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Original Article

Electromagnetic information delivery as a new tool in translational medicine

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Abstract: Some experimental evidences of the procedure defined as electro-magnetic information delivery, mediated through aqueous system, have accumulated in the last two decades. The present work is based on the hypotheses that an aqueous system like those enfolded in livings, could play an additional synergic role in modulating biological functions. Aqueous system could generate dissipative structures under appropriate patterns of electromagnetic signals providing basis for storing and retrieving biologic activities. External electro-magnetic stimuli in resonant conditions with some of the coherent domains of water can induce dipole moments re-patterning in a way that these structure start to oscillate coherently each other generating a new phase correlation. This procedure allows to an external electro-magnetic stimulus to be stored, translated and transferred by the aqueous systems to the biological target, driving selectively their endogenous activity mimicking the effect of a specific source molecule. Signals from a chemical differentiation agent such as Retinoic Acid (RA) was captured and transferred to the target culture medium of Neuroblastoma Cell Line (LAN-5) and the proliferation rate was assessed, in order to investigate cell responses to electromagnetic information system.

Keywords: LAN-5, neuroblastoma cell line, electromagnetic medicine, biophysical information therapy, water coherence domains, electro-magnetic Information delivery

Introduction

Aqueous system is universally assumed as the basis for any living process. Szent-Gyorgyi suggested that water could be considered as the forgotten matrix of life [1] and very likely it plays a key role in the development of many pathologies including cancer [2]. Nevertheless only recently the role of water has been reconsidered as more than a simple solvent and has been established that aqueous system could play an active role in the architecture and function of cell and tissues [2-5].

Our hypotheses is that an aqueous system, such one of those enfolded in livings, could play an additional role in modulating biological functions providing basis for processing, storing and retrieving information mediated by electromagnetic signals mimicking the effect of a specific drug or driving a specific endogenous function [6, 7].

Liquid water shows many anomalies in its thermodynamics properties such as compressibility, density variation and many others. Some of these features are more evident at low temperatures but they are still present at room temperature were living systems exert their biological activities. At ambient conditions our traditional view of water is a homogeneous distribution of tetrahedral structure hydrogen bonded. In spite of this very simple description a more complex picture arises from recent report identifying inhomogeneous structures even at ambient condition [8-10]. This picture could fit the concept of coherent domains as previously described by The Italian physicist Giuliano Preparata applying the Quantum Electrodynamic Theory (QED) to the understanding of water and of biological systems behaviour [11].

According to QED liquid water can be viewed as equilibrium between two components: the coherent and the incoherent one. The coherent

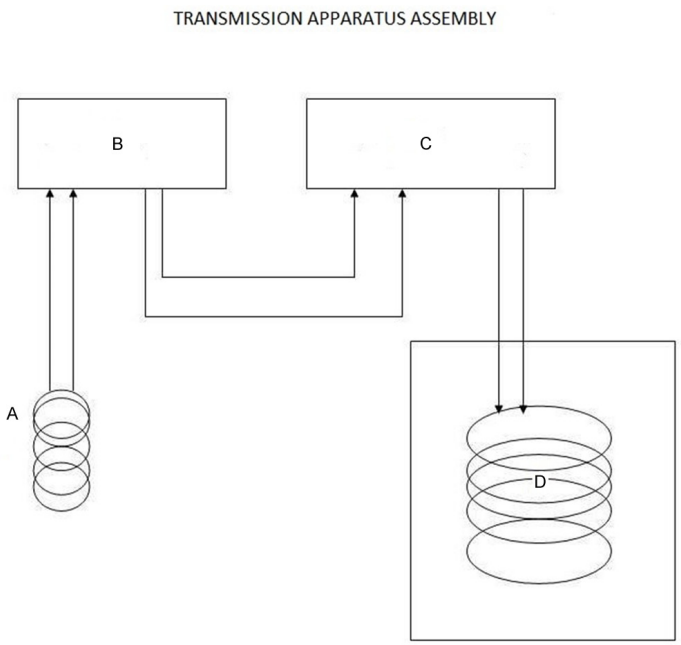

Figure 1. Transmission apparatus assembly. A. Input coil; B. Wave generator; C. Amplifier; D. Output coil.

component is contained within spherical, so called "Coherence Domain" (CD), where all water molecules synchronously oscillate with the same phase and emit coherent electromagnetic radiations. Coherence Domains are surrounded by the incoherent component where water molecules oscillate in random phases. In this framework an aqueous system such one enfolded in livings could play an additional role in modulating biological functions by generating dissipative structures able to became a resonant antenna, providing basis for processing, storing and retrieving information mediated by electro-magnetic signals [6, 7, 11, 12].

