Trehalose Activates Autophagy and Prevents Hydrogen Peroxide-Induced Apoptosis in the Bone Marrow Stromal Cells

Shahram Darabi a*, Ali Noori-Zadeh b, Hojjat Allah Abbaszadeh c, d and Farzad Rajaei a

a Cellular and Molecular Research Center, Qazvin University of Medical Science, Qazvin, Iran. b Department of Clinical Biochemistry, Faculty of Paramedicine, Ilam University of Medical Sciences, Ilam, Iran. c Hearing Disorders Research Center, Loghman Hakim Medical Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. d Department of Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Abstract

Bone marrow stromal stem cells (BMSCs) play a significant role in cell therapy. These cells quickly die after transplantation to the affected area due to oxidative stress. The natural disaccharide, trehalose which can be known as autophagy inducer. The present study aimed to investigate the role of trehalose in preventing BMSCs from oxidative stress caused by H2O2. BMSCs were isolated from the adult rats. The cells were divided into three groups: (a) control; (b) 100 µM H2O2; (c) 100 µM H2O2 and trehalose 3%. The morality rate was analyzed by viability test. Immunocytochemistry and Western blot was used in order to evaluate p62 protein and LC3II/LC3I ratio, respectively. In order to evaluate apoptosis, cleaved caspase-3 protein was used. In viability test, the survival rate for BMSCs after 8 h were 82%, 72%, 49%, and 39% (for groups who received 50, 100, 200, and 400 µM H2O2, respectively) compared to the control group. Pre-treatment with the use of trehalose 3% increased cell survivals. The levels of p62 protein, were increased in the cells under H2O2 treatment, while the levels of p62 protein in the cytoplasm, as autophagy inclusions, reduced for the group with trehalose pre-treatment. In addition, trehalose caused to increase LC3II/LC3I ratio and decreased the expression of cleaved caspase-3. Trehalose decreased apoptosis and increased the autophagy and survival levels of the cells against H2O2. Due to the unique properties of trehalose and its low toxicity, it can be used as a pharmaceutical agent in cellular transplantation to reduce oxidative stress.

Keywords: Stress oxidative; Autophagy; Apoptosis; Bone marrow stromal cells; Trehalose.

Introduction

BMSCs are able to differentiate into other ectodermal, mesodermal, and endodermal cell lines in laboratory culture medium (1-3). These cells, nowadays, play a significant role in cell and gene therapy (3), restorative medicine (4), and damaged tissue regeneration (5, 6). These cells are quickly dead after transplantation to the affected area due to hypoxia conditions, oxidative stress, and serum deprivation (7, 8). These cells, after transplantation to the injured spinal cord or the ischemic area of the brain, have very short lifespan due to oxidative factors and inflammatory cells (9-11). The ideas that prolong the cell life-span, by drugs therefore, play an important role in prolonging the survival...
of these cells after transplantation (12).

Trehalose is present in many microorganisms, plants, fungi, and insects (13). It is simply produced in the yeast, in response to environmental stressors including heat, cold, pressure, and lack of water (14). Trehalose is assumed to be a scaffold molecule that directly prevents protein aggregation (15). Trehalose was described as a chemical chaperone which stabilizes proteins, and prevents protein misfolding and protein aggregation (15). It was initially considered to be a rare sugar, but nowadays it is widely used in nature, pharmaceutical, and food industries. This sugar, due to its physiological and chemical properties, received a significant attention by many researchers (16). This sugar cannot simply pass through the cell membrane, while it can enter into cells through endocytosis and pinocytosis mechanisms, and has anti-inflammatory, anti-aggregation, and anti-aging properties (15, 17). Many studies have shown the role of trehalose in autophagic induction for neurodegenerative diseases (18).

