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Impact of Biofield Energy Treated DMEM on Mitochondrial Biogenesis Using Myoblast Cell Line, C2C12: Implications for Metabolic Disease

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Abstract

Mitochondria play an important role in the body, and its dysfunction lead to the defective cellular energy production results to heterogeneous group of disorders such as early aging, type-2 diabetes, Alzheimer's disease, and many more metabolic diseases. The study was aimed to examine the effect of Consciousness Energy Healing based DMEM on myoblasts cells (C2C12) for mitochondrial mass content. The test item, DMEM was divided into three parts. First part did not receive any sort of treatment and defined as the untreated DMEM group. The second and third portions were treated with the one-time and two-times Biofield Energy Treatment by a renowned Biofield Energy Healer, Mahendra Kumar Trivedi and coded as the one-time Biofield Energy Treated DMEM (BT-I) and two-times Biofield Energy Treated DMEM (BT-II) groups, respectively. Cell viability of the test items by MTT assay showed more than 72% viable cells, which suggested that the test items were nontoxic and safe in nature. Moreover, the mitochondrial mass content in terms of fluorescence unit (FU) was significantly ($p \leq 0.05$) increased by 43.11% and 64.16% in the BT-I and BT-II groups, respectively in C2C12 cells compared to the untreated DMEM group. Overall, the experimental data suggested that two-times treated DMEM (BT-II) was found more significant improvement in mitochondrial mass content compared with the one-time treated DMEM (BT-I) group and results in better respiratory capacity. Thus, data envisaged that the Biofield Energy Treated DMEM has a potential to improve respiratory activity, which could be used against various metabolic diseases, such as insulin resistance, type-2 diabetes, and cardiovascular diseases, etc.

Keywords: Biofield Energy; Mitochondrial Biogenesis; Metabolic Disorders; Myoblast Cell; DMEM; C2C12; NAO Assay

Abbreviations: CAM: Complementary and Alternative Medicine, NCCAM: National Center for Complementary and Alternative Medicine; DMEM: Dulbecco's Modified Eagle's Medium; FBS: Fetal Bovine Serum, BT-I: One-time

Biofield Energy Treated DMEM; BT-II: Two-times Biofield Energy Treated.

Introduction

Mitochondria are one of the complex cellular organelles, which govern most of the metabolic processes such as fatty acid oxidation, Krebs cycle, oxidative phosphorylation in the electron transport chain (ETC), and many others. Mitochondrial disease denotes to a collection of disorders that mainly affects the ETC, which ultimately results in hamper the production of adenosine-triphosphate (ATP), the major cellular energy carrier [1]. However, recent research on genetic showed that the mitochondrial dysfunction is being recognized to be even more complex than initial concern. Consequently, mitochondrial pathophysiology was defined in terms of primary mitochondrial disease (PMD) and secondary mitochondrial dysfunction (SMD). Mitochondria produce energy from basic components, which undergo fusion, fission, transport, and degradation, all of the process is vital to maintain a healthy mitochondrial population [1]. However, mitochondrial biogenesis process involved an increased and controlled mitochondrial mass with number that helped to produce a greater production of ATP as a response to greater energy expenditure [2]. Genetic, physiologic, metabolic, and pathologic factors along with morphological and functional adaptability are the vital factors to regulate the process of mitochondrial biogenesis [3]. Mitochondrial biogenesis can control several therapeutic diseases such as metabolic syndrome, neurodegenerative disorders, sarcopenia, cardiac pathophysiology and physiological processes like aging [4]. Nonyl-acridine orange (NAO) is a non-fluorescent metachromatic dye that converts into the fluorescent dye in the presence of oxidative species, which is one of the gold standard assays to detect the mitochondrial mass alteration [5]. Therefore, mitochondrial mass of cells can measure by analyzing accumulation of the fluorescent dye in the mitochondria. Some alternative therapies such as nuclear gene were reported to regulate total mitochondrial mass in response to mitochondrial dysfunction [6].