Any electro-magnetic signals, both endogenous and exogenous, when became resonant with the frequencies of the coherent domains of water can induce a dipole moments repatterning therefore inducing these structure to oscillate coherently each other generating a new phase correlation defined as super-coherent [13]. This procedure could allow to an external pattern of electro-magnetic signals to be stored, translated and transferred by the water structure of the aqueous systems toward the biological target selectively modulating their activity. Actually, some experimental evidence of the process defined as electro-magnetic information delivery mediated through aqueous system has accumulated in the last two decades [14-21].

Neuroblastoma (NB) is the most common malignant solid tumor of childhood and the most common cancer in infancy. This cell line is an appropriate "*in vitro*" model for investigating the mechanisms of neuronal death and their relation to differentiation. In particular, several papers have shown, that the Retinoic Acid is able to reduce the tumorogenicity of these cells through modulation of their neuronal differentiation and cell proliferation [22].

In order to confirm and clarify our previous hypotheses that aqueous system could be able to store and transfer specific information to a biological target mediated by electromagnetic protocols we report in these paper additional evidences [20, 21, 23].

Signals from a chemical differentiation agent such as Retinoic Acid was captured and transferred to the target culture medium of Neuroblastoma Cell Line (LAN-5) and the proliferation rate was assessed, in order to investigate cell responses to electromagnetic information system.

Materials and methods

Cell culture

The LAN-5 neuroblastoma cells were grown in monolayer culture on 25 cm^2 plastic culture flasks, using RPMI supplemented with 10% fetal bovine serum (Euroclone), 2 mM glutamine (Sigma), 1.0 unit/ml penicillin (Sigma), and 1.0 mg/ml streptomycin (Sigma), in a humidified incubator with 95% air and 5% CO_2.

Growth curves

Cells were seeded at density of 10 x 10^3 cells/cm^2 on 25 cm^2 plastic culture flasks. After 24 h the signals from a standard Retinoic Acid solution was captured and transferred to the target culture medium by a commercially available oscillator (Vegaselect 719). For each experimental condition, cells were grown for 4 days. At day 1, 2, 3 and 4, cells were harvested with

