every 2 days up to 6-7 days.

After replating the neurospheres dissociated cells on cover slips in a 24-well plate at a density of $2 \times 10^4/cm^2$, incubated in neurobasal medium supplemented with 5% FBS, 1%l-glutamin, 1% none essential amino acids, 1% N2 supplement and 2% B27 for 1 week (the growth factors and supplements are all from Gibco BRL, Paisley, UK).[32]

Depending on the purpose of the experiments, the cells were cultured in neural induction medium with or without of 0.5 μM Sertraline in treated and control groups respectively for 7 days.

MTT assay

For assessment of cell viability and proliferation rate in Sertraline-treated neuronal precursor cells, MTT assay was performed at 2, 4 and 6 days after Sertraline treatment. Differentiated cells with PBS were washed; The MTT assay was performed as previously described.[32] Briefly, 2×10^3 cells/well, were seeded on 96-well plates and grown in the presence of Sertraline at 0.5 μM or absence of Sertraline. 100 μl of DMEM and 10 μl of a MTT solution (0.5 mg/ml) were added to each well and incubated for a 4 h. The MTT solution was removed from cell cultures and 100 μl of dimethyl sulfoxide added to extract the MTT formazan. The absorbance of each well was measured by microplate reader at 540 nm.[32]

Immunocytochemistry staining

The induced cells were fixed in 4% paraformaldehyde for 20 min at room temperature, after rinsed twice with PBS, the cells permeablized using PBS containing 2%

Triton X-100 at room temperature for 30 min. primary antibodies diluted in blocking solution consistent 10% goat serum and 1 mg/ml bovine serum albumin (BSA) for 2 h at 37°C. Then, the cells were incubated with primary antibodies overnight at 4°C in the dark. After washing with PBS, the cells were incubated with fluorescein isothiocyanate (FITC) conjugated secondary antibodies for 2 h at 37°C. The differentiated cells were reacted with antibodies against mouse anti-Nestin (1:300, Abcam, Cambridge, MA, USA), mouse anti-microtubule-associated protein 2 ([MAP2], 1:300, Abcam, Cambridge, MA, USA), mouse anti-glial fibrillary acidic protein ([GFAP], 1:600, Abcam, Cambridge, MA, USA)and antimouse FITC-conjugated immunoglobulin antibody (1:500, Abcam, Cambridge, MA, USA).For quantitative analysis, the cells were incubated with 4, 6-diamidino-2-phenylindole ([DAPI] 1:1000) for 2 min at room temperature. Preparations were examined using a fluorescence microscope (Olympus BX51, Japan). The numbers of positive cells for each antigen were counted as percentages of the total DAPI-stained cell population. For quantitative assessment of cell differentiation in control and Sertraline treated cells, the relative numbers of cells expressing different markers like mature neurons (MAP2), astrocytes (GFAP) and neural progenitor cells (Nestin) were counted as percentages of the total DAPI-stained cell population. Image J software was used for merging the pictures.[32]

Statistical analysis

Cell proliferation and neural differentiation data were analyzed using one-way analysis of variance (ANOVA) (SPSS Inc., Chicago, IL). All data were shown as means ± standard error of the mean. Experiments with two groups were subjected to a one-way ANOVA, $P < 0.05$ was taken as significant and $P < 0.001$ was taken as highly significant to indicate levels of statistical significance.

RESULTS

Morphologic changes of hADSCs following neurogenic differentiation

To assessment changes in cell morphology following Sertraline treat, we analyzed morphology of neurogenic induced cells for 2, 4 and 6 days. Images were viewed using bright field and phase contrast microscopy (Nikon Eclipse TS100). After two or three passages hADSCs appeared by their spindle-shaped fibroblastic morphology [Figure 1a].

Then, cells were cultured in induction medium for 7 days, the cells floated in suspension as small aggregates (neurospheres) [Figure 1b].

After 9 days treated with Sertraline, the induced cells forming contracted cell bodies with long cytoplasmic processes [Figure 1c] and the cell bodies became bipolar and multipolar appearance on 10 day [Figure 1d].

Effect of Sertraline on proliferation rate

To investigate whether Sertraline affects the proliferation of isolated ADSCs, cell proliferation assay was carried out by MTT assy. The cells exposed to 0.5 μM Sertraline for 2, 4 and 6 days, there were no significant difference mean of absorbance of Sertraline in treated group as compared with the control group for 2 and 4 days post-induction. While, in compared with the control group, exposure to 0.5 μM Sertraline resulted in a significant increase of cell proliferation after 6 days ($P = 0.02$) [Figure 2].

