Mitochondria morphology and membrane potential under stress conditions

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Abstract — Mitochondria, organelles found in the cytoplasm of almost all eukaryotic cells, generate large quantities of energy in the form of adenosine triphosphate (ATP). In addition to producing energy, mitochondria store calcium for cell signaling activities and play an important role in maintenance of ionic balance. Mitochondria, however, are highly sensitive to any kind of stress in which they mainly response by disturbance of respiration, reactive oxygen species (ROS) production and release of cytochrome c into the cytoplasm. Osmotic stress, in particular, generates ROS that degrade lipids, proteins, and DNA. High levels of salt concentration can cause an imbalance in cellular ion homeostasis that results in ion toxicity and osmotic stress. This study aims to investigate possible effects of KCl and NaCl on Bone marrow-derived (MSCs) mitochondria membrane potential (MMP) and morphology. The results indicated that KCl and NaCl of salt concentration can cause an imbalance in cellular ion homeostasis that results in ion toxicity and osmotic stress. This study aims to investigate possible effects of KCl and NaCl on MSCs mitochondria membrane potential (MMP) and morphology. The results indicated that KCl and NaCl decreased the potential and changed the morphology of mitochondria membrane compared to cells growing in normal condition.

Keywords — Mitochondria membrane potential, Mitochondria morphology, Osmotic stress, Mitochondria fusion.

1. INTRODUCTION

Mitochondria are membrane-bound cell organelles that generate most of the chemical energy needed to power the cell's biochemical reactions. Chemical energy produced by the mitochondria is stored in a small molecule called adenosine triphosphate (ATP). Cells use this energy to synthesize proteins, power mechanical motion, sustain homeostasis, and perform other life-supporting functions. Mitochondria contain a double membrane. The outer membrane allows large molecules to flow into the mitochondrial intermembrane space, and the highly invaginated inner membrane, which has a large surface area, is responsible for oxidative phosphorylation. Diverse environmental stresses often induce similar kinds of cellular damage. For example, many, or even most, environmental stresses induce oxidative stress and protein denaturation. As a consequence, diverse stresses often illicit similar cellular adaptive responses, such as the production of stress proteins, up-regulation of oxidative stress protectors, and accumulation of protective solutes. In many cases, mitochondria are key sites of damage during environmental stress, especially mitochondrial electron transport. The mitochondria are highly networked and can change shape and size in response to stress. They also have been shown to join together in response to stress [1], which is known as mitochondria fusion. Mitochondrial fusion play critical role in maintaining functional mitochondria when cells experience metabolic or environmental stresses. Fusion helps mitigate stress by mixing the contents of partially damaged mitochondria as a form of complementation [2], [3].

Researches show that high levels of salt concentration can cause an imbalance in cellular ion homeostasis that results in ion toxicity and osmotic stress. This stress generates reactive oxygen species (ROS) that degrade lipids, proteins, and DNA. Potassium chloride (KCl) is a naturally occurring salt

derived from the ground or sea. It's a potassium-based salt that food manufacturers mostly use to replace sodium chloride, or table salt. KCl may affect the integrity of mitochondria by breaching the electrostatic force between the lipids and proteins. the mitochondrial membrane, cristae, and the matrix proteins appear altered under the influence of KCl. Sodium chloride (NaCl), also known as salt, is an essential compound our body uses to absorb and transport nutrients, maintain blood pressure, maintain the right balance of fluid, transmit nerve signals and contract and relax muscles. Human body needs NaCl to function, but too little or too much salt can be harmful to your health. Hypertonicity, induced by high NaCl, decreases cell volume, increases cytosolic osmolality, and changes mitochondrial osmotic equilibrium, which could affect mitochondrial function [4].