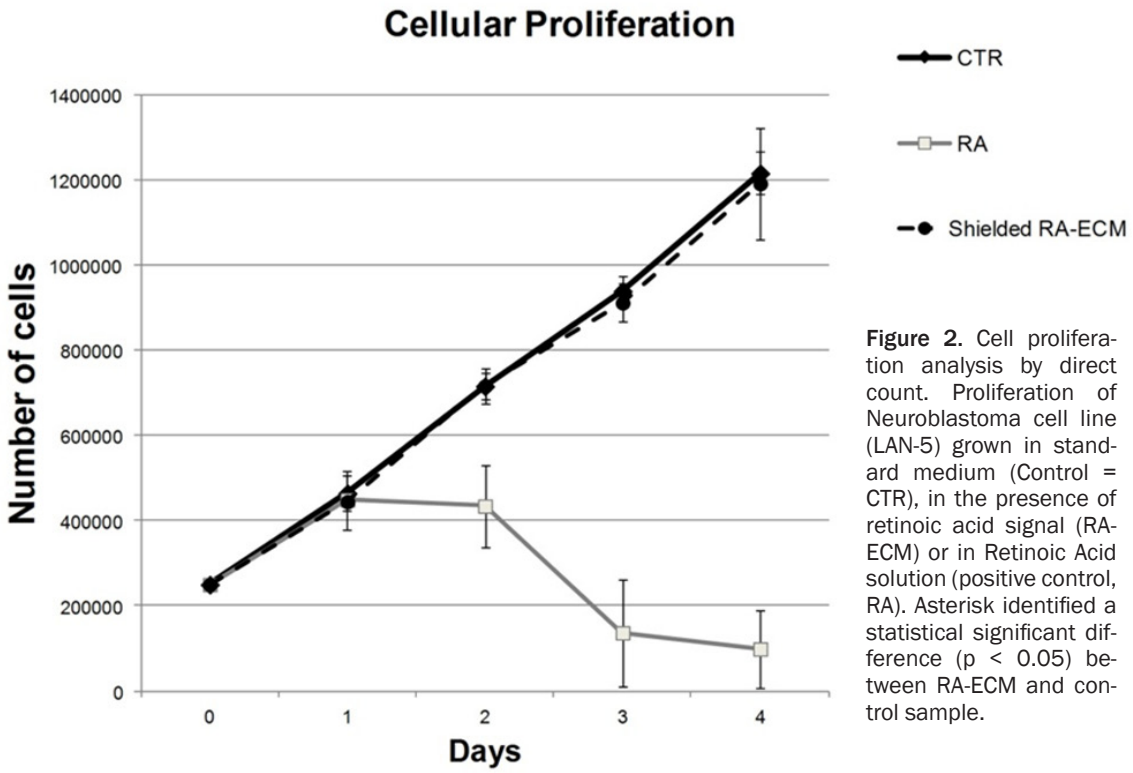

Figure 2. Cell proliferation analysis by direct count. Proliferation of Neuroblastoma cell line (LAN-5) grown in standard medium (Control = CTR), in the presence of retinoic acid signal (RA-ECM) or in Retinoic Acid solution (positive control, RA). Asterisk identified a statistical significant difference ($p < 0.05$) between RA-ECM and control sample.

0.1% trypsin-EDTA (Sigma), washed twice with PBS and the total number of nucleated and viable cells was counted by Trypan Blue dye (0.4%) (Sigma) exclusion assay using a Bürker hemocytometer chamber. Each experiment was repeated three times and the significance level adopted for all analyses was $P < 0.05$.

Transmission apparatus and cell medium conditioning

For the transmission experiments to cell's medium, the input coil was operated at room temperature and was coupled via a homemade amplifier (Gain 0.25 dB from 1 to 100 Hz maximum output voltage 20 V p-p, maximum output current 1A, Max Power 20 W rms) to a commercial available wave generator (VEGA Select 719). Into the output (target) coil was placed the cell's culture medium. The target coil was made of 85 turns of 2 mm copper wire, 17 cm long and 9.5 cm width and fed at 100 mV from the wave generator. The source tube containing 5 μM RA was placed inside the input coil. The signal from the Retinoic Acid (RA) solution in the coil (**Figure 1A**) was fed into the electronic amplifier (**Figure 1B**), then from the electronic amplifier (**Figure 1C**) the signal was transferred to the wave generator. In the wave generator the electronic signal corresponding to RA was superimposed over a 7 Hz sinusoidal frequency carrier modulated at 3 kHz as previously reported [20, 21]. From the wave generator then, the signal was delivered to the culture medium (**Figure 1D**). During the entire experimental procedure all the electrical parameters remained constant.

Statistical analysis

Statistics was performed with Student's t-test with $P < 0.05$ as the minimum level of significance.

Results

Electromagnetic signals from Retinoic Acid solution affect cell proliferation

In order to investigate cell responses to electromagnetic information system, signals from a standard Retinoic Acid solution was captured and transferred to the target culture medium by a commercially available oscillator (Vegaselect 719).

Neuroblastoma cell line (LAN-5) was grown up for 4 days in standard medium (Control = CTR) or in the presence of Retinoic Acid signals

