Viability of C2C12 Mouse Myoblast Cells  
Under Chemical Hypoxic Condition

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ABSTRACT

The present study was to use the MTT assay in evaluating the viability of C2C12 cells under the chemical hypoxia which mimics muscle cells death process in anoxia after animal bleeding. C2C12 cells were seeded at the density of $0.5 \times 10^4$, $1 \times 10^4$, $1.5 \times 10^4$, $2.5 \times 10^4$ per well of 96-well plates to test correlation between MTT assay and direct cell counting and cells were seeded at the density of 5000 cells per well of 96-well plates to construct cells growth curve. C2C12 cells were treated with DMEM (serum withdraw), 1 mM, 10 mM and 50 mM sodium azide (NaN3 in DMEM) and returned to the incubator for 12 hours, 24 hours, 48 hours and 72 hours. MTT assays were performed using cells harvested from the time points indicated and the responses of C2C12 cells to serum deprivation and sodium azide-mediated (NaN3) chemical hypoxia were determined by optical density at 490 nm. Our results demonstrate that there is a linear correlation between directly counted C2C12 cell number and MTT absorbence. The cells growth curve showed that the period of before 24 hours is the lag phase and the period of after 24 hours is the exponential phase. The present in vitro model with 1 mM sodium azide (NaN3) or serum deprivation is well applicable to mimic the hypoxia-induced apoptosis.

Key words: Apoptosis, C2C12 cells, Chemical hypoxia, MTT assay, Sodium azide (NaN3)

I. INTRODUCTION

C2C12 cells are a mouse myoblast cell line, originally obtained through serial passage of myoblasts cultured from the thigh muscle of C3H mice after a crush injury. This cell line is an excellent cell culture model to study the proliferation and differentiation of muscle satellite cell-derived myoblasts (Sun et al., 2007). MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) is a yellow water-soluble tetrazolium salt. The dye is converted to water-insoluble purple formazan on the reductive cleavage of its tetrazolium ring by the succinate dehydrogenase system of the active mitochondria. Thus, the amount of formazan formed can be determined spectrophotometrically and serves as an estimate of the number of mitochondria and hence the number of living cells in the sample. During the harvesting process of meat there is a generalized cell death often referred to as necrosis or apoptosis (Fidzianska et al., 1991). This process of cell death has been well studied with respect to various physiological functions in vivo but its role in post-mortem meat quality has received little attention. In all meat animal species and whatever the technology of stunning used, the last phase of the slaughter process is bleeding. After animal bleeding, all cells will be in anoxia and will receive no more nutriments. In such conditions, each cell can decide to die by initiating the apoptotic process. Apoptosis induced a series of biochemical and structural changes in dying cells which will be very likely found in postmortem muscle (Ouali et al., 2006). Sodium azide (NaN3) as a mitochondrial respiratory chain complex IV inhibitor was used to achieve chemical hypoxia and induce myocyte cell death (apoptosis) in many experiments (Chen et al., 1998; Inomata & Tanaka, 2003; Kositprapa et al., 2000). Herein hypoxia or lack of oxygen supply to C2C12 mouse myoblast cells which
were treated with sodium azide (NaN₃) or serum deprivation and these conditions model the ATP depletion and reductive stress of anoxia ("chemical hypoxia"). Cell killing, oxygen consumption were determined in C₂C₁₂ mouse myoblast cells after glycolytic and respiratory inhibition. Glycolysis was inhibited with serum withdraw, and mitochondrial electron transfer was blocked with sodium azide (NaN₃). Our experiment was to use the MTT assay in evaluating the death process of C₂C₁₂ cells under the chemical hypoxia which mimics muscle cells death process in anoxia after animal bleeding. However, in all circumstances, the extrapolation of in vitro data back to the whole animal is limited. Whatever, the use of in vitro systems for determining the developmental biology of muscle cells has resulted in considerable, useful data and knowledge of the changes during cell death process is the first step towards a positive outcome of this goal. In the present study, we attempt to investigate the cell viability under the hypoxia condition in cultured C₂C₁₂ mouse myoblast cells, using changes in cellular respiration as an indicator of metabolic inhibition to mimics muscle cells death process in anoxia after animal bleeding.

II. MATERIALS AND METHODS

1. Drugs used and laboratory wares

Unless specified otherwise, all chemicals and laboratory wares were purchased from Sigma- Aldrich Chemical Co. (St. Louis, MO, USA) and Falcon Labware (Becton-Dickinson, Franklin Lakes, Nj, USA), respectively.

2. Cell culture

C₂C₁₂ cells were brought from American Type Culture Collection (ATCC-CRL 1772), and cultured in growth medium (Dulbecco’s modified Eagle’s Medimj, DMEM) containing 10% fetal bovine serum, 100 IU/mL penicillin, and 100 µg/mL streptomycin) in a humidified incubator at 37°C with 5% CO₂. Cells were re-fed every three days. Cells cultivated in growth medium were subcultured after they became approximately 80% confluent, and cells of five passages were used for the current study.

3. Cell seeding and experimental design

C₂C₁₂ cells were trypsinized from culture flasks and suspended to create a single cell suspension. Cell number was determined by direct counting with a hemacytometer (Fisher scientific, #026715).

To test correlation between MTT assay and direct cell counting, C₂C₁₂ cells (100µL/well) were seeded at seeding densities of 5×10⁴, 1×10⁵, 1.5×10⁵ or 2.5×10⁵ cells/ mL into 96 well micro plates and allowed to adhere for 24 h, and then did MTT assay. The absorbency values were plotted against the counted cell numbers to establish a calibration curve.

To evaluate cell growth using the MTT assay, C₂C₁₂ cells were plated in 96-well plates with 100 µL of cells which had previously been resuspended to 5×10⁵/mL. After an overnight incubation, MTT assays were performed using cells harvested from 12 hours, 24 hours, 48 hours and 72 hours. The absorbance at 490 nm and 570 nm were measured with a plate reader followed the recommended protocol (some protocol recommended at 490 nm and others recommended at 570 nm, thus we test at both wavelength). The growth curve was constructed by plotting absorbance against time.

To analyze DMEM and Sodium azide (NaN₃) induced MTT reduction in C₂C₁₂ cells, cells were seeded at the density of 5000 cells per well of 96-well plates. The cells were left to adhere overnight and then treated with DMEM (serum withdraw), 1 mM, 10 mM and 50 mM sodium azide (in DMEM) and returned to the incubator for 12 hours, 24 hours, 48 hours and 72 hours. MTT assays were performed using cells harvested from the time points indicated, and were determined by optical density at 490 nm.

4. MTT assay

The MTT assay was performed according to the method of Mosmann (1983). Cell viability was assessed on time points by adding 20 µl of filter sterilised MTT (5 mg/mL in PBS) to a single row