CRIMSON PUBLISHERS
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Orthopedic Research Online Journal

ISSN: 2576-8875

Research Article

Anti-Rheumatoid Arthritis (RA) Action of Biofield Energy Treatment Using Synovial Sarcoma Cell Line (SW982)

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Submission: January 03, 2019; **Published:** February 01, 2019

Abstract

The aim of the present research investigation was to monitor the effect of Consciousness Energy Healing based DMEM medium in a synovial sarcoma cell line (SW982) for evaluation of bone health (anti-rheumatoid arthritis) potential using inflammatory parameter interleukin-8 (IL-8). The test item (DMEM) was divided into two parts. One part was represented as the untreated test item without any Biofield Energy Treatment, while the other portion was defined as the Biofield Energy Treated test item, which received the Biofield Energy Healing Treatment by a renowned Biofield Energy Healer, Dahryn Trivedi. MTT assay was used for testing the cell viability and the results showed that the cell viability of SW982 cells was more than 100% with a safe and non-toxic profile. Further, the test item showed a significant ($p \leq 0.001$) inhibition of IL-8 secretion by 70.05% under the stimulation of IL-1β in SW982 cells as compared to the untreated DMEM group. Overall, the experimental data revealed the significant potential of the test item against rheumatoid arthritis to reduce inflammation in joints. Thus, it is concluded that Consciousness Energy Healing Treatment significantly inhibits the pro-inflammatory cytokine (IL-8), which showed a potential to act as an anti-rheumatic action in arthritis and other bone inflammatory disorders such as osteoporosis, Paget's disease of bone, rickets, deformed bones, osteomalacia, osteoma, aging, bone loss, and fractures.

Keywords: Biofield energy; Interleukin-8; Rheumatoid arthritis; DMEM; Synovial cell line; Bone health

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

Introduction

Rheumatoid arthritis (RA), a chronic autoimmune bone disorder reported to having a high prevalence worldwide [1]. The severe inflammation in RA leads to joint stiffness, pain, and symmetrical synovitis of diarthrodial joints such as the knee joint, which results in the functional decline in joint movements, articular destruction, and extensive co-morbidity related with the physiological systems including cardiovascular, neurologic, and metabolic changes. Immune cells such as B and T cells are present in the RA synovial membrane, and sometimes plasma cells and activated macrophages are present in the synovial fluid with neo-vascularization process [2]. However, host tissue cells are involved in the destruction of the joints and cartilage results in the perpetuation of the inflammation. Cells recruitment, initiation, activation, inflammation, etc. all are mediated with the wide network of cytokines, which are one of the major sign of RA. Nuclear factor (NF)-kB and activator protein (AP)-1 is the various transcriptional factors that are responsible for gene regulation during inflammatory reactions, along with the inflammatory cytokines (interleukins), MMPs, and COX-2. Pro-inflammatory cytokines have been reported with significant roles such as synovial fluid interleukin-8 (IL-8) and neutrophil in RA pathogenesis [3]. Triggering factors play a significant role in the initiation of the diseases, it may be internal such as chemotactic factors like leukocytes released in the inflammation area or might be some external injuries that activate the internal inflammatory mechanism. Time course of leukocyte immigration and kind of cellular infiltration were determined by various triggering factors [4]. IL-8 was found to have high selectivity for neutrophils formed by the mononuclear phagocytes and different tissue cells, on stimulation with interleukin-1 (IL-1), tumor necrosis factor (TNF), and bacterial endotoxins [5]. Currently, various synthetic drugs such as methotrexate and sulfasalazine have been recommended using therapeutic approaches on the disease-modifying anti-rheumatic drugs (DMARDs). However, these drugs have been reported with very partial clinical benefits and largely related to high toxicity. Thus, in order to find some golden method using alternative treatment approach, Biofield Energy Healing was used in this study as the treatment approach in RA using human synovial sarcoma cell line with standard experimental protocols. SW982 (human synovial sarcoma cell line) is characterized by expression of inflammatory cytokines and MMP genes. SW982 cells express

genes encoding IL-1β, IL-6, cyclooxygenase (COX)-2, and MMPs [6]. Dexamethasone has been used as one of the best approaches for the treatment of RA and is reported to inhibit secretion of IL-6 and IL-8 in SW982 cells [7]. Hence, in the present study, inhibition of cytokine secretion (IL-8) by SW982 cells against IL-1β stimulated levels suggested beneficial effects of Biofield Energy Treatment in DMEM against RA.