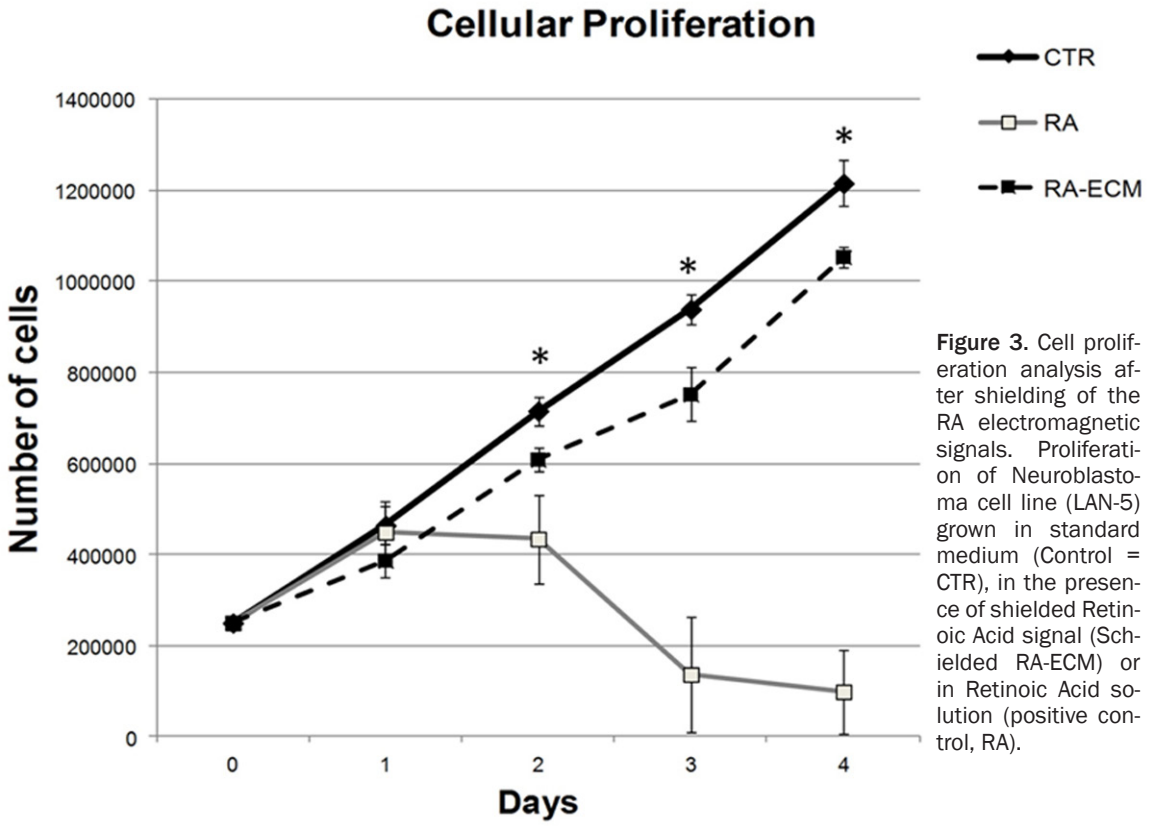

Figure 3. Cell proliferation analysis after shielding of the RA electromagnetic signals. Proliferation of Neuroblastoma cell line (LAN-5) grown in standard medium (Control = CTR), in the presence of shielded Retinoic Acid signal (Schielded RA-ECM) or in Retinoic Acid solution (positive control, RA).

(RA-ECM); Retinoic Acid molecules were also used to treat the cells as positive control (RA). Cell proliferation was analysed by direct cells count (**Figure 2**). The results showed that treatment with retinoic acid dramatically decreased LAN-5 cell proliferation. Interestingly, the cells cultured in an electronically conditioned medium, therefore receiving physical electro-magnetic information of RA, presented a statistical significant reduction in the proliferation rate of about 20% compared to control cells.

Shielding of the electromagnetic signals remove the inhibition of cell proliferation

To further confirm the electromagnetic nature of the conditioned medium effect on cell proliferation, we shielded the electromagnetic information signal that very likely is emitted from a standard Retinoic Acid solution. LAN-5 neuroblastoma cell line was grown up for 4 days in standard medium (CTR) or in the presence of shielded retinoic acid signal (Shielded RA-ECM); Retinoic Acid molecule was used as positive control (RA). Cell proliferation was then analyzed by direct cells count (**Figure 3**). The results showed that LAN-5 cultured with the shielded electronically conditioned medium didn't present any changes in the proliferation rate compared to control.

Electromagnetic signals from Retinoic Acid do not affect cell viability

We found out that reduction in cell proliferation rate is correlated with the electromagnetic information system, while did not correlate with an increase in cell death. LAN-5 neuroblastoma cell line was grown up for 4 days in standard medium (CTR) or in the presence of Retinoic Acid signals (RA-ECM) while Retinoic Acid molecule was used as a positive control (RA). Cellular mortality was analyzed by Trypan blue exclusion test (**Figure 4**). The results showed a sustained increase of cellular mortality in Retinoic Acid treated cells as compared to control ones. Moreover the cells cultured in the electronically conditioned medium, receiving physical electro-magnetic information from RA, displayed no differences in cellular mortality compared to control.