The objective of the present study is to investigate the mitochondrial membrane potential and morphology of MSCs in stress response during different ionic stress caused by KCl and NaCl. To find the optimum non-toxic concentrations of the salts, MTT [3–4,5-dimethylthiazol-2yl (2,5diphenyl-2H-tetrazoliumbromide] assay was used. The cells then stained with JC-10 dye (AAT Bioquest, Inc) for investigating the MMP and MitoLite Red FX600 (AAT Bioquest, Inc) for determining the cells mitochondrial morphology. The morphology of the stained cells were studies using confocal fluorescent microscopy. The results suggested that KCl and NaCl caused reduction in MMP and morphological changes in the mitochondria membrane. The results also indicated that KCl may induce the mitochondria fusion under different stress conditions after 3 and 24 hours.

2. MATERIALS AND METHODS

2.1 CELL CULTURE

MSCs was used for this study and cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37°C in a 5% CO2 (carbon dioxide). The culture medium was changed every 2 days. For biological experiments, cultured cells were detached by trypsinization, suspended in a new culture medium and used for designed experiments.

2.2 CELL VIABILITY ASSAYS

To examine cell viability, MSCs cells were plated 24 hours before the experiment in 96-well plates at a density of 3×10^4 cells/well. The cells were then treated for 24 hours with KCl and NaCl at several concentrations. Then, MTT assay was used to study the viability of MSCs. For the measurement, medium was removed and replaced with 100 µL of MTT reagent (Sigma-Aldrich, United States) (5 mg/ml) and left at 37°C for 3 h; MTT solution was removed and the formazan crystals were then dissolved in solubilizing solution and transferred into a new 96-well plate. The plate then was scanned at 570 and 650 nm (for background subtraction) using a Portable Biotek Epoch Microplate Spectrophotometer.

2.3 MEASUREMENT AF MITOCHONDRIAL MEMBRANE POTENTIAL

Effects of KCl and NaCl on mitochondrial membrane potential in MSCs cells was investigated by JC-10 staining, which is an indicator of cell health. MSCs were incubated with 1 µM of the dye at 37°C for 30 minutes. JC-10 is capable of selectively entering into mitochondria, and reversibly changes its color from green (monomeric form) to orange (aggregate form) as membrane potentials increase.

2.4 MITOCHONDRIAL MORPHOLOGY

Mito-Lite Red FX600 reagent was used for determining mitochondrial morphology. Cells were plated at 5×10^4 cells on confocal dish for quantitating fluorescence. The medium was removed, and cells were washed with PBS before staining. Cells were incubated with 2 µl of 500 X MitoLite stock solution in

1000 µl of HBSS for 20 min at 37°C. MitoLite Red FX600 has excitation/emission maxima of approximately 580/598 nm.

3. RESULTS AND DISCUSSION

3.1 CELL VIABILITY ASSAYS

Mitochondria are fundamental organelles in animal and plant cells for energy supply by synthesis of ATP via oxidative and are thus highly sensitive to impact of stress, disease or ageing. In this experiment different concentrations of KCl and NaCl were used. The cell viability after KCl and NaCl exposed was determined by MTT test against the MSCs. According to **Fig.1 a** and **b** cell death was begun after incubation with the solution of 200 mM NaCl and KCl respectively, which indicates the cytotoxicity of these solutions. After 24 hours of incubation, MSCs were death completely in the medium contained 380 mM NaCl and 450 KCl. Therefore, 180 mM NaCl and 150 mM KCl were used for MMP measurement and mitochondrial morphology analysis.