Biofield Energy Healing Therapies purport to sense and control the body's subtle energies, which are reported with a wide range of effectiveness concerning reduced tension level, anxiety, and pain. These therapies are used as a complement to other types of medical care with a wide range of significant outputs in living and non-living things due to the absence of any associated side-effects [8]. Biofield Energy Healing is one of the best approaches in Complementary and Alternative Medicine (CAM) because a human can harness energy from the universal and can transmit it to any living organism(s) or nonliving object(s) around the globe. It was accepted and recommended by National Center for Complementary and Alternative Medicine (NCCAM) [9] due to several advantages in addition to other therapies, medicines and practices such as natural products, Tai Chi, chiropractic/osteopathic manipulation, healing touch, movement therapy, deep breathing, yoga, Qi Gong, meditation, Reiki, cranial sacral therapy, massage, special diets, homeopathy, progressive relaxation, guided imagery, acupressure, acupuncture, relaxation techniques, hypnotherapy, essential oils, and aromatherapy, naturopathy, Ayurvedic medicine, traditional Chinese herbs and medicines, mindfulness, [10,11]. Biofield Energy Healing Treatment (The Trivedi Effect®) contains putative bioenergy, which is channeled by a renowned practitioner from a distance. Biofield Energy Healing as a CAM showed significant results in biological studies [12]. However, NCCAM had well-defined and categorized the Biofield therapies under the class of Energy Therapies [13]. The Trivedi Effect®- Consciousness Energy Healing Treatment has been reported with a significant revolution in materials science, agriculture, microbiology, biotechnology, improved bioavailability, skin health, nutraceuticals, cancer research, bone health [14-35], human health and wellness.

In this consequence, the authors evaluate the impact of the Biofield Energy Treatment on DMEM for bone cell development with respect to the assessment of various bone health-related parameter, inhibition of cytokine secretion (IL-8) by SW982 cells against IL-1β stimulated effect using the standard *in vitro* assay in SW982 cells.

Material and Methods

Chemicals and reagents

Antibiotics solution (penicillin-streptomycin) was procured from HiMedia, India, while 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium) (MTT), Direct Red 80, and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma, USA. Fetal bovine serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Life Technology, USA. All the other chemicals used in this experiment were analytical grade procured from India.

Cell culture

SW982 (Synovial cell line) was used as a test system in the present study. The SW982 cell line was maintained in DMEM growth medium for routine cell culture supplemented with 10% FBS. Growth conditions were maintained at 37°C, 5%CO_2, and 95% humidity and subcultured by trypsinization followed by splitting the cell suspension into fresh flasks and supplementing with fresh cell growth medium. Three days before the start of the experiment, the growth medium of near-confluent cells was replaced with fresh phenol-free DMEM, supplemented with 10% charcoal-dextran stripped FBS (CD-FBS) and 1% penicillin-streptomycin [36].

Experimental design

The experimental groups consisted of group 1 (G-I) with cells with untreated DMEM. Group 2 (G-II) contained cells with Biofield Energy treated DMEM group.

Consciousness energy healing treatment strategies

The test item, DMEM was divided into two parts. One part each of the test item was treated with the Biofield Energy by a renowned Biofield Energy Healer (also known as The Trivedi Effect®) and coded as the Biofield Energy Treated DMEM, while the second part did not receive any sort of treatment and referred as the untreated DMEM group. This Biofield Energy Healing Treatment was provided by Dahryn Trivedi remotely for ~5 minutes through the Healer's unique Energy Transmission process to the test sample. 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. Dahryn Trivedi in this study never visited the laboratory in person, nor had any contact with the test item (DMEM medium). Further, the control group was treated with a "sham" healer for better comparative purposes. The "sham" healer did not have any knowledge about the Biofield Energy Treatment. After that, the Biofield Energy Treated and untreated samples were kept in similar sealed conditions for experimental study.