Effects of Sertraline on neurogenic differentiation of hADSCs

Evaluation of the percentage of neural markers, Nestin, GFAP and MAP2 determined by immunofluorescence technique. [Figure 3] indicated that after treatment with 0.5 μM sertraline the induced cells were not significant difference for MAP2 positive cells, in comparison with the control group, while the mean percentage of GFAP positive cells was significantly decreased in Sertraline treated group relative to the control group ($P < 0.05$).

Quantification of immunostaining revealed that 2.6 ± 1.2% of inducing treated cells were positive for Nestin, which was not significantly different from the control group (10 ± 3.7%). Immunostaining results showed the mean percentage of GFAP-positive cells was 2 ± 1.1 in Sertraline treated group compared with 32 ± 18 in the control group, which demonstrated that GFAP decreased significantly in treated group. Finally, the mean percentage of MAP 2 positive cells was 36 ± 28 near to the control group (38 ± 31) [Figure 4].

DISCUSSION

Previous studies have shown that antidepressants drugs increase neural cell proliferation and enhance differentiation in neural precursors derived from human embryonic stem cells.[1,19,33] Antidepressants also increase proliferation and differentiation in hippocampal progenitor cell both *in vivo* and *in vitro*.[5,17,34-37]

Several studies showed that hippocampus undergoes decrease of size and neuron numbers in stress condition.[38-42] Therefore, commonly use of antidepressants are useful in depression.[43,44]

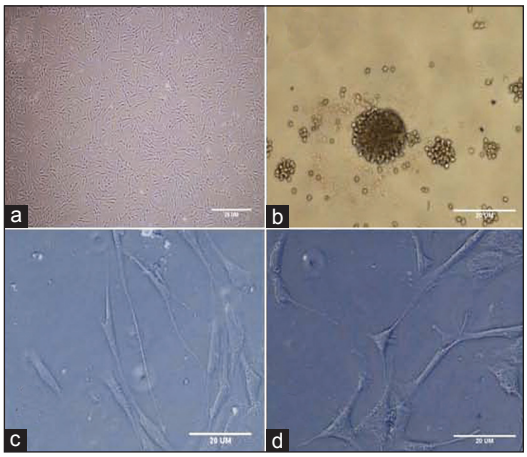

Figure 1: Morphological changes of adipose-derived stem cells following differentiation with 0.5 μM Sertraline *in vitro*. (a) Undifferentiated adipose-derived stem cells. (b) After 6-7 days, cultured in neurogenic induction medium. The cells were exhibited sphere shape. (c) After 9 days, the induced cells with 0.5 μM sertraline forming contracted cell bodies with long cytoplasmic processes. (d) After 10 days, the cell bodies became bipolar and multipolar appearance

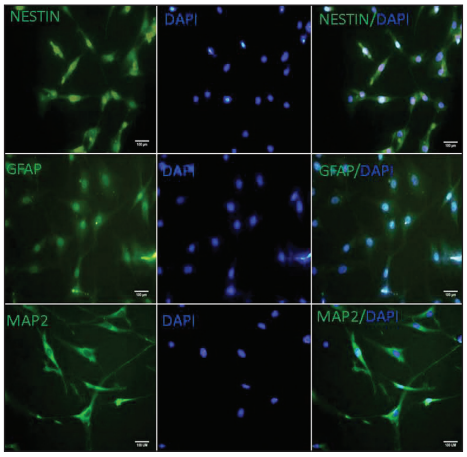

Figure 3: Immunocytochemical staining for specific markers in neurogenic differentiated cells (Netin, GFAP and MAP2) treated with 0.5 μM Sertraline. In each experiment, the nuclei were counterstained with6-diamidino-2-phenylindole. Scale bar = 100 μm

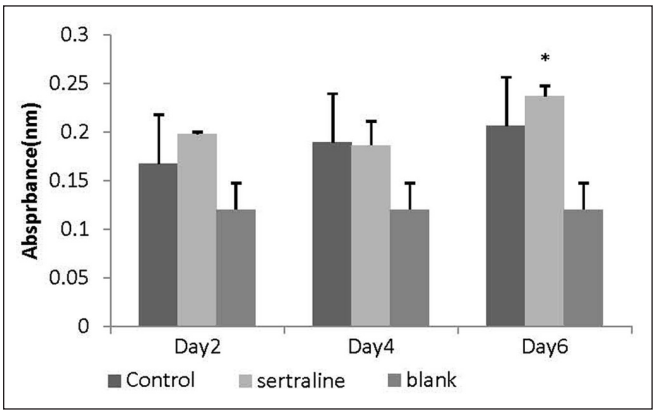

Figure 2: Determination of the absorbance for neuronal precursors treated with 0.5 μM Sertraline in order to assessment of proliferation rate at 540 nm in 2, 4 and 6 days after induction. Compared with the control group, exposure to 0.5 μM Sertraline resulted in a significant increase of cell viability after 6 days (*$P \leq 0.05$)