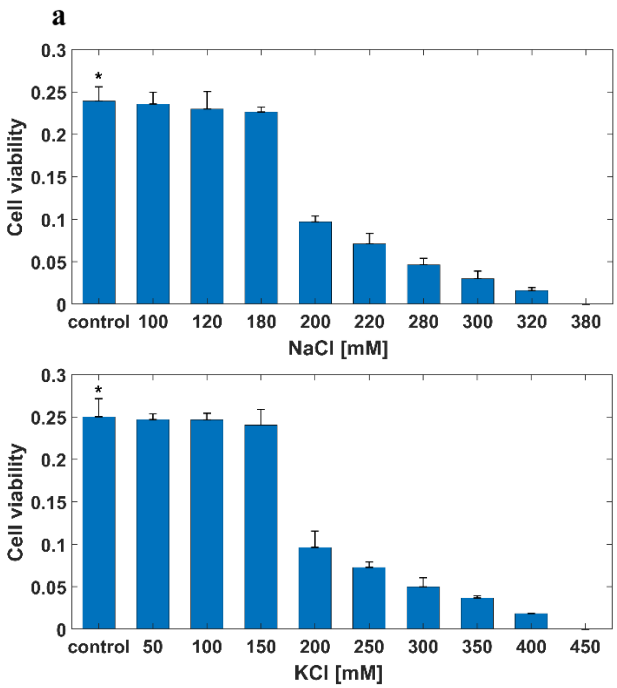

Figure 1: Cell viability on MSCs with MTT assay after 24 h treatment of MSCs with various concentrations of NaCl (a) and KCl (b). The control shows MSCs cells without the exposure to NaCl and KCl. Data are presented as mean ± SD; n = 3. *p<0.05 control compared to different concentration of NaCl and KCl (the unequal variances two-sample t-test, or Welch's t-test was used).

3.2 STUDY OF MITOCHONDRIAL MEMBRANE POTENTIAL

One of the most common methods to detect mitochondrial dysfunction is to monitor the mitochondrial membrane potential. Mitochondria are critical oxygen sensors linked to various protective effects, such as enhancement of antioxidant defense, cell survival and anti-apoptosis to determine the mitochondrial membrane potential in MSCs (control) and MSCs exposed KCl and NaCl was assessed using membrane-permeant dual-emission potential-sensitive JC-10 dye. Confocal image analysis consisted of calculating mean red intensity (amount of J-aggregates) in each group using the MATLAB software. According to confocal images in the MSC cells, increasing osmolality by adding NaCl causes decreases mitochondrial membrane potential and reduces cell volume after 3 and 24 hours compared to control. However, there was no significant difference between the samples exposed to NaCl after 3 and 24 h. The same results were observed with the cells treated with KCl, however, KCl effect on MMP was

significantly higher than that of NaCl. In contrast to the cells treated with NaCl, a significant difference was observed between the exposed cells to KCl after 3 and 24 h. (**Fig 2)**.

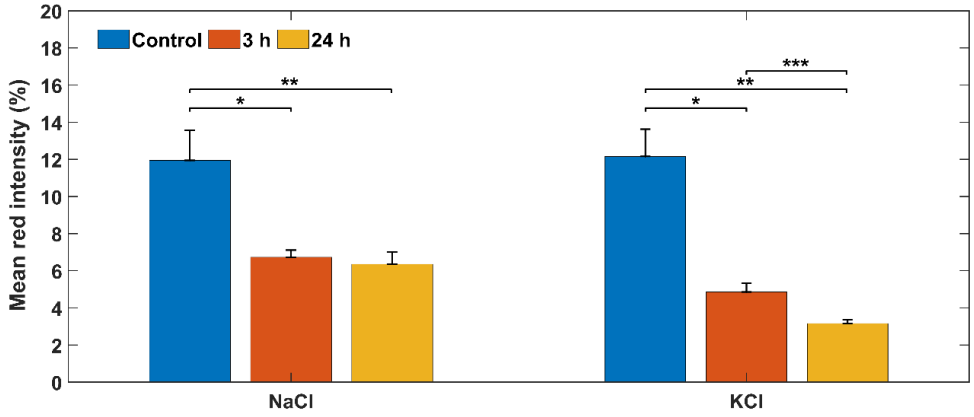

Figure 2: Mitochondrial membrane potential assessment of MSCs exposed to 180 mM NaCl and 150 mM KCl for 3 and 24 h. Data are presented as mean ± SD; n = 3. *p<0.05 control compared to NaCl and KCl after 3 h, **p<0.05 control compared to NaCl and KCl after 24 h, ***p<0.05 KCl (3h) compared to KCl (24h). The unequal variances two-sample t-test, or Welch's t-test was used.