Autophagy is a lysosomal pathway that degrades proteins and organelles, and recycles intracellular organelles and proteins to maintain energy homeostasis times of cellular stress (19, 20). In autophagy, the organelles and degraded fragments fall into double-membraned autophagosome. Autophagy is a process for maintaining cellular homeostasis under oxidative stress conditions. There is a relationship between deficiency in autophagy, and many neurodegenerative, cardiovascular diseases, aging and cancer (21). Some stressful states such as hypoxia and cell’s serum deprivation can induce autophagy and prolong cell life.

p62, a scaffold protein, has several domains whose function involve in the signal transferring process, proliferation, cell survival, death, tumor formation, and the response to oxidative stress (22). In the cell, ubiquitin–protein aggregation can cause neurodegenerative and cardiovascular disease. Lysosomal-autophagy process plays an essential role in removing cells from ubiquitin aggregation through the p62 ubiquitin-binding protein (22). p62, in addition to binding to ubiquitin protein, can be bound to LC3 protein directing the ubiquitinated complex to the autophagosome to be degraded (23). LC3 is a substrate for initiating autophagy and forming autophagosome. In the studies conducted in this regard, the expression of LC3 was investigated as an indicator of autophagy (24). The conversion of LC3I to LC3II represents the autophagy process, and higher amount of LC3II shows increased formation of autophagic vacuoles in the cells (25).

The caspase-3 protein is a member of the cysteine–aspartic acid proteases family whose activation plays an important role in apoptosis (26-28). Caspases are formed as proenzymes and zymogens which are converted into large and small active subunits during proteolytic activity after activation (26).

In this study, we indicated that oxidative stress caused by H$_2$O$_2$ can reduce autophagy (reduced LC3II/LC3I ratio and increased p62) and increase apoptosis (expression of caspase-3 protein). However, the pre-treatment with the use of trehalose 3% can increase the autophagy and reduce apoptosis. Therefore, trehalose 3% can regulate the cellular defense system against cell death by activating autophagy.

**Experimental**

**BMSCs cultivation**

In the present study, four 6-8-week-old adult female Wistar rats from Pastor Institute were used. Observing ethical rules, rats were kept in a 12-hour light and dark under standard conditions of Qazvin University of medical sciences’ Animal House. After BMSCs isolation from the long bones of the lower limb, they were washed with sterile phosphate-buffered saline (PBS). The cells were cultured in Dulbecco’s modified Eagle’s medium: F12 (DMEM/F12: Gibco) containing, 100 U/mL penicillin/streptomycin (Gibco, BRL) and 10% heat-inactivated fetal bovine serum (FBS: Gibco, BRL) and incubated at 37 °C, 5% CO2, and 95% relative humidity (RH) until the third passage.

**Immunocytochemistry**

In order to investigate the mesenchymal origin of BMSCs at the third passage, 5000 cells were seeded equally into each well of the 24-well cell
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Culture plate. The immunocytochemistry stages were performed according to the recommended ones with minor modifications (29). In summary, the cells were placed in paraformaldehyde solution 4% for 20 min and after being washed with phosphate buffered, the cells were placed in 0.3% Triton X for 15 min. After being washed with PBS, the cells were exposed to the primary antibody for 24 h at 4 °C. The primary antibodies include CD31 (endothelial stem cells marker), CD90 (mesenchymal stem cells marker), CD34 (hematopoietic stem cells marker), and p62 (autophagy marker) from ABCAM Company. The samples was washed with PBS and incubated with the secondary antibody conjugated with FITC (1:100; Chemicon) for 2 h at the room temperature. Nuclei were counterstained with PI (propidium iodide). Immunostaining was visualized under a fluorescent microscope (Olympus). The total number of the cells (PI staining) were estimated after counting at least three separate fields in the middle of each coverslip (200 cells per field counted).

Viability test
Prior to the study, viability test was performed by Trypan blue on cells in order to determine H$_2$O$_2$ toxicity and the protective effect of trehalose. The cells were divided into 1000 cells per well in the 96-well plate. In order to determine the H$_2$O$_2$ toxicity, survival rate for BMSCs was measured at different doses (0, 50, 100, 200 and 400 µM) for 8 h. After obtaining the lethal dose, the cells were divided into three groups: (A) control; (B) H$_2$O$_2$ and (C) H$_2$O$_2$ + trehalose. In order to examine the protective effects of trehalose, the cells were pretreated by trehalose 3% before being exposed to H$_2$O$_2$ medium (H$_2$O$_2$ + trehalose group). In order to analyze the survival rate of the cells, a volume of cell suspension and an equal volume of trypan blue were mixed and the cells were counted using the neobar lam under a microscope. In this method, the stain penetrates into the dead cells and turns the their color into blue. The non-stained cells represent the living cells. The percentage of the living cells can be obtained by counting the total number of the cells and the stained cells. Each cell group was counted three times using a microscope.

