INDIBA®

BIOMEDICAL RESEARCH ON THE INDIBA® SYSTEM AT RAMÓN Y CAJAL HOSPITAL, MADRID

SUMMARY UPDATE SEPTEMBER 2015

Sixteen years of research to further support INDIBA® medical technology that has been and continues to be a benchmark in Rehabilitation Therapy, Beauty and Aesthetic Medicine. This research underpins the valuable contribution of INDIBA® to the health and well-being of our patients.[1]

INTRODUCTION:

One of the distinguishing features of the INDIBA® system, applied in a biomedical environment, is the technical and scientific support that guarantees its continued efficacy, security and safety.

For over 16 years INDIBA® has been supporting a major research project in the BioElectroMagnetica Department of the Ramón y Cajal Hospital in Madrid.

This research, due to its high specialisation, tends to be restricted to very specific biohealth specialist fields and rarely reaches a wider target audience. However, it is of enormous importance for understanding the mechanism of action of the INDIBA® system in order to understand the reasons for the differences compared to many other technologies that claim to be, yet importantly are not, "equivalent" to INDIBA®. Furthermore this research is able to ascertain that our technology goes far beyond the specific aesthetic or medical applications in which it is currently used.

Indeed, by influencing the basic mechanisms of our natural physiology and to the extent that we are increasing our knowledge base, the INDIBA® system harnesses great potential to not only improve current results, but also for future applications that will undoubtedly contribute to improve the quality of life of our patients and users.

We present in this summary document the state of the art in the completed and ongoing investigations by the Ramón y Cajal Hospital, led by Dr. Alejandro Úbeda and Dr. María Luisa Hernández – Bule, with the aim of providing all users and stakeholders with further knowledge around the INDIBA® system.

We hope that this will be of interest to our readers and we offer all our clients this important scientific support that confirms INDIBA® the leader in biomedical research for radiofrequency technology.

Sant Quirze del Vallés, September 2015

1. EXPERIMENTAL BIOMEDICAL RESEARCH ON INDIBA® ELECTROTHERMAL THERAPY

The INDIBA® system is based on medical applications of electro thermal technology known as Capacitive-Resistive Electrical Transfer (CRET). Basically, it is a non-invasive therapy consisting of raising the temperature of targeted organs or tissues during the treatment program through the action of radiofrequency electrical currents. The empirical evidence accumulated over more than three decades has clearly demonstrated the effectiveness of INDIBA® treatments in rehabilitation, regenerative and aesthetic medicine. However the evolution of concepts in biomedicine require empirical data on the effectiveness of any therapeutic agent or strategy to be supported by knowledge of the mechanisms and processes that, at the cellular and molecular level, explain and justify both the clinical efficacy of treatment and its safety. This knowledge is only attainable through translational biomedical research. Consistent with the above, INDIBA® has been supporting since 1998 investigations into the molecular and cellular processes involved in the medical effects of its therapies.

The results obtained so far indicate that INDIBA® exerts its therapeutic action through two types of biophysical phenomena:

- Phenomena of a thermal nature, by the Joule effect due to the passage through tissues treated with electric current at a relatively wide range of radio frequencies. Although still under investigation, the therapeutic properties of heat treatments are widely described in scientific literature.
- Phenomena of an electrical nature, which generate critical responses, although more subtle than those that are thermally induced as they exert their action at the cellular and molecular level. These responses occur mainly or solely with currents with a frequency that falls within a more specific and restricted range than the radio frequencies inducing thermal effects (in the range kHz- MHz).

INDIBA®, using a frequency of 448 kHz, is capable of combining both effects.

[1] «Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity.»
Preamble to the Constitution of the World Health Organisation (WHO) as adopted by the International Health Conference, New York, 19-22 June 1946; signed on 22 July 1946 by the representatives of 61 States (Official Records of the World Health Organization, Nº 2, p. 100), and entered into force on 7 April 1948.

This makes INDIBA® a unique, electro-thermal therapy, which is distinguished from other therapies which are focused exclusively on thermal effects.

