

Effects of Biofield Therapy on Calcium Release in Immortalized Mouse Keratinocyte HaCaT Cells

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Abstract-Objective: Observe the response on the release of calcium into mouse keratinocyte HaCaT cells subjected to biofield therapy (BT) through a pranic healing technique. **Design:** This was a pilot experimental study. **Settings/location:** The study was conducted in a laboratory at Simon Bolivar University. **Subjects:** Mouse keratinocyte HaCaT cells. **Interventions:** The intervention consisted of a 15-minute biofield therapy using a pranic healing technique. **Outcome measures:** Cells were loaded with 5 μ M calcium indicator Fura 2-AM to monitor changes in intracellular calcium concentration. Cell population was separated into two groups: a control group where cells received no stimulation and the other experimental group where pranic healing was applied. **Results:** The cells that were treated with pranic healing showed a significant increase in intracellular calcium concentration as compared with untreated cells. Such increase in calcium concentration is consistent with the depletion of intracellular stores. By the action of Thapsigargin (TG) peak, calcium release is equivalent to cells exposed to pranic healing in comparison to control cells. One possible explanation for this observed result is that store of intracellular calcium had been stimulated by pranic healing and hence the resulting drain is lower. These results allow us to infer that pranic healing has an action on intracellular calcium storage but does not allow us to clarify how calcium has been stimulated.

Keywords- Fura-2 AM; Mouse Keratinocyte HaCaT Cells; [Ca]²⁺; Issuing Biofield Therapy (BT); Pranic Healing Technique; Information Theory; Biofield

I. INTRODUCTION

It is difficult to define complementary and alternative medicine (CAM, for short), because this is a very broad field constantly evolving. The terms of complementary/alternative/nonconventional medicine are used interchangeably, to define a set of practices that do not meet the requirements of “main stream” evidence based medicine. The World Health Organization (WHO) considers traditional medicine as: the sum of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures (whether explicable or not), used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses [1]. Some countries around the world have established institutions and governmental agencies in order to address these issues. The best example is the United States of America with the creation and operation of the National Centre for Complementary and Alternative Medicine (NCCAM) [2], which is a dependency of the National Institutes of Health (NIH). According to the NCCAM Complementary, alternative medicine is defined as a set of systems, practices and products that, in general, not considered a part of conventional medicine.

NCCAM considers Energy Based Medicine as a kind of therapeutic approach which involves the manipulation of various energy fields that affect the health. Practices based on “assumptions” of energy fields, also called biological fields (biofield), usually reflect the concept that human beings are engaged in subtle forms of energy [1, 2]. It includes procedures based on external bioenergy emission (EBE), which brings the things together: Qi gong, therapeutic touch, reiki, pranic healing and other techniques. The existence of these fields has not yet been scientifically proven, thus the basic science to give support for such practices is needed. In this article, we propose a biological model that could be used as a biosensor to prove and test such biofields.

One of the difficulties presented by the study of the therapeutic approaches of CAM and in particular the energy-based medicine has been the action of the placebo effect and the effect of suggestion on the individual. Therefore studies on Biofield Therapies (BT), such as Pranic Healing (PH), must apply the same standards to using in the design of experiments in physics, chemistry and other scientific disciplines focusing on the use of biomodels, such as cells, tissues and experimental animals [1, 2].

Energy Based Medicine posits that a human's physical body is really composed of two parts: the visible physical body and the invisible energy body. The visible physical body is the part we see and touch, the energy body is invisible, interpenetrating the visible physical body and extending beyond this [3]. The human being is a system of energies that are in constant vibration, i.e., molecules that are composed of atoms and their subatomic particles are in constant motion and interaction. For our purposes, this energy designated as prana, ki, qi, life force, pneuma, mana, ruach, “breath of life” among other terms, will be

designated with the Greek letter χ (Xi capital letter).

