Available online at SciVarse ScienceDirect

Chinese Herbal Medicines (CHM)

ISSN 1674-6384

Journal homepage: www.tiprpress.com E-mail: chm@tiprpress.com

Original article

Protective Effects of Hydroxysafflor Yellow A against Oxidative Damage of β-Mercaptoethanol During Neural Differentiation of Mesenchymal Stem Cells

Xiao-qing Song[1], Li-ning Su[1], Hui-ping Wei[1*], Ying-hui Liu[2], Hai-feng Yin[1]

1. Basic Medical College, Hebei North University, Zhangjiakou 075000, China

2. Agriculture and Forestry College, Hebei North University, Zhangjiakou 075000, China

ARTICLE INFO

Article history

Received: January 19, 2017

Revised: Fabruary 25, 2017

Accepted: April 1, 2017

Available online:

July 17, 2017

DOI:

10.1016/S1674-6384(17)60105-9

ABSTRACT

Objective To study the protective effects of hydroxysafflor yellow A (HSYA) against the oxidative damage caused by β-mercaptoethanol (BME) during neural differentiation of mesenchymal stem cells (MSCs) *in vitro*. **Methods** When the confluence reached 50%-60%, 4th passage MSCs were divided into three groups to culture. G1: normal group which was cultured using basic medium (DMEM containing 10% FBS all the time); G2: unprotected group which was continuously cultured using basic medium for 24 h, and then cultured using pre-induction medium (DMEM containing 10% FBS and 1 mmol/L BME); G3: protected group which was firstly cultured using protective medium (DMEM containing 10% FBS and 160 mg/L HSYA) for 24 h, and then cultured using pre-induction medium for 24 h. After these treatments as above, cell viability, relative levels of SOD/GSH and apoptosis rate were respectively detected. The expression of Bcl and Bax was examined by Western blotting. After HSYA protection and BME pre-induction, neural induction was performed. The expression of NSE and MAP-2 was respectively analyzed on cellular and molecular levels. **Results** Compared with unprotected group, 160 mg/L HSYA could obviously improve cells viability, maintain high level of SOD and GSH in MSCs, reduce apoptosis rate and improve the ratio of Bcl/Bax. After protection with 160 mg/L HSYA, the survival time of neuron-like cells could be extended. Immunocytochemical staining showed that after 10 h of neural induction, the differentiated neuron-like cells in protected group were still in a good state, and the mRNA levels of NSE and MAP-2 were increased during the induction course checked. **Conclusion** HSYA could improve the resistance of cells to the oxidative damage caused by BME.

Key words

hydroxyl safflower yellow A; β-mercaptoethanol; mesenchymal stem cells; neural differentiation; protective effects

* **Corresponding author: Wei HP** E-mail: whp123456@sina.com

Fund: Major Projection of Hebei North University (No. ZD201413)

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss of substantia nigra dopaminergic neurons, which leads to chronic dyskinesia and severely affect the life quality of patients and their family. Replacing lost cells with the differentiated neurons in a good state may be an increasingly promising method to repair the damaged brain neurons. Nowadays, many classes of cells, such as embryonic stem cells (Montzka et al, 2009) and neural stem cells (Lee et al, 2014) can be induced into neurons. However, tissue resource limitations and socio-ethical problems restrict their clinical application. Mesenchymal stem cells (MSCs) are one type of adult stem cells characterized by self-replication and multipotential differentiation. For they are very easily obtainable (Kim et al, 2014; Jin et al, 2015; Han et al, 2015) and have no significant immunogenicity (Shi et al, 2012), MSCs have been regarded as attractive candidates in clinical application for the repair or regeneration of damaged tissues.