2. THE THERMAL BASES OF TREATMENTS WITH INDIBA®

- **Capacitive Mode (CAP):** In this mode, the active electrode surface in contact with the patient has an insulating layer serving as the dielectric of a capacitor. The sine wave electrical current (flow of electrons) comes from the generator and charges the capacitor. In the positive half-cycle of the wave, the metal part of the active electrode has a positive charge and the skin has a negative charge. When, in the negative half-cycle, the polarity of the applied wave is reversed, the electrons flow in the opposite direction, and what before was positively charged is now negatively charged. The rate of change of polarity is given by the frequency of the wave or signal. When the electron current reaches the tissues of the patient, these electronic currents become ionic currents. It is this flow of ions and charged molecules which collide with the immobile particles of the tissues that induces an increase in the temperature of the treated region. The temperature reached will depend on the current intensity and on the electric and anatomical characteristics of the treated tissues. The capacitive technique allows mainly access to more superficial and better vascularised anatomical areas.
- **Resistive Mode (RES)**: In this mode the active electrode does not have an insulating layer serving as the dielectric of a capacitor. Thus, the electric current supplied by the generator results in ionic currents in the tissues, producing a temperature increase by the Joule effect. In this case, hyperthermia reaches localised tissues at a greater depth than under capacitive treatments. The current in these conditions allows more fibrotic tissues to be treated, with greater resistance-impedance to current flow and worse hydration-vascularisation.

3. THE ELECTRICAL BASES OF ATHERMAL EFFECTS IN TREATMENTS WITH INDIBA®

3.1. Studies on the cell response to stimulation with INDIBA®; applications in security and safety assessment

A key priority in the development and use of any therapy is to ensure the safety of its implementation in the middle to long term. In the case of INDIBA®, the Spanish Agency for Medicinal Products and Medical Devices (AEMPS) of the Department of Health historically believed that the focus of interest with regard to possible harmful side effects should centre around the potential action at the cellular level of weak sub thermal currents, to which the tissues around the treated organ or area are exposed. AEMPS was particularly interested to study the effects of sub thermal currents in human cancer cells as this could be perceived as a serious risk with this type of technology. The BioElectroMagnetics (BEM) Department at Ramón y Cajal Hospital in Madrid was therefore commissioned to conduct experimental research to assess the potential action of INDIBA® electric currents on tumour promotion. *In vitro* studies carried out over more than 8 years on human liver cancer cells and neuroblastoma have revealed that INDIBA® currents, in athermal or subthermal conditions, do not promote proliferation in those cells, but, on the contrary, actually induce anti-proliferative responses in both tumour cell types.

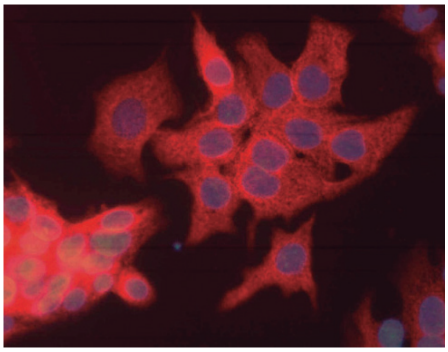

Fig. 1. Photomicrograph of HepG2 cells in culture

These responses, which would be used to support previous empirical observations on the potential effectiveness of INDIBA® as adjuvant therapy in cancer treatment, would suggest some effectiveness in slowing of the cell division cycle.

3.2. Studies on the cell response to stimulation with INDIBA®; applications in tissue regeneration of injuries and improvement of healing

INDIBA® therapy is used routinely in physical rehabilitation and sports medicine for the treatment of musculoskeletal injuries, including muscles, ligaments, joints and bones. INDIBA® has also been shown to be effective in the treatment of vascular diseases. Unlike other therapies used in regenerative medicine, INDIBA® does not induce side effects such as oedema, dermal or epidermal burns. Several clinical studies have shown that INDIBA® treatment promotes acceleration of injury repair, generally reducing the extent of the damaged area, together with analgesia and anti-inflammatory effects. These effects observed in patients could be attributable to an enhancement at the cellular level of the phenomena involved in regenerative processes. Thus *in vitro* studies using stem cells from healthy adult volunteers have shown that the electrical, sub thermal stimulation with INDIBA® significantly increases the proliferation of these cells. This increase in the population of stem cells promotes tissue regeneration by increasing the amount of cells available to repair damaged tissues. Furthermore, unlike other methods capable of proliferating stem cells, stimulation with INDIBA® does not affect the capacity of these treated cells to differentiate into (specialise in) the specific cell type of the tissue to be repaired.