MTT assay for cell viability

MTT assay was used for the assessment of cell viability in SW982 cells of untreated and Biofield Energy Treated test samples. The details methodology of cell viability assay was followed by Lorraine et al. [37] with few modifications [37]. The cytotoxicity of each tested concentration of the test items was calculated with the help of Equation (1):

$$\% \text{ Cytotoxicity} = \{(1-X)/R\}*100 \quad \ldots\ldots (1)$$

Where, X = Absorbance of treated cells; R = Absorbance of untreated cells

The percentage of cell viability corresponding to each treatment group was calculated by Equation (2):

$$\% \text{ Cell Viability} = (100 - \% \text{ Cytotoxicity}) \quad \ldots\ldots (2)$$

The concentration exhibiting ≥70% cell viability was defined as non-cytotoxic [38].

Assessment of IL-8 using ELISA

How to cite this article: Dahryn T, Snehasis J. Anti-Rheumatoid Arthritis (RA) Action of Biofield Energy Treatment Using Synovial Sarcoma Cell Line (SW982).Ortho Res Online J. 5(1). OPROJ.000602.2019. DOI: 10.31031/OPROJ.2019.05.000602

SW982 cell suspension in DMEM medium containing 10% FBS was plated at a density of 0.1 x 10^6 cells/well/mL in 12-well-plates. The cells were incubated in a CO_2 incubator for 24 hours at 37 °C, 5% CO_2, and 95% humidity. The cells were sera starved by replacing with the DMEM medium and 1% FBS for 24 hours. After 24 hours of sera starvation, a medium was removed, treatments were provided with the test items. A test item with 900 µL was added to each well along with inflammatory stimulus IL-1β at the final concentration of 0.25ng/mL (100µL from 2.5ng/mL). After treatment, cells were incubated in a 5% CO_2 incubator for 24 hours. The level of a cytokine (IL-8) in culture supernatants of SW982 cells was determined using ELISA as per manufacturer's instructions. The absorbance of each well was taken using Synergy HT microplate reader, BioTek, the USA at 450nm.

Statistical analysis

Data were expressed as Mean ± SEM (standard error of the mean) of three independent experiments. The statistical analysis was done by statistical software, Sigma Plot (v11.0). Between two groups comparison, Student's *t*-test was performed followed by post-hoc analysis using Dunnett's test. Statistically significant values were set at the level of $p \leq 0.05$.

Results and Discussion

Cell viability assay

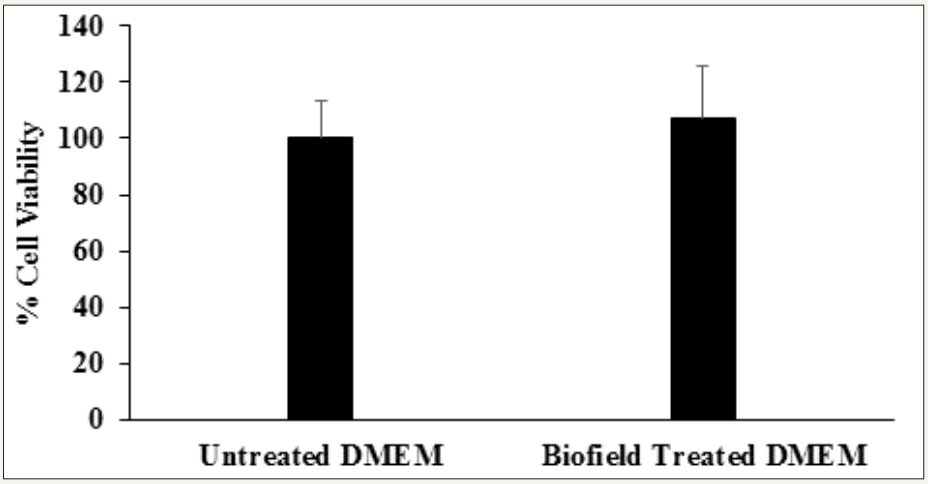

Figure 1: Evaluation of non-cytotoxic concentration of the test items (DMEM) using MTT cell viability assay in SW982 cells.

Cell viability test was performed using MTT assay in SW982 cells for testing the effect of test item on percentage cell viability. The results of MTT assay data in terms of percentage values are presented in Figure 1. The MTT cell viability assay results showed that the test items were found to have significant cell viability with more than 100%. Overall, experimental MTT data suggested that the Biofield Energy Treated DMEM was found as safe and non-toxic in nature. Thus, the test items were used to study the bone health parameter, cytokine IL-8 in SW982 cells.