In order to alter the mitochondrial mass content, some alternative treatment approach without any associated side effect is required. Biofield Energy Healing Therapies are the emerging field which aims to provide a scientific foundation in order to understand the complex homeodynamic regulation of living systems. “Biofield” as the unifying concept which defines and acts as a bridge between traditional and contemporary explanatory energy medicine models for both clinical practice and scientific research that focus on energy fields of the body [7]. Biofield Energy is a para-dimensional, infinite, and can freely flow between the environment and human. Biofield Energy Healing is categorized as one of the Complementary and Alternative Medicine (CAM) and agreed worldwide for the various ailments. It was also well accepted by National Center for Complementary and Alternative Medicine (NCCAM) [8]. Biofield Energy can transfer into both nonliving and living object(s) in the universe through healers intentional thought transmission process. The Biofield Energy recipient always receives the energy from the ionosphere of the earth, the “universal energy field” and responds in a useful way. This process is known as The Trivedi Effect® - Consciousness Energy Healing Treatment. Previously, many energy healing practices have been reported with an outstanding results in various fields both clinical and non-clinical trials, which includes Tai Chi, external qigong, pranic healing, Johrei, Reiki, therapeutic touch, acupuncture, yoga, Qi Gong, polarity therapy, chiropractic/osteopathic manipulation, deep breathing, progressive relaxation, guided imagery, hypnotherapy, homeopathy, meditation, massage, Rolfing structural integration, relaxation techniques, acupressure, special diets, Ayurvedic medicine, healing touch, mindfulness, movement therapy, pilates, traditional Chinese herbs and medicines in biological systems both *in vitro* and *in vivo* [9]. The Biofield Treatment has been reported with a significant revolution in different fields like materials science [10-12], agriculture [13,14], microbiology [15-17], biotechnology [18,19], improved bioavailability of low bioavailable compounds [20-22], improved skin health [23,24], enhanced nutraceuticals potency [25,26], cancer research [27,28], bone health [29-31], and human health and wellness. Concerning with the exclusive benefits of Biofield Therapy, here authors evaluate the impact of The Trivedi Effect® on DMEM for the assessment of mitochondrial mass content by NAO assay in murine myoblasts cells (C2C12).

Material and Methods

Chemical Requirement

Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were obtained from Life Technology, USA. Ethylenediaminetetraacetic acid (EDTA) and antibiotics solution (penicillin-streptomycin) were purchased from HiMedia, India. All the other chemicals used in this experiment were analytical grade procured from India.

Cell Culture

C2C12 (murine myoblasts) was used as a test system in the present study. The C2C12 cell line was maintained in DMEM growth medium for routine culture supplemented with 10% FBS. Growth conditions were maintained at 37°C, 5% CO_2, and 95% humidity and subcultured by trypsinisation followed by splitting the cell suspension into fresh flasks and supplementing with fresh cell growth medium. Before initiation of the experiment, cells were incubated in DMEM + 2% horse serum (HS) for 3 days to allow the cells to differentiate into myotubes.

Experimental Design

The experimental groups consisted of group 1 (G-I) with untreated DMEM. Group 2 (G-II) included one-time treated DMEM medium (BT-I), and group 3 (G-III) included two-times treated DMEM and denoted as BT-II.

Consciousness Energy Healing Treatment Strategies

The test item, DMEM was divided into three parts. First part did not receive any sort of treatment and defined as the untreated DMEM group. The second and third parts were treated with the one-time and two-times Biofield Energy Healing Treatment by a renowned Biofield Energy Healer (The Trivedi Effect®) Mahendra Kumar Trivedi remotely for ~3 minutes under laboratory conditions through unique energy transmission process and coded as the one-time Biofield Energy Treated DMEM (BT-I) and two-times Biofield Energy Treated DMEM (BT-II) groups, respectively. "The Trivedi Effect® is a natural and only scientifically proven phenomenon in which a person can harness this inherently intelligent energy from the Universe and transmit it anywhere on the planet through the possible mediation of neutrinos" [32]. The Biofield Energy Healer was located in the USA, while the test item was located in the research laboratory of Dabur Research Foundation, New Delhi, India. Healer in this study never visited the laboratory in person, nor had any contact with the test item (DMEM medium). Further, the untreated DMEM group was treated with a "sham" healer for comparative purposes. The "sham" healer did not have any knowledge about the Biofield Energy Treatment. After that, the Biofield Energy Treated and untreated test items were kept in similar sealed conditions for experimental study.

MTT Assay

MTT assay was used for the evaluation of viable cells in C2C12 cell line. The details procedures were followed according to Dahryn, et al. 2018 [29]. The cytotoxicity of the test items at each concentration were performed with the help of following equation (1):

% Cytotoxicity = (1-X/R)*100 ---------- (1)

Where, X = Absorbance of the Biofield Treated cells; R = Absorbance of untreated cells

The percentage cell viability concerning to each treatment was obtained using the following equation (2):

% Cell Viability = (100 - % Cytotoxicity) ---------- (2)

The concentrations exhibiting more than 70% cell viability was considered as non-cytotoxic.