Pranic Healing is an ancient science of healing, using χ to heal the whole physical body. Pranic Healing is based on two laws: the law of self-healing and the law of prana (or vital energy) [3].

- The law of self-healing states that the body is able, to some extent, to heal. If a person has a wound or burn, the body will heal and recover.
- The vital power law states that the body must have χ for life to exist. The healing process can be accelerated by increasing the amount of χ in the parties' concern.

One of the major obstacles scientific researchers faced with on alternative medical practices is the placebo controversy. According to Fabrizio Benedetti and colleagues [4]: “the placebo effect is a psychobiological phenomenon that can be attributable to different mechanisms, including the expectation of clinical improvement and pavlovian conditioning”. According to [4], “subjective” constructs such as expectation and value have identifiable physiological bases, and these bases are powerful modulators of perceptual, motor, and internal homeostatic processes. The physiological bases of the placebo effect have been supported by multiple authors [5-8]. Without discussion of the inherent physiological pathways for placebo, the underlying mechanism of operation can be summarized as follows: An inducer or motivator (substance, device, expectation, conditioning, ritual, etc.), will interact with the mind of a patient, producing a “subjective construct” (SC). SC will trigger a neurohormonal response, producing a physiological outcome (Fig. 1).

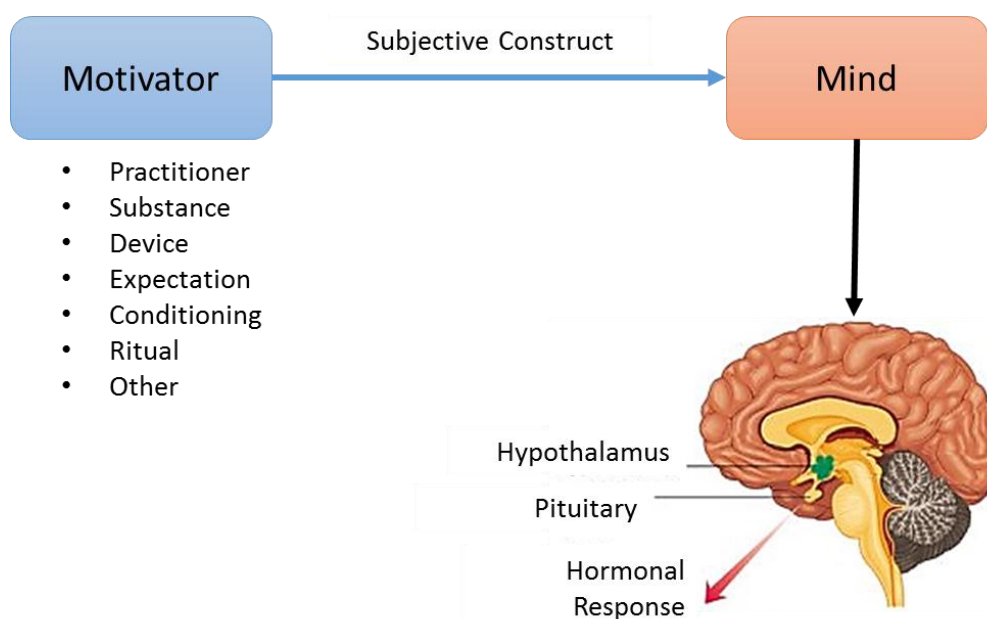


Fig. 1 Effects of cognitive expectations on neural chemical functions and physiological response (placebo effect)

As seen from Fig. 1, all of the traditional medical practices and CAM could fit in the category of motivators. In the case of traditional evidence based medicine, there is a general consensus on the fact that “medicine” interacts directly with the metabolism, producing a physiological outcome; therefore, the “motivational” aspect of medicine falls onto a second plane. The case of “Energy Based Medicine” and BT, is critical, since the sole element of therapy is a practitioner who claims that he/she can “project” a form of energy that will interact with the patient and produce a response. It is assumed that BT is purely “motivational” or nothing more than placebo. This is what we define as the Placebo Controversy, In order to break apart from this controversy; we need to strip BT from “motivational effects”, in a sense, we need to strip them from mind/body interactions.