3.3 MITOCHONDRIAL MORPHOLOGY

Mitochondria are highly dynamic organelles and attain different shapes reflecting different cellular states, during the lifetime of a cell. In most mammalian cells, mitochondria are tubular in shape under normal conditions but may attain various forms during various cellular perturbations. During apoptosis mitochondria are extensively fragmented and form small punctuate or round structures, while during necrosis mitochondria usually swell and become distended. Fusion is another behavior of cell mitochondria during abnormal condition [5] (**Fig 3)**. Fusion helps mitigate stress by mixing the contents of partially damaged mitochondria as a form of complementation and as a result maintain one of the most important vital functions, namely respiration. According to our confocal images, after 3 hours of incubation with KCl and NaCl, no significant changes in the cells mitochondria morphology was observed compared to control **(Fig 4 a)**. However, in some regions the early stage fusion was seen. After 24 hours of incubation, the morphological changes were significantly noticeable. The mitochondria were completely round shape and fusion between them was obvious (**Fig 4 b, c, d, e)**. These results are in line with previous reports which suggested that mitochondrial fusion and formation of protuberances of the outer mitochondrial membrane are induced by elevated levels of KCl and NaCl. According to the literature [6], the higher concentration of KCl may affect the integrity of mitochondria by breaching the electrostatic force between the lipids and proteins. In addition, adding NaCl decreases mitochondrial membrane potential by causing NADH redistribution out of mitochondria [7].

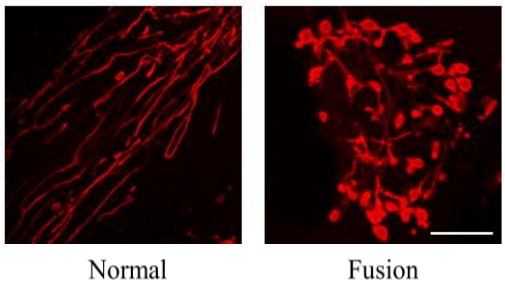

Figure 3: Confocal images of cells in normal (left) and abnormal condition (right). Scale bar 8 μm.

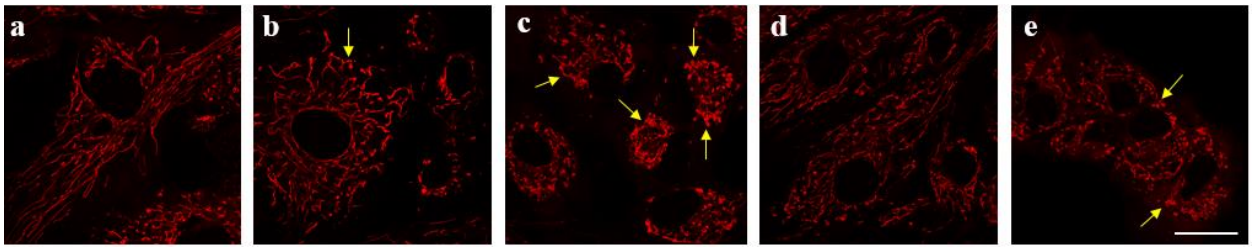

Figure 4: Confocal fluorescence microscopic images of mitochondria in MSCs in control (a), 3 h (b) and 24 h (c) after treatment with KCl and 3 h (d) and 24 (e) after treatment with NaCl. Yellow arrows show locations where mitochondria fusion can be seen. Scale bar 30 μm.

4. CONCLUSION

This study aims to investigate the possible morphological and membrane potential changes of MSCs cells mitochondria under stress conditions. In this regard, the cells exposed to NaCl and KCl provided the osmotic condition for the cells. Based on the obtained results, it was found that both NaCl and KCl strongly decreased mitochondrial membrane potential after 24 hours. In addition, the mitochondria morphology analysis confirmed that in some cases, the mitochondria normal structure (tubular shape) changed to round shape under the stress condition. The results also confirmed that putting mitochondria under environmental stress condition may lead to their fusion to large networks may help the organism to cope with the stress situation.

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