Assessment of Mitochondrial Content

For the assessment of mitochondrial mass, the cells were counted using an hemocytometer and plated at 4500 cells/well in dark walled 96-well plates in DMEM supplemented with 2% HS. The cells were incubated overnight under standard growth conditions to allow the cell recovery and exponential growth, which were treated by the test items in different groups followed by incubation with the test items for 72 hours. After incubation with the test items, mitochondrial content was determined by 10-N-nonyl acridine orange (NAO) dye. 50nM dye was added to each well and the cells were incubated for 30 minutes at 37˚C and 5%CO_2. After 30 minutes of incubation, media was discarded and cells were washed with phosphate buffer saline (PBS). 150µL of PBS was added to each well and fluorescence was read at 485/20 excitation, 528/20 emission filter using synergy HT microplate reader. The percentage increase in mitochondrial content was calculated using following equation-

Percentage increase = [Average FU_{sample} - Average $FU_{baseline}$]*100/Average $FU_{baseline}$------(3)

Where, FU denotes Fluorescence unit

Statistical Analysis

Study data were stated as Mean ± SEM (standard error of mean) of three independent experiments. For two groups comparison Student's *t*-test was used. For multiple group comparison, one-way analysis of variance (ANOVA) was used followed by post-hoc analysis by Dunnett's test. Statistically significant values were set at the level of $p \leq 0.05$.

Results and Discussion

Cell Viability using MTT Assay

The cell viability using MTT assay was performed to study the cytotoxicity of the test items in C2C12 cells (Figure 1). The cell viability (%) in the untreated DMEM, one-time Biofield Energy Treated DMEM, and two-times Biofield Energy Treated DMEM groups was 72.32%, 129.64%, and 123.41%, respectively (Figure 1). However, data also showed that percentage of cell viability was significantly ($p \leq 0.001$) improved in the Biofield Energy Treated DMEM groups compared with the untreated DMEM group. The study data suggest that the test items were observed as safe in C2C12 cells, which were further used for the estimation of mitochondrial mass content, which indicate extend of respiratory activity.

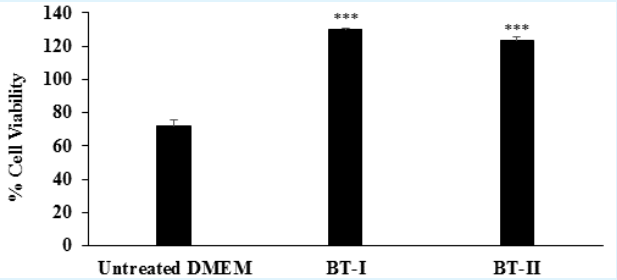

Figure 1: Effect of the test item on cell viability assessed by MTT assay in myoblast cell (C2C12). BT-I: One-time Biofield Energy Treated DMEM; BT-II: Two-times Biofield Energy Treated DMEM. ***p≤0.001 vs. Untreated DMEM group.

Mitochondrial Mass Content

The study was performed to evaluate the influence of Biofield Energy Healing Treatment on mitochondrial content in C2C12 cells using NAO dye assay. For the actual assessment of mitochondrial health is very crucial to understand their role in disease. There are two fluorescent probes extensively used to assess the mitochondrial mass like MitoTracker green (MTG) and nonylacridine orange (NAO). The basic mechanism is that the accumulation of this NAO dye in the mitochondrial membrane and to alter the trans-membrane potential as well. NAO can present in mitochondria, where it binds to cardiolipin [33]. The result in terms of average fluorescence unit (FU) in mitochondrial mass content among different groups is shown in Figure 2. Increase NAO dye accumulation in muscle cells indicate an increase FU *i.e.,* the mitochondrial mass content. The untreated DMEM showed 272.3 ± 12.14 FU. On the other hand, the one-time Treated DMEM group (BT-I) and two-times Treated DMEM group (BT-II) showed 43.11% and 64.16% increase the level of mitochondrial mass in terms of FU, respectively compared to the untreated DMEM group (Figure 2).