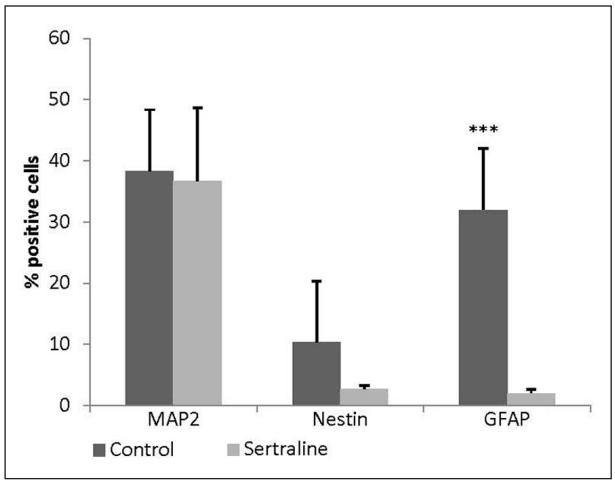

Figure 4: The mean percentage of immunoreaction positive cells for Nestin, microtubule-associated protein2 (MAP2) and glial fibrillary acidic protein (GFAP) in neuronal precursors treated with 0.5 μM Sertraline in compared with the control group. The mean percentage of MAP2-positive cells was close to control group. While, the mean percentage of Nestin-positive cells was decreased in the Sertraline treated group compared with the control group, but the mean percentage of GFAP positive cells in the treated group significantly decreased relative to the control group (***$P \leq 0.001$)

ADSCs have been defined on cells with the capacity to differentiated in to multiple cell lineage, furthermore, these cells can be extracted from donor by a safety procedure.[45] Therefore, these cells can be the suitable candidate for neurogenesis in neurodegenerative diseases.[46-48]

Previous investigate showed that Sertraline increased cell proliferation in the human hippocampus, also this study demonstrated that Sertraline could promotes differentiation of neural stem cells (NSCs), which dissociated from hippocampus to neurons, but inhibits its differentiation to glial cells.[35]

In vitro culture of NSCs from the hippocampus of fetal rats was demonstrated that sertraline could not increase proliferation and viability of NSCs. Furthermore, by decreasing the expression of proinflammatory cytokine, Sertraline might provide neuroprotection.[17]

Sutcigil *et al*. showed that Sertraline treat might have decline in the proinflammatory cytokine *interleukin (*IL)-12 and elevate transforming growth factor beta (TGF-β1) and anti-inflammatory cytokines IL-4, which can influence neurogenesis widely.[49]

We showed that 0.5 μM of Sertraline increased the proliferation rate. Our results were disagreement with the findings of Peng *et al*.,[17] which showed that 1 μM of Sertraline cannot effect on proliferation of cells in compare with the control group. They confirm that high concentrations of Sertraline (20 μM and 50 μM) inhibit the proliferation of ADSCs.

Previous studies showed that other antidepressants, such as fluoxetine (1 μM),[2,19,50] imipramine, venlafaxine (1 μM)[2] and paroxetine (1-5 μM)[37] increased the proliferation rate of with different sources.

Furthermore, Peng *et al*. demonstrated that Sertraline induces NSCs to differentiate into neurons, but inhibits its differentiation to glial cells. These different in findings may have resulted from differences in cell lines, concentration and type of drugs or the duration of treatment.[2,17,19,37,50]

Our results could be due to Sertraline by decreasing proinflammatory cytokines induced cell proliferation and through TGF-β1 could be inhibit gliogenesis.

Overall, results of our study show that sertraline can be enhancing proliferation rate during neurogenic differentiation of hADSCs. However, Sertraline treatment can be decreased glial markers (GFAP+) and neural progenitor cells (Nestin+) as compared with the control group, but there were no significant difference in the expression of mature neuron marker (MAP2+) between control and treated groups. However, the molecular mechanisms of Sertraline on ADSCs proliferation and differentiation are not known yet. Moreover, the mechanisms and factors effective of Sertraline function needs to be further investigated.

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