Western Blot
The protective effect of trehalose against H$_2$O$_2$ damage was evaluated by analyzing apoptosis (Cleaved Caspase-3) and autophagy (LC3). After treatment of the cell groups as described, the protein of the cells was extracted and frozen at -80 °C for further use. The proteins were separated using SDS-PAGE gel (15%), and transferred to the polyvinylidene fluoride (PVDF) membrane. The PVDF membrane was blocked by fat-free dry milk (5%) for 30 min. Then, it was washed three times with TBST buffer and incubated in the solution containing peroxidase-conjugated secondary goat anti-mouse or -rabbit IgG (1:1000-1:2000) for 1 h. The membrane was washed three times using TBST buffer and the membrane image was developed using the enhanced chemiluminescence kit (ECL kit). Densitometric analysis was performed using Image J software.

Results

BMSCs Analysis
BMSCs, after being isolated, had the appearance of round and spherical. They were stuck to the bottom of the flask after 48 h and after being washed with PBS, the floating cells that were not stuck on the bottom of the floor, were washed. After the third passage, BMSCs were evaluated by analyzing immunocytochemical surface markers in terms of mesenchymal origin. The cells were negative to CD31 and CD34, while indicating the positive immune response to CD90 and CD106 (Figure 1). The fluorescence light is associated with the conjugated secondary antibody to the FITC, which looks green. In order to count the number of the cells, PI dye was used, as it stains the nuclei (red color).

BMSCs Viability
While testing the viability, the BMSCs at the third passage without the H$_2$O$_2$ treatment were considered as the control group. The cells were then treated with H$_2$O$_2$ at different concentrations for 8 h. The survival rate after 8 h was 82%, 72%, 49%, and 39% of the control group in
the group receiving 50, 100, 200, and 400 μM of H$_2$O$_2$, respectively (Figure 2). The highest mortality rate belonged to the cell groups which received 200, 400 μM of H$_2$O$_2$, with a survival rate below 50%, and a significant difference ($p < 0.05$) with the control group. Such fatal doses were subsequently eliminated from the study (Figure 2).

To evaluate the protective effect of trehalose, the BMSCs were pretreated with 3% trehalose 2 h before being exposed to H$_2$O$_2$ with the previous concentrations. The results showed that the pretreatment with trehalose increased cell survival. The survival of the cells in the medium containing trehalose 3% and H$_2$O$_2$ at concentrations of 50, 100, 200, and 400 μM was 92%, 84%, 75%, and 48%, respectively (Figure 3). The comparison of H$_2$O$_2$-exposed cells with and without trehalose 3%, as shown in Figures 2 and 3, reveals that trehalose significantly increases cell survival and decreases cell death compared to H$_2$O$_2$.

**H$_2$O$_2$ caused autophagosomal vesicles formation containing p62**

p62 protein is an autophagy marker. p62

![Figure 1. Characteristics of the isolated bone marrow stromal cells (BMSCs). (A–D) show BMSCs labeled with mouse monoclonal primary antibodies, incubated with anti-IgG mouse secondary antibody conjugated with FITC (secondary antibody), and counterstained with propidium iodide. (A) The primary antibodies used were anti-CD31, (B) anti-CD90, (C) antiCD106 and (D) anti-CD34.](image)

![Figure 2. Dose-dependent cell death induced by H$_2$O$_2$ in BMSCs. Cell viability was determined by trypan blue assay. The survival rate after 8 h was 82%, 72%, 49% and 39% of the control group in the group receiving 50, 100, 200 and 400 μM of H$_2$O$_2$, respectively.](image)
connects to LC3 and then decomposes through the lysosomal pathway of autophagy. Therefore, high levels of p62 represent a problem in autophagy. In autophagy, p62 is low. The study shows that p62 increases with raising the H$_2$O$_2$ concentrations such that the highest p62 in the H$_2$O$_2$ without trehalose treatment was shown after 8 h, indicating the inhibition of autophagy (Figure 4). Moreover, in order to evaluate the autophagy in BMSCs, the expression of p62 protein was scrutinized through immunocytochemistry in different groups (Figure 4). The formation of autophagosomes containing p62 characterizes the autophagic activity (30). First, the BMSCs, as the control group, were evaluated in terms of p62 expression at an environment free of H$_2$O$_2$. As shown in Figure 4A, in the control group, the expression of p62 protein was given in a basic and low form, with a diffuse distribution pattern throughout the cytoplasm. However, for the BMSCs exposed to 100 μM of H$_2$O$_2$ for 8 h, the expression of p62 increased and p62 vesicles were formed as glowing spots in the cytoplasm (Figure 4B). These points containing glowing vesicles indicate the formation of autophagosomes after the oxidative stress, and they outnumber the other groups according to the counting. In the H$_2$O$_2$ group pretreated with trehalose, the number of glowing spots reduced compared to the H$_2$O$_2$ group without pretreatment, indicating an increase in the autophagy (Figure 4C). After counting autophagic inclusions containing p62 in each cell, their number in the control groups, H$_2$O$_2$, and H$_2$O$_2$ + trehalose was 4%, 30%, and 15%, respectively (Figure 4D).