So far, MSCs have been applied in neural differentiation with different agents, such as bone morphogenetic proteins (BMPs) (Tio et al, 2010; Woodbury et al, 2000), all-trans retinoic acid (RA) (Jin et al, 2015), brain-derived neurotrophic factor (BDNF) (Shi et al, 2012), traditional Chinese medicines (Huang et al, 2013; Ruan et al, 2015; Fu et al, 2014) and so on. Compared to others, the neural induction method using BME combined with growth factors (such as bFGF or EGF) was almost a typical one. High differentiation rate is its advantage, but the survival time of neuron-like cells differentiated from it was shorter than others. So a new method which can rapidly induce MSCs into desired neurons with a long-term survival rate is urgently needed. In order to improve cell activity, Chinese scholar Xu et al (Xu et al, 2010) used grape seed proanthocyanidins to protect MSCs before BME induction treatment, and the results showed that the protective effects was very significant. In view of the protective effects of hydroxysafflor yellow A (HSYA) on nerve injuries (Lu et al, 2008; Zhu et al, 2005; Yang et al, 2010), we used DMEM containing 160 mg/L HSYA (Lu et al, 2008) to culture MSCs before BME treatment to explore the protective effects of HSYA against oxidative damage caused by BME and to obtain the highly differentiated neuron-like cells.

2. Materials and methods

2.1 Isolation and culture of MSCs

In our experiment, 4-week-old male SD rats (purchased from Animal Center of Hebei North University and treated under experimental conditions following the National Institutes of Health Guide for the Care and Use of Laboratory Animals) were anesthetized through ip injection of 0.5 g/kg chloral hydrate. The femurs were flushed to harvest MSCs with basic medium L-DMEM (GIBCO) containing 10% FBS (Gibco) using 5 mL disposable syringes. After being stirred repeatedly, isolated cells were planted in 25 cm^2 culture flasks for culture. The culture conditions of cell incubator were maintained at 37 ^oC in a humidified atmosphere plus 5% CO_2. After 48 h of culture, the medium was replaced with the fresh and later changed every 72 h. When the cell confluence reached 80%−90%, 0.05% (w/v) trypsin in 0.1% (w/v) EDTA was used to trypsinise cells. The cell suspension was divided into two flasks for further culture until 4th passage.

2.2 Determination of cell-surface antigens by FCM

Flow cytometry (FCM) was carried out to analyze the surface markers of MSCs. The density of 4th passage MSCs was mediated to about 10^6 cells/mL; Then 10 μL rat-specific antibodies: CD29, CD34, CD45 and CD90 (BD Pharmingen) were respectively added into the bottom of tubes in which MSCs were contained. The mixture was kept at room temperature for 30 min in the dark, and positive cells were detected by FCM. Rat IgG_1 (BD Pharmingen) were taken as isotype controls.

2.3 Detection of cell viability and apoptosis

MSCs with 10^5 cells/mL density were seeded into 96-well plate and cultured using basic medium at 37 ^oC in cell incubator containing 5% CO_2. When the cell confluence reached 50%−60%, all wells containing MSCs were divided into three groups, G1: normal group cultured in basic medium all the time; G2: unprotected group continuously cultured in basic medium for 24 h, and then cultured in pre-induction medium (L-DMEM containing 10% FBS and 1 mmol/L BME) for 24 h; G3: protected group firstly cultured in protective medium (L-DMEM containing 10% FBS and 160 mg/L HSYA) for 24 h, and then cultured in pre-induction medium for 24 h.

After the treatments mentioned as above, cell viability of all groups was measured by MTT assay (Wang et al, 2001). In brief, the culture medium was replaced with 5 mg/mL MTT. After 4 h of incubation at 37 ^oC in cell incubator, MTT medium was removed and the cells were washed for three times with PBS, and 150 μL DMSO was subsequently added to each well containing cells to solubilize the formazan. The absorbance of each well was measured at 570 nm by ELISA reader. In addition, in order to further check whether HSYA could lower the rate of cell apoptosis or not, double fluorescent staining was performed. That is, the cells of these three groups were respectively stained with Hoechst 33258 and Propidium Iodide (PI) to count the rate of cell apoptosis.

2.4 Measurement of SOD and GSH

To assess the anti-oxidative ability of MSCs, the relative levels of two main antioxidases SOD and GSH were measured. In short, the cultured MSCs were firstly planted into 6-well plate to culture until 50%−60% confluence. Then, the treatments following the methods mentioned in "2.3"