Study of the test Item on IL-8 in SW982 Cells

Inflammation is one of the major factors responsible for rheumatoid arthritis (RA). Joints inflammation results in the synovial membrane and proliferation in its lining results in a severe pathological condition in RA. The inflammations of joints lead to erosion of the cartilage and bone. Macrophage-like synoviocytes and the fibroblast-like synoviocytes are the major contributors in the joint destructive process [39]. IL-8 is one of the novel cytokine, which has an important role in arthritis. IL-6 and IL-8 are the major mediators in inflammation that results in RA [40,41]. Pro-inflammatory mediator's expression was initiated by the IL-1β formed by the synovial lining macrophages [42]. The RA physiology can be controlled using a decreased level of IL-8 in joints and synovial fluids, which can be achieved using some medication or alternative therapies. Thus, the present experiment was carried out to evaluate the alteration in the level of IL-8 secretion in SW982 cells against IL-1β stimulation after 24 hours. The test item (DMEM) was treated with the Biofield Energy and results are presented in Figure 2 about the inhibitory effect of the test items on IL-8 secretion in SW982 cells. The Biofield Energy Treated DMEM group was significantly ($p \leq 0.001$) inhibits the level of IL-8 by 70.05% compared to the untreated DMEM group. As per the experimental data using SW982 cells, the results established that Biofield Energy Healing Based test item has shown significant inhibition of IL-1β stimulated IL-8 secretion that would be a beneficial and important measure in bone-health and diseases-related with joints such as osteoporosis. The Trivedi Effect®-Energy of Consciousness Healing based DMEM can be significantly used as an anti-RA activity to improve overall bone health.

Conclusion

The bone health study for anti-RA activity using SW982 cells demonstrated significant results from Biofield Energy Healing based DMEM in bone health. MTT assay showed significant improvement

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of cell viability with more than 100% that suggested that the test items were safe and non-toxic in nature. The Biofield Energy Treated test item showed significant inhibition of interleukin-8 (IL-8) by 70.05% in SW982 cells after 24 hours stimulation with the IL-1β as compared to the untreated DMEM group. Overall, the experimental data showed that the Biofield Energy Treated (The Trivedi Effect®) DMEM has a significant impact to improve the pathophysiology of rheumatoid arthritis and could significantly improve the overall bone health. Thus, the Biofield Energy Treated DMEM might be suitable for an alternative media for cell growth. It can also be useful for the management of various bone-related disorders (such as rickets, osteomalacia, osteoporosis, Paget's disease of bone, etc.), hormonal imbalance, and aging. Besides, it might be useful various immune-related disease conditions (*viz*. Irritable Bowel Syndrome, Pernicious Anemia, Ulcerative Colitis, Multiple Sclerosis, Aplastic Anemia, Alzheimer's Disease, Dermatitis, Asthma, Hashimoto Thyroiditis, Hepatitis, Graves' Disease, Diabetes, Atherosclerosis, Myasthenia Gravis, Parkinson's Disease, Systemic Lupus Erythematosus, stress, etc.).

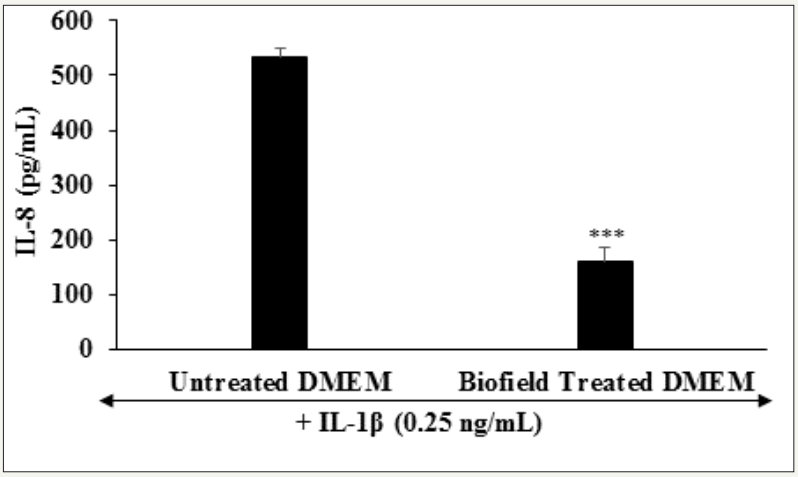

Figure 2: Effect of the test item on the level of IL-8 under stimulation with IL-1β in SW982 cells after 24 hours. $p \leq 0.001$ *vs*. untreated DMEM group.

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