**Discussion**

In summary, the study revealed that (a) 3% trehalose pre-treatment protects BMSCs against H$_2$O$_2$-induced cell death. The survival of the cells in the medium containing trehalose 3% and H$_2$O$_2$ at concentrations of 50, 100, 200, 400 μM was 92%, 84%, 75%, and 48%, respectively.

**The Role of trehalose in the autophagy induction and apoptosis prevention**

BMSCs, with and without trehalose, were exposed to H$_2$O$_2$ at 100 μM of concentration for 8 h to figure out if the oxidative stress caused by H$_2$O$_2$ activates the pathway of autophagy. The level of autophagy was assessed upon the determination of LC3II as the value of the LC3I. The ratio of LC3II to LC3I decreased in 8 h after being treated with 100 μM of H$_2$O$_2$, but in the medium of 3% trehalose and 100 μM of H$_2$O$_2$, this ratio increased (Figure 5). In order to examine the apoptosis in BMSCs, the proteolytic activity of cleaved caspase 3 was evaluated in different groups by means of Western Blot. Trehalose reduced the activity of the apoptotic marker of cleaved caspase 3 compared to H$_2$O$_2$. Figure 5 shows that trehalose decreased the activity of cleaved caspase 3 while its lack increased it and cleaved into two subunits. Caspase-3 was formed from a 32 kDa zymogen (inactive) that was cleaved into 17 kDa and 12 kDa subunits (active forms) (Figure 5).
Effect of trehalose on autophagy in the cultured BMSCs. p62 immunostaining using an anti-p62 antibody showed the cytosolic accumulation of p62 in the BMSCs. (A) BMSCs, as the control group, were evaluated in terms of p62 protein expression at an environment free of H$_2$O$_2$. The expression of p62 protein was given in a basic and low form, with a diffuse distribution pattern throughout the cytoplasm. (B) BMSCs exposed to 100 μM of H$_2$O$_2$ for 8 h, the expression of p62 increased and p62 dot-like vesicles were formed in the cytoplasm. (C) BMSCs were pretreated with 3% trehalose, the number of glowing spots reduced compared to the H$_2$O$_2$ group. (D) After counting autophagic inclusions containing p62 in each cell, their number in the control groups, H$_2$O$_2$, and H$_2$O$_2$ + trehalose was 4%, 30%, and 15%, respectively. BMSC were immunolabeled with anti-p62 primary antibody, incubated with FITC-conjugated secondary antibody.

However, if autophagy cannot reactivate the basic hemostasis of the cell and adapt the cell to stress conditions, the path of apoptosis will be activated (32). The appropriate concentration of trehalose increases and regulates autophagy, but upon increasing trehalose concentration, the unwanted pathways of the cell which lead to its death are activated (33). Therefore, in this study, 3% trehalose was used because of the findings of the previous studies.