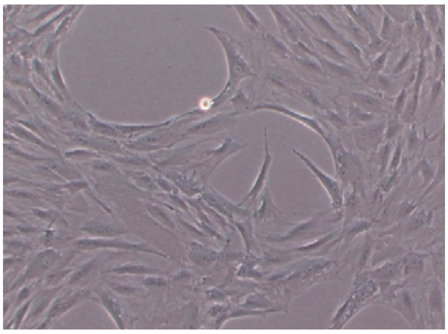

Fig. 2. Photomicrograph of phase contrast of stem cells in culture

3.3. Studies on the cell response to stimulation with INDIBA®; applications in regeneration of lesions of the musculoskeletal system

3.3.1. Regeneration of cartilage Cartilage is a tissue that, due to its intrinsic characteristics such as low proliferation rate and poor vascularisation, is highly resistant to standard regenerative treatments. Therefore, the search for new therapeutic strategies for the regeneration of cartilage is currently a matter of serious interest in medicine. Given that there is indicative empirical data that INDIBA® is effective in repairing cartilage lesions, it is reasonable to ask whether, in addition to its general effect of inducing a proliferative response in stem cells, INDIBA® could favour regeneration of cartilage by promoting: A) the differentiation of stem cells into actual cartilage cells, known as chondrocytes, and/or B) synthesis by chondrocytes present in the tissue of specific macromolecules of the extracellular matrix of cartilage. Experimental results have provided evidence that stimulation with INDIBA® could exert both actions at the cellular level. Thus, it has been observed that after electrical treatment stem cells have an increased expression and activation of Sox5, one of the key molecules involved in chondrogenesis and cartilage formation. It was also found that in human stem cells INDIBA® promotes macromolecular synthesis of the cartilage matrix, such as chondroitin sulphate and other glycosaminoglycans (GAG). The induction of both responses, either simultaneous or sequential, linked to the proliferative effect described above, would mean significant acceleration of wound repair in tissues with very poor capacity for regeneration.

3.3.2. Regeneration of bone The same reasoning outlined in the previous section is largely valid in posing the possibility that INDIBA® accelerates the repair of bone tissue damage, whether traumatic or degenerative. The preliminary results in this area have shown that in stem cells undergoing differentiation into osteoblasts (bone cells), stimulation with INDIBA® increases alkaline phosphatase activity and the formation of calcium deposits in cultures. Since both responses are clearly indicative of tissue mineralisation, it is proposed that INDIBA® treatment could promote bone repair through the formation of bone tissue mediated by mineralisation acceleration.

3.4. Studies on the cell response to stimulation with INDIBA®; applications in anti-cellulite and anti-adipogenic treatments

Empirical evidence shows that INDIBA® is also effective in aesthetic medicine treatments including reduction and modelling of abdominal adiposity or cellulite reduction. The cellular and molecular processes underlying the response to such treatments are studied experimentally in stem cells obtained from the adipose tissue of healthy adult donors. The aim of the studies is to establish whether INDIBA® treatment is capable of: A) interfering with the formation of new fatty tissue (adipogenesis) and/or B) enhance the destruction of already formed adipose tissue (lipolysis). The results obtained *in vitro* so far have shown that INDIBA® treatments reduce the amount of fatty acids synthesised and accumulated by stem cells during the early stages of their differentiation into fat cells. This reduction of fatty acids is mediated by a subexpression (decrease in expression, or depression in function) of molecules that promote the formation of fatty tissue such as PPAR (P-pair gamma). Moreover, besides the above, in more advanced stages of differentiation of stem cells it has been found that the INDIBA® stimulus induces the reduction of the expression of perilipin, a protective molecule of fat vesicles present in the cell cytoplasm. This result is an indication that the INDIBA® treatment could also induce degradation or mobilisation of fat in mature adipose tissue. In fact, the drop in the expression of perilipin would facilitate the action of lipases, enzymes that degrade lipids in fat cells, thus reducing fat in the treated tissues.