Scientists have made several approximations [9, 10] to explain the concept of biofield and energy projection. The focus on BT raises interesting challenges to the scientific community to try to find relationships between the responses elicited in biological structures as an effect of stimulus from BT. Cellular studies with immortalized cell lines of T-lymphocyte culture show that BT application increases intracellular calcium [11], and that this fact is not related to the activation of heat shock proteins [12]. These experiments are directed towards assessing cause and effect, but the means of this cause effect relation is yet to be understood.

II. MATERIALS AND METHODS

For this study, we utilized immortalized mouse keratinocyte HaCaT cells [13]. HaCaT is a spontaneously immortalized human keratinocyte cell line, which develops through long-term culture of normal human adult skin keratinocytes at reduced calcium concentration and elevated temperature [14].

A. $[Ca^{2+}]_i$ Determinations

For the $[Ca^{2+}]_i$ measurements, the fluorescent ratiometric Ca^{2+} indicator Fura 2 was used, because its excitation spectrum depends on the concentration of the cation, while its emission peak remains invariable.

The cells were cultured in a defined culture medium containing calcium-free DMEM, 9% FBS and EGF [15]. The cells were seeded on tissue culture plastic and expanded using standard conditions for a period of two weeks. Cells showed the typical basal keratinocyte morphology. After expansion, the cells were detached by trypsinization. Isolated cells were incubated in a solution containing the following composition: 20 mM Hepes, 120 mM NaCl, 5 mM KCl, 1 mM MgCl, 1.5 mM $CaCl_2$, 1 mg / ml glucose and sodium pyruvate. pH: 7.4. Excess solution was removed and cells were incubated with 5 μ M of a fluorescent indicator, Fura 2- AM [16] in 400 ml of the same buffer for 45 min in darkness and with constant stirring. Cells were centrifuged and placed in 500 ml of the same buffer and transferred into cuvettes for the measurement [17, 18].

For the experimental setup, two cuvettes were used: a control, where cells were not stimulated; and a test cuvette, where an experienced PH practitioner projected vital energy (VE) into it. The procedure used to energize the cells is called the palms technique, where the practitioner has the intention to absorb VE with one hand, and also has the intention of projecting this energy with the other [3]. In this case, the practitioner's right hand was at an approximate distance of 10 cm from the test cuvette for a period of 15 min. As reported by the PH expert, his intention was to energize the cells inside the cuvette.

After this, the cuvette was placed for measurement of fluorescence in a fluorescence spectrometer Perkin Elmer LS-55 provided with an acquisition system for excitation ratio measurements at 29 °C with continuous agitation in a stirred cuvette. $[Ca^{2+}]_i$ were evaluated by applying the equation $[Ca^{2+}]_i = K_d \times [(R-R_{min})/(R_{max}-R)] \times F_{min}/F_{max}$, as reported by [16]; R is the fluorescent emission ratio obtained after excitation at 340 nm/380 nm; R_{max} and F_{max} are the ratios of excitation fluorescence at 340 nm/380 nm and the fluorescence of Fura 2 at 380 nm, respectively, under saturated Ca^{2+} concentrations; and R_{min} and F_{min} are the ratios of excitation fluorescence at 340 nm/380 nm and the fluorescence of Fura 2 at 380 nm, respectively, in the absence of Ca^{2+} . Maximum and minimum values were obtained after the addition of 30 μ M Thapsigargin (TG). TG is a lactone, non-competitive inhibitor of the sarco/endoplasmic reticulum Ca^{2+} ATPase pumps. TG raises intracellular calcium concentration by blocking the ability of the cell to pump calcium into the sarcoplasmic and endoplasmic reticula.