In this study, 3% trehalose increased the cellular survival. In this regard, previous studies have shown that the increase of the concentration of trehalose causes changes in osmolality of cells and their death (34). Other our finding was the rise in the autophagy activity because of trehalose. Trehalose is likely to prevent cells from death by increasing autophagy and eliminating the organs and proteins damaged by H$_2$O$_2$. In this study, H$_2$O$_2$ with a concentration more than 100 μM increased cell death. A variety of studies has shown the sensitivity of the cells to H$_2$O$_2$. For example, retinal pigment epithelia’s (RPE) are resistant to the concentrations more than 400 μM of H$_2$O$_2$ even until 24 h (35, 36), but neurons are highly susceptible to H$_2$O$_2$-induced oxidative stress and 10-50 μM of H$_2$O$_2$ leads to the toxicity of a neuron and so the autophagy, instead of playing a protective role, leads to cell death (37). In this study, in the trehalose-free environment and 100 μM of H$_2$O$_2$, the cells expressed the cleaved caspase 3 protein after 8 h, and the ratio of LC3II/LC3I, which is the autophagic index, decreased. The activation of caspases plays a major role in the cell death through apoptosis (38). In apoptosis, the executioner caspases (caspase-3/7 and caspase-6) begin with autophagy inhibitors (38). Many studies have shown the role of autophagy and apoptosis in stressful conditions and cellular signals leading to apoptosis in BMSCs. Caspase 3 is a protein from the family of cysteine-aspartic acid proteases, the activation of which plays an important role in apoptosis (38). The caspases (32 kDa) are pro-enzymes and zymogens which convert into two small (12 kDa) and large (17 kDa) forms.
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kDa) active subunits through a proteolytic process after activation (39). After being broken, the enzyme activates caspases 6 and 7, and it, in turn, is activated by caspases 8, 9, and 10. This protein is the main caspase in beta-amyloid decomposition in Alzheimer’s disease (40). The caspases kill the cell through two outer (apoptotic ligands) and inner pathways (mitochondria). Caspase 3 is engaged in the formation of the brain by chromatin condensation and DNA fragmentation via apoptosis (41). The increased subunit of P17 in the bloodstream indicates myocardial infarction. Caspase 3 contributes to the differentiation of the stem cells of the blood and embryos (42). In previous studies, it has been shown that trehalose plays an important role in preventing apoptosis (43).

In many of the neurodegenerative diseases such as Huntington, 2% trehalose, after having been orally administered, traversed the blood barrier of the brain, and prevented the possible damage resulted from the poly-glutamine and poly-alanine in the cerebellum, leading to the improvement of movement and longevity (44).

In fact, trehalose prevents the poly-glutamine-mediated protein aggregation. The ubiquitination of the p62 protein, which is an LC3-linked protein, generates protein compositions which could be removed with autophagy. Phosphatidyl ethanol amine, while being attached to L3, turns into LC3II, which is located in the internal and external membranes of autophagosomes, and it is completely eliminated after the attachment of autophagosomes to a lysosome. p62 plays a variety of cellular signals against stress, inflammation, and cell survival, and plays a role in the formation of ubiquitin-containing inclusions. Our study showed that trehalose reduced p62 levels in the cells.

Trehalose is a non-mTOR autophagy inducer that has been shown to reduce the toxic protein accumulation in the cells of many neurodegenerative diseases such as Alzheimer’s, Parkinson’s, Huntington’s through the induction of autophagy, and in the clinic, it has been shown to increase the life expectancy and relative improvement of patients. The special properties of trehalose and its low toxicity turned it into a pharmaceutical agent for the disorders requiring long-term medicine taking. In a study, it was revealed that trehalose protected Candida albicans against the oxidative stress caused by H$_2$O$_2$ (45).

**Discussion**

In summary, the study revealed that (a) 3% trehalose pre-treatment protects BMSCs against H$_2$O$_2$, and induces autophagy and decreases the death of the cells; (b) in the culture medium without trehalose, H$_2$O$_2$ causes apoptosis in the BMSCs in 8 h.

![Figure 5. Western blot analyses of LC3, p62 and caspase-3 protein expression in control (lane1), H$_2$O$_2$ (lane2) and H$_2$O$_2$+trehalose (lane3) groups. Autophagic flux was examined by means of LC3 I/II conversion with the use of Western blot with LC3 antibody. LC3I, LC3II, p62 and Cleaved caspase 3 were detected using specific antibodies. GAPDH was used as an internal control. Caspase-3 was formed from a 32 kDa zymogen (inactive) that was cleaved into 17 kDa and 12 kDa subunits (active).](image)

**Conclusion**

In the current study, the BMSCs subjected to cell death after exposing to H$_2$O$_2$, which was intensified after increasing the H$_2$O$_2$ concentrations. Furthermore, the protein expression in p62 in the cell cytoplasm increased, in accordance with other studies, indicating a decrease in autophagy level and an increase in the expression of antioxidant pathway genes.
References

(1) Menabde G, Gogilashvili K, Kakabadze Z and Berishvili E. Bone marrow-derived mesenchymal stem cell plasticity and their application perspectives. *Georgian Med. News* (2009) 167: 71-6.