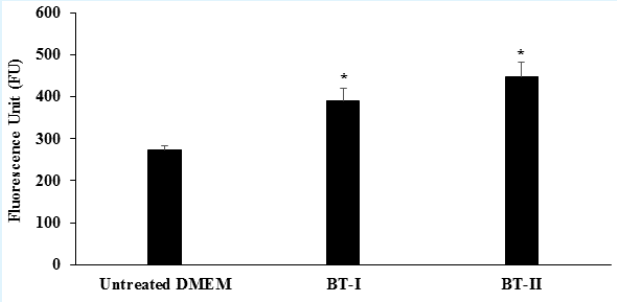

Figure 2: Effect of the test items for the change in mitochondrial mass on C2C12 cell line. BT-I: One-time Biofield Energy Treated DMEM; BT-II: Two-times Biofield Energy Treated DMEM. Data are expressed as mean ± SEM of three independent experiments.

*p≤0.05 vs. Untreated DMEM group.

Mitochondria produce most of the vital energy is required for the cellular function through oxidative phosphorylation. Energy requirement of a cell is directly proportional by the number and amount of mitochondria present in a cell. However, the mitochondrial mass content or its biogenesis process showed a significant effect against various metabolic diseases. Mitochondrial biogenesis would improve the cell capacity to control and maintain the cell metabolism, signal transduction, and regulation of mitochondrial reactive oxygen species (ROS) production [2]. Mitochondrial biogenesis might reduce, which results in mitochondrial dysfunction and results in mitochondrial oxidative stress, which leads to various diseases [34]. Mitochondrial mass content results an improved production of ATP as a results to more energy expenses [35].

In energy metabolism mitochondria play a negative impact on body weight. Mitochondria are dynamic organelles whose main function is production of ATP through oxidative phosphorylation. Apart from ATP generation, it also regulates various cellular functions such as apoptosis, calcium homeostasis, and production of reactive oxygen species (ROS) [36]. Numerous factors like nutritional factors, physical exercise, etc. has been

reported to improved mitochondrial mass content, more glucose uptake by muscles, increased metabolic enzymes level for glycolysis, oxidative phosphorylation, and ultimately a more mitochondrial metabolic capacity [37]. Aging leads to a reduce level of mitochondrial mass content and that results in several disorders like obesity, type-2 diabetes, enhanced aging, cardiovascular diseases, insulin resistance, etc. [38]. Thus, overall experimental data suggested that two-times Biofield Treated DMEM group showed a significant improved mitochondrial mass content, which results in better respiratory functions as compared to the one-time Biofield Treated DMEM group. Overall, The Trivedi Effect® has the excellent capacity to improve and maintain overall Quality of Life with an improved respiratory capacity and mitochondrial content.

Conclusions

The MTT assay data showed a significant improvement in cell viability by 123% and 129% in the one-time treated DMEM (BT-I) and two-times treated DMEM (BT-II) groups, respectively in C2C12 cells. Besides, the mitochondrial mass content was significantly ($p \leq 0.05$) increased by 43.11% and 64.16% in the BT-I and BT-II groups, respectively compared to the untreated DMEM group. Overall, the Biofield Energy Treated DMEM significantly improved mitochondrial mass content, and that results in the better respiratory activity. Thus data anticipated that it could be used to improve the energy level, endurance, body energy, which can be utilized against many metabolic diseases such as aging, diabetes, cancer, depression, hypertension, cardiovascular disease, and physical strength. Thus, the Biofield Energy Healing based DMEM might be a suitable alternative for cell growth and development. It can be further useful for the management of numerous disorders such as Systemic Lupus Erythematosus, Addison Disease, Reactive Arthritis, Multiple Sclerosis, Dermatomyositis, Rheumatoid Arthritis, Graves' Disease, Hashimoto Thyroiditis, Pernicious Anemia, Aplastic Anemia, Scleroderma, Crohn's Disease, Psoriasis, Diabetes, Myasthenia Gravis, Chronic Fatigue Syndrome, Vitiligo, Alopecia Areata, and Vasculitis, Irritable Bowel Syndrome, Asthma, Alzheimer's Disease, Dermatitis, Parkinson's Disease, Hepatitis, Ulcerative Colitis, Atherosclerosis, and Diverticulitis. Further, Biofield Energy Healing Treatment can also be used in the prevention of immune-mediated tissue damage in cases of organ transplants (for example kidney transplants, heart transplants, and liver transplants), stress, anti-aging, etc.

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