The control cuvette remained under the same conditions of temperature, pressure and light, at the same period of time (15 min), a student placed his hand about 10 cm above the cells, with the goal of "shading" the cells from the light. Shading from the light was the excuse used by the researchers, in order to replicate conditions similar to experimental conditions. Fig. 2 presents a schematic of the experimental setup.

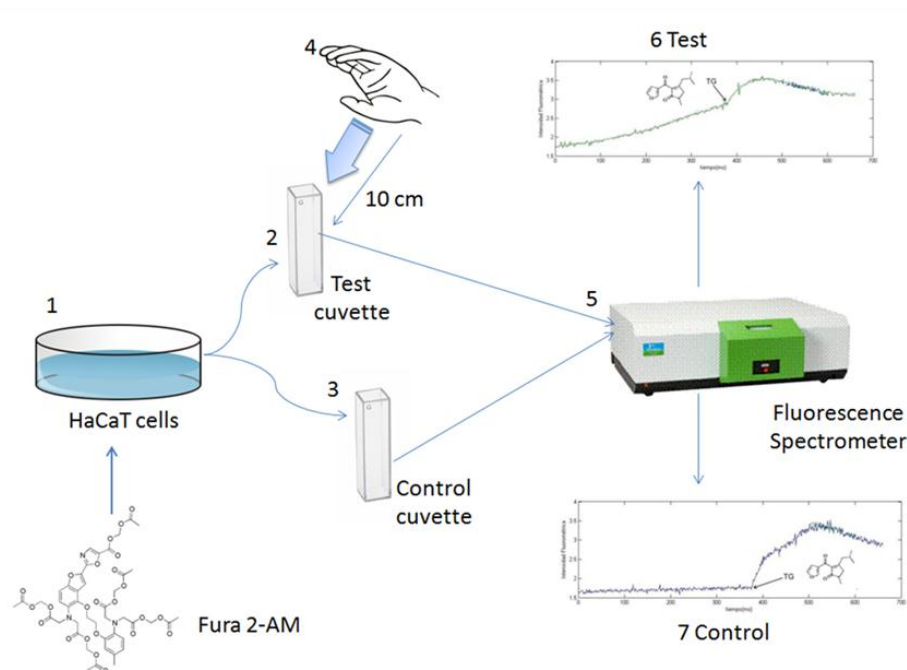


Fig. 2 Schematic of the experimental setup: Tripsinized HaCat cells loaded with Fura 2-AM (1) were placed into two cuvettes (2, 3). VE (4) was projected onto test cuvette (2). Both cuvettes (2, 3) were placed in a fluorescence spectrometer Perkin Elmer LS-55 (5). Fluorescent measurement results for test (6) and control (7). The black arrow indicates the application of Thapsigargin (TG) for both samples

The study was double blinded. Cell culture and cell preparation for fluorescent measurements were performed by a graduate student in cell biology who was not aware of the methods and procedures of BT or PH techniques. Once the cuvettes were prepared, they were handled by the authors, with the participation of the PH practitioner, and submitted to a fluorescent

technician for measurement. The technician was also not aware of the performed procedures.

III. RESULTS

Fig. 3 shows two superimposed curves, corresponding to one test and one control cuvette. Cells were loaded with 5 μM calcium indicator Fura 2-AM to monitor changes in the concentration of intracellular calcium. Cells that have not been energized (control cells, B) produced lower fluorescence intensity compared with cells that have previously been energized (test cells, A), before the application of TG.