(2) Ivanovska IL, Shin JW, Swift J and Discher DE. Stem cell mechanobiology: Diverse lessons from bone marrow. *Trends Cell Biol.* (2015) 25: 523-32.

(3) Darabi S, Tiraihi T, Delshad A, Sadeghizadeh M, Khalil W and Taheri T. In-vitro non-viral murine pro-neurotrophin 3 gene transfer into rat bone marrow stromal cells. *J. Neurol. Sci.* (2017) 375: 137-45.

(4) Moradian H, Rafiee A and Ayatollahi M. Design and fabrication of a novel transplant combined with human bone marrow mesenchymal stem cells and Platelet-rich fibrin: New horizons for periodontal tissue regeneration after dental trauma. *Iran. J. Pharm. Res.* (2017) 16: 1370-8.

(5) Naderi N. The perspectives of mesenchymal stem cell therapy in the treatment of multiple sclerosis. *Iran. J. Pharm. Res.* (2015) 14: 1-2.

(6) Rabbani S, Soleimani M, Sahebjam M, Imani M, Haeri A, Ghiasiuddin A, Nassiri SM, Majd Ardakani J, Tajik Rostami M, Jalali A and Ahmadi Tafti SH. Simultaneous delivery of wharton’s jelly mesenchymal stem cells and insulin-like growth factor-1 in acute myocardial infarction. *Iran. J. Pharm. Res.* (2018) 17: 426-41.

(7) Pezzi A, Amorin B, Laureano A, Valim V, Dahmer A, Zambonato B, Sehn F, Wilke I, Bruschi L, Silva M, Filippi-Chiela E and Silla L. Effects of hypoxia in long-term in-vitro expansion of human bone marrow derived mesenchymal stem cells. *J. Cell Biochem.* (2017) 118: 3072-9.

(8) Das R, Jahl H, van Osch GJ and Farrell E. The role of hypoxia in bone marrow-derived mesenchymal stem cells: Considerations for regenerative medicine approaches. *Tissue Eng. Part B Rev.* (2010) 16: 159-68.

(9) Parr AM, Kulbatski I, Wang XH, Keating A and Tator CH. Fate of transplanted adult neural stem/progenitor cells and bone marrow-derived mesenchymal stromal cells in the injured adult rat spinal cord and impact on functional recovery. *Surg. Neurol.* (2008) 70: 600-7.

(10) Borlongan CV, Glover LE, Tajiri N, Kaneko Y and Freeman TB. The great migration of bone marrow-derived stem cells toward the ischemic brain: Therapeutic implications for stroke and other neurological disorders. *Prog. Neurobiol.* (2011) 95: 213-28.

(11) Aramvash A, Rabbani Chadegani A and Loffti S. Evaluation of apoptosis in multipotent hematopoietic cells of bone marrow by anthracycline antibiotics. *Iran. J. Pharm. Res.* (2017) 16: 1204-13.

(12) Naderi Y, Parvardeh S, Moini Zanjani T and Sabetkasaei M. Neuroprotective effect of paroxetine on memory deficit induced by cerebral ischemia after transient bilateral occlusion of common carotid arteries in rat. *Iran. J. Pharm. Res.* (2018) 17: 215-24.

(13) Zhu Y, Zhang J, Xing L and Li M. Progress on molecular biology of trehalose synthase--A review. *Wei Sheng Wu Xue Bao* (2009) 49: 6-12.

(14) Petitjean M, Testa MA, Leger-Silvestre I, Francois JM and Parrou JL. A new function for the yeast trehalose-6P synthase (Tps1) protein, as key pro-survival factor during growth, chronological ageing, and apoptotic stress. *Mech. Ageing Dev.* (2017) 161: 234-46.

(15) Li Y, Luo Y, Luo T, Lu B, Wang C, Zhang Y, Piao M, Feng C and Ge P. Trehalose inhibits protein aggregation caused by transient ischemic insults through preservation of proteasome activity, not via induction of autophagy. *Mol. Neurobiol.* (2017) 54: 6857-69.