In short, experimental stem cell studies have shown evidence that, in adipose tissue formation, INDIBA® inhibits the synthesis and accumulation of intracellular fat, whilst in adipose tissue more advanced in maturation; the electrical treatment may favour the destruction of fat present inside the cells.

3.5. Studies on the cell response to stimulation with INDIBA®; applications in other reaffirmation and remodelling treatments

The effects described in the previous sections, promotion of the differentiation and proliferation of stem cells, or extracellular matrix synthesis, could, by deepening specific research in the respective fields, provide basic evidence to INDIBA® applications in other therapies, such as those aimed at reaffirming and/or remodelling tissues, for treatment due to injuries, degeneration or pre/post surgical procedures.

ADDENDA

A1. Specificity of the INDIBA® frequency

We have said before that capacitive-based therapies use thermal signals covering a wide frequency range from kHz to several MHz, while the therapeutic effects of electrical stimulation would take place on a more restricted range of frequencies. The INDIBA® frequency, capable of combining thermal and electrical action, is at the specific frequency of 448 kHz. Thus, experimental studies *in vitro* have been conducted, in athermal conditions, comparing the cell response to two different frequencies within the range of application of INDIBA®. While a 570 kHz signal induced an exclusively anti-proliferative response in human neuroblastoma cells, a frequency of 448 kHz caused, besides the anti-proliferative effect, a cytotoxic response (death by apoptosis) in the same cell type. This data would support the specificity of the frequency in the induction of cell responses of an athermal or subthermal nature.

A2. The anti-proliferative response in human cancer cells versus stem cell proliferative response - A contradiction?

In vitro studies with INDIBA® stimulation have shown two different effects on two different types of human cells: anti-proliferative effects on human cancer cells and proliferative effects on stem cells. At face value these effects could be misinterpreted as antagonistic or contradictory.

However, it must be highlighted that regulation of proliferation is altered in cancer cells. Importantly, cancer cells lack the mechanisms controlling cell division possessed by normal cells, resulting in uncontrolled growth of cancer tissue and tumour formation. INDIBA® treatment acts by enhancing the expression of key molecules for cell cycle control, such as p53, thereby slowing down the cycle of division of these cancer cells. In contrast, stem cells in the mechanisms of cell cycle regulation are still intact and keep the proliferation rate under control. In these normal stem cell cultures INDIBA® treatment enhances proliferation through activation of the P-ERK1/2 protein without disrupting normal cell cycle regulation and the ability of treated cells to differentiate (multipotency) to the corresponding specialised cell forms.

The parallelism in the responses of the two cell types is further validated when the INDIBA® treatment is applied to senescent stem cells, which have developed genetic abnormalities and show disorders in the control of their division cycle. Under these conditions, which are similar to those of the cancer cells, the treatment induces in stem cells an anti-proliferative effect similar to that presented by cancer cells.

A3. The electro-thermal synergy. It should be recalled in this point that the experimental studies summarised herein relate primarily to the electrical, not thermal, effects of INDIBA®. We have seen that these effects constitute signs of cell responses to therapeutic applications in oncology, in the repair and healing of various tissues, and in medical aesthetic treatments to reduce adiposity. But apart from its action of an electrical nature, INDIBA® is also a heat treatment and applications of hyperthermia in oncology, tissue regeneration and removal of subcutaneous fat, have been known for some time. Accordingly, it is proposed that the outstanding efficiency of INDIBA® in its applications for the therapies mentioned here could be attributed to synergies between the two simultaneous actions of INDIBA® treatment, that of an electrical origin and that of a thermal nature.

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