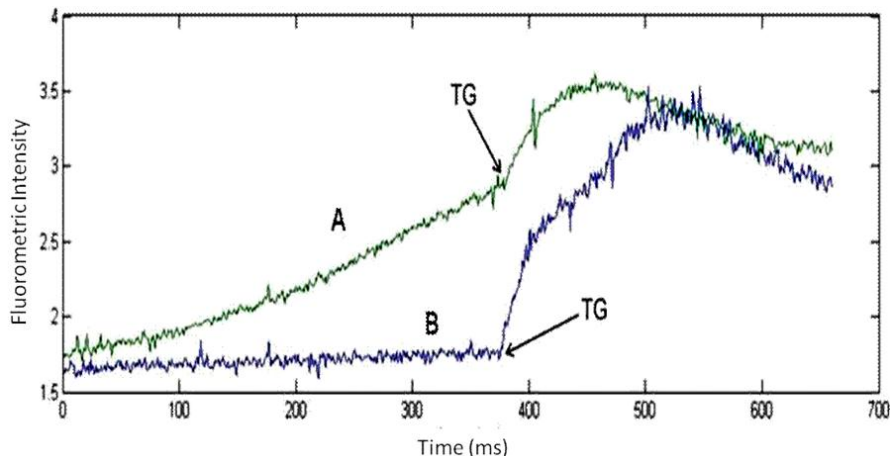


Fig. 3 Fluorescent measures for HaCaT cells loaded with Fura 2-AM. Control cells (B) and cells treated with BT (A). The black arrow indicates the application of Thapsigargin (TG)

Note that the fluorescence intensity of control cells is stable up to the point where TG is applied. With the application of TG, an increase of fluorescence intensity is observed. The quantity of intracellular calcium was computed by using the conversion factors found in [18] with $k_d = 224 \text{ nm}$. Fig. 4 shows the result of four experiments. Three points are compared: The amount of intracellular Ca at the beginning of the measurement, the amount of Ca after 400 ms, and the maximum amount of Ca^{2+} after TG application. At 400 ms, the amount of Ca^{2+} for the control group is $380,80 \pm 18,29 \text{ nm}$ while test group is $694,40 \pm 70,84 \text{ nm}$ ($R^2 1,4063\text{E-}124$), the mean slope (400 ms window) for control group is $0,1260 \pm 0,0705 \text{ nm/ms}$ while test group is $0,7000 \pm 0,0970 \text{ nm/ms}$.

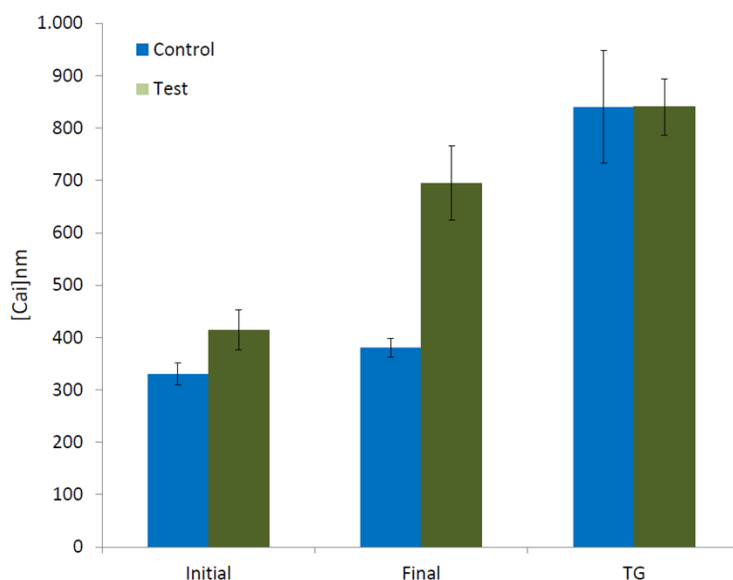


Fig. 4 Comparison of mean Ca^{2+} concentration at three points of the fluorescent measurements for four consecutive experiments: at the beginning of the experiment, 400 ms later and the maximum amount of Ca after TG application

IV. DISCUSSION

In this study, the intracellular calcium concentration $[\text{Ca}^{2+}]$ for immortalized mouse keratinocyte HaCaT cells was monitored with the fluorescent indicator Fura 2-AM. Changes in fluorescence intensity are proportional to calcium fluctuations

and can be computed using an intensity conversion factor. Cells exposed to VE showed higher basal calcium concentration compared with control cells (untreated cells). By including the student hand in the vicinity of the cells, it can be shown that the human hand is not enough to elucidate the response and that some type of stimulus is provided by the PH Practitioner. Calcium concentration increased steadily in cells treated with BT (approximately 0.7 nm/ms) as compared with control cells which remained steady until TG addition.