(16) Martinetti D, Colarossi C, Buccheri S, Denti G, Memeo L and Vicari L. Effect of trehalose on cryopreservation of pure peripheral blood stem cells. *Biomed. Rep.* (2017) 6: 314-8.

(17) Pagliassotti MJ, Estrada AL, Hudson WM, Wei Y, Wang D, Seals DR, Zigler ML and LaRocca TJ. Trehalose supplementation reduces hepatic endoplasmic reticulum stress and inflammatory signaling in old mice. *J. Nutr. Biochem.* (2017) 45: 15-23.

(18) Tang Q, Zheng G, Feng Z, Chen Y, Lou Y, Wang C, Zhang X, Zhang Y, Xu H, Shang P and Liu H. Trehalose ameliorates oxidative stress-mediated mitochondrial dysfunction and ER stress via selective autophagy stimulation and autophagic flux restoration in osteoarthritis development. *Cell Death Dis.* (2017) 8: e3081.

(19) Ha J and Kim J. Novel pharmacological modulators of autophagy: An updated patent review (2012-2015). *Expert Opin. Ther. Pat.* (2016) 26: 1273-89.

(20) Shariatpanahi M, Khodagholi F, Ashabi G, Aghazadeh Khasrangi A, Azimi L, Abbollahi M, Ghaehremani MH, Ostad SN, Noorbakhsh F and Sharifzadeh M. Ameliorating of memory impairment and apoptosis in amyloid beta-injected rats via inhibition of nitric oxide synthase: Possible participation of autophagy. *Iran. J. Pharm. Res.* (2015) 14: 811-24.

(21) Shams Nooraeei M, Noori-Zadeh A, Darabi S, Rajaei F, Golmohammadi Z and Abbasszadeh HA. Low level of autophagy-related gene 10 (ATG10) expression in the 6-hydroxydopamine rat model of parkinson’s disease. *Iran. Biomed.* (2017) 22: 15-21.

(22) Liu H, Dai C, Fan Y, Guo B, Ren K, Sun T and Wang W. From autophagy to mitophagy: The roles of P62 in neurodegenerative diseases. *J. Bioenerg. Biomembr.* (2017) 49: 413-22.

(23) Peng H, Yang J, Li G, You Q, Han W, Li T, Gao D, Xie X, Lee BH, Du J, Hou J, Zhang T, Rao H, Huang Y, Li Q, Zeng R, Hui L, Wang H, Xia Q, Zhang X, He Y, Komatsu M, Dikic I, Finley D and Hu R. Ubiquitylation of p62/sequestosome1 activates its autophagy receptor function and controls selective autophagy upon ubiquitin stress. *Cell Res.* (2017) 27: 657-74.

(24) Huang R and Liu W. Identifying an essential role of nuclear LC3 for autophagy. *Autophagy* (2015) 11: 852-
The Role of Trehalose on the Apoptosis and Autophagy

(25) Jiang P and Mizushima N. LC3- and p62-based biochemical progression in mammalian cells. Methods (2015) 75: 13-8.

(26) Munoz-Pinedo C and Lopez-Rivas A. A role for caspase-8 and TRAIL-R2/DR5 in ER-stressed-induced apoptosis. Cell Death Differ. (2017) 25: 226.

(27) Gregoraszczuk EL, Rak-Mardy A, Rys J, Jakubowicz J and Urbanski K. Effect of chemotherapeutic drugs on caspase-3 activity, as a key biomarker for apoptosis in ovarian tumor cell cultured as monolayer. A pilot study. Iran. J. Pharm. Res. (2015) 14: 1153-61.

(28) Hajimahdi Z, Safizadeh F and Zarghi A. QSAR analysis for some 1, 2-benzisothiazol-3-one derivatives as caspase-3 inhibitors by stepwise MLR method. Iran. J. Pharm. Res. (2016) 15: 439-48.

(29) Haratizadeh S, Nazm Bojnordi M, Darabi S, Karimi N, Naghikhani M, Ghasemi Hamidabadi H and Seifi M. Condition medium of cerebrospinal fluid and retinoic acid induces the transdifferentiation of human dental pulp stem cells into neuroglia and neural like cells. Anat. Cell Biol. (2017) 50: 107-14.

(30) Cha-Molstad H, Yu JE, Feng Z, Lee SH, Kim JG, Yang P, Han B, Sung KW, Yoo YD, Hwang J, McGuire T, Shim SM, Song HD, Ganipisseti S, Wang N, Jang JM, Lee MJ, Kim SJ, Lee KH, Hong JT, Ciechanover A, Mook-Jung I, Kim KP, Xie XQ, Kwon YT and Kim BY. Formation of high molecular weight caspase-3 complex in neonatal rat brain. Neurochem. Int. (2015) 6: 1153-42.

(31) Revuelta M and Matheu A. Autophagy in stem cell aging. Aging Cell (2017) 16: 912-5.

(32) Nowikovsky K and Bergmann M. Autophagy regulates apoptosis on the level of the death-inducing signalling complex. FEBS J. (2017) 284: 1967-9.

(33) Ichihara H, Kuwabara K and Matsumoto Y. Trehalose liposomes suppress the growth of tumors on human lung carcinoma-bearing mice by induction of apoptosis in-vivo. Anticancer Res. (2017) 37: 6133-9.

(34) Lan DM, Liu FT, Zhao J, Chen Y, Wu JJ, Ding ZT, Yue ZY, Ren HM, Jiang YP and Wang J. Effect of trehalose on PC12 cells overexpressing wild-type or A53T mutant alpha-synuclein. Neurochem. Res. (2012) 37: 2025-32.

(35) Kaamiranta K, Sinha D, Blasiak J, Kauppinen A, Vereb Z, Salminen A, Boulton ME and Petrovski G. Autophagy and heterophagy dysregulation leads to retinal pigment epithelium dysfunction and development of age-related macular degeneration. Autophagy (2013) 9: 973-84.

(36) Kim MH, Chung J, Yang JW, Chung SM, Kwag NH and Yoo JS. Hydrogen peroxide-induced cell death in a human retinal pigment epithelial cell line, ARPE-19. Korean J. Ophthalmol. (2003) 17: 19-28.

(37) Higgins GC, Devenish RJ, Beart PM and Nagley P. Autophagic activity in cortical neurons under acute oxidative stress directly contributes to cell death. Cell. Mol. Life Sci. (2011) 68: 3725-40.

(38) Nicholson DW. Caspase structure, proteolytic substrates, and function during apoptotic cell death. Cell Death Differ. (1999) 6: 1028-42.

(39) Cui J, Chen B, Wang H, Han Y, Chen X and Zhang W. Glucosidase II beta-subunit, a novel substrate for caspase-3-like activity in rice, plays as a molecular switch between autophagy and programmed cell death. Sci. Rep. (2016) 6: 31764.

(40) D’Amelio M, Cavallucci V, Middei S, Marchetti C, Pacioni S, Ferri A, Diamantini A, De Zio D, Carrara P, Battistini L, Moreno S, Bacci A, Ammassari-Teule M, Marie H and Cecconi F. Caspase-3 triggers early synaptic dysfunction in a mouse model of alzheimer’s disease. Nat. Neurosci. (2011) 14: 69-76.

(41) Kuros K, Saeki M and Kamasaki Y. Formation of high molecular weight caspase-3 complex in neonatal rat brain. Neurochem. Int. (2004) 44: 199-204.

(42) Boehm D, Mazurier C, Giarratana MC, Darghouth D, Faussat AM, Harmond L and Douay L. Caspase-3 is involved in the signalling in erythroid differentiation by targeting late progenitors. PLoS One (2013) 8: e62303.

(43) Lu H, Zhu Z, Dong L, Jia X, Sun X, Yan L, Chai Y, Jiang Y and Cao Y. Lack of trehalose accelerates H2O2-induced Candida albicans apoptosis through regulating Ca2+ signaling pathway and caspase activity. PLoS One (2011) 6: e15808.

(44) Tanaka M, Machida Y, Niu S, Ikeda T, Jana NR, Doi Kurosu K, Saeki M and Kamasaki Y. Formation of high molecular weight caspase-3 complex in neonatal rat brain. Neurochem. Int. (2015) 6: 1153-42.

(45) Alvarez-Peral FJ, Zaragoza O, Pedreno Y and Arguelles JC. Protective role of trehalose during severe oxidative stress caused by hydrogen peroxide and the adaptive oxidative stress response in Candida albicans. Microbiology (2002) 148: 2599-606.

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