Both the extracellular Ca^{+2} and the one located within intracellular deposits, can contribute to increase in cytosolic concentration of the cation in response to external stimuli such as VE. Although the objective of this study was not to characterize the signaling pathways of calcium, but to know whether this elevation of intracellular calcium observed in cells stimulated with VE was related to intracellular or extracellular stores. Therefore, Figs. 3 and 4 show that the depletion of intracellular stores by blocking Ca^{+2} ATPase pumps through the action of TG, is diminished in cells that have been exposed to VE in comparison to control cells, while the final Ca^{+2} concentration is equivalent. The explanation for this observed result is that stores of intracellular calcium had been stimulated by VE and hence the resulting concentration increase is the result of stored calcium drainage and not of extracellular input. These results allow us to infer that BT has an effect on intracellular calcium but does not allow us to clarify how calcium has been stimulated. Further studies are needed with emphasis on Tris Inositol Phosphate (IP3) and Ryanodine Receptor (RyR), as these are important biological triggers for intracellular calcium pathways [17].

Calcium is an important second messenger and is involved in many cellular signaling processes; therefore the study of intracellular calcium release is an important indicator for cellular response. The involvement of intracellular calcium mobilization with implementation of BT may be a good start for experimental strategies and to provide an explanation for previously reported results [11, 12].

V. CONCLUSIONS

These studies show that the application of pranic healing can have an effect on intracellular calcium and help understand the effect of complementary therapies on cell regeneration. The article also proves that VE is not the result of motivation induced in the patient, by avoiding the Placebo Controversy. It is important to emphasize the importance of this finding since keratinocytes are part of 90% of the epidermis. The effect of pranic healing on intracellular calcium release, as a signaling mechanism for cell regeneration, suggests that the results observed by other researchers [11], [12] associated with cell regeneration may be mediated by this channel.

Pranic healing provides VE as a function of time and the effect of this energy produces a linear increase of intracellular calcium up to 400ms after initial stimulation. This VE increases the amount of intracellular calcium, hence increasing the rate of metabolism and acting as a powerful biocatalyst that can accelerate the regeneration rate. This idea is consistent with the two laws of PH: the law of auto recovery and the law of vital energy, which are both combined to say that the physical body is able to heal itself and uses vital energy for this purpose [3]. Beverly Rubik suggests that Biofields are extremely weak electromagnetic fields capable of transmitting electromagnetic bioinformation [10]. The use of HaCaT Cells as a biosensor for BT, should serve to test Rubik's or any other hypothesis about the nature of VE. Additional parameters of the VE effect should be explored, e.g. is it time-dependent? distance-dependent? reproducible by other healers? In summary, the correlation, relationship between BT and Ca^{+2} release needs to be strengthened.

ACKNOWLEDGMENTS

This work is partially supported with a grant from Venezuela Ministry of Science and Technology, called "Studies for the scientific validation", the use of Pranic Healing as monotherapy or complementary therapy on conventional animal models. Project PG-2007001522, Subproject 3. Dr. Ricardo Silva is member of the Prometeo Program, sponsored by the Secretariat for Higher Education, Science, Technology and Innovation (Senescyt) in the Republic of Ecuador.

CONFLICT OF INTEREST

When this research was performed, Dr. Herrera was Medical - Scientific Advisor in the area of Urology and Central Nervous System for GSK-Venezuela, however GSK was in no way involved or in any way related with the present research. Senescyt did not provide any additional support for the development of this research. Experiments were performed independently and the authors of the article in no way altered or modified the measured results.

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