Discussion

Low frequency electromagnetic fields at 50 or 60 Hz indeed are reported to stimulate nerve

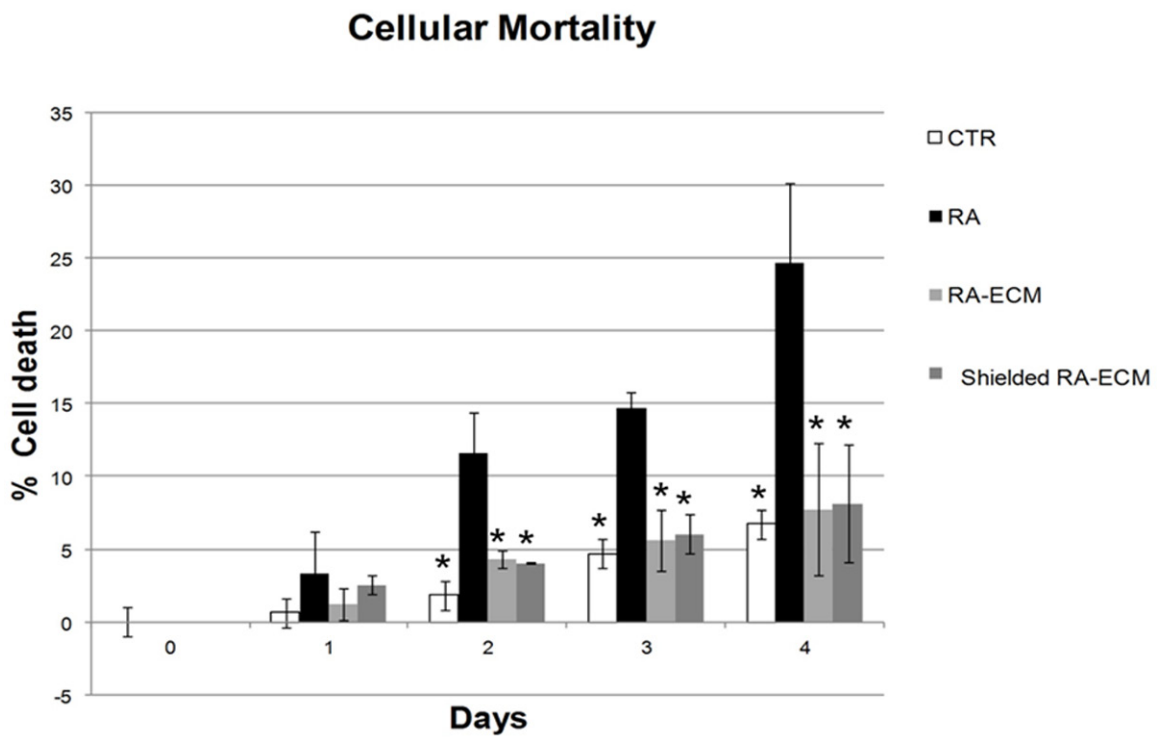

Figure 4. Cellular mortality analysis by direct count. Mortality rate of Neuroblastoma cell line (LAN-5) grown in standard medium (Control = CTR), in the presence of retinoic acid signal (RA-ECM), shielded Retinoic Acid signal (shielded RA-ECM) or in Retinoic Acid solution (positive control, RA). Asterisk identified a statistical significant difference ($p < 0.05$) referred to RA sample.

regeneration, alter gene transcription and they may also play a synergistic role in cellular processes that are already activated, such as cell proliferation [20]. Despite an increasing number of publications demonstrating an effect of very low frequencies EM field on biological systems, other in vivo and in vitro studies suggest opposite results; in addition the possible interaction mechanism is not yet completely understood.

Possible mechanisms evoked to explain the mechanism of EM field action to biological system is involving Ca^{2+} transport across cell membrane, to trigger the signal transduction cascade [20].

Electromagnetic therapeutic potential can be seen in the proven efficacy of low-energy pulsed magnetic fields in non-union bone fracture healing, confirming that under certain conditions non-ionising electro-magnetic energy can influence physiological processes in organisms. Physiological paradigms for non-ionising radiation effects are required. Clues may be found in the mechanisms by which EM field interacts with cultured cells under controlled laboratory conditions and by correlating in vivo evidence with in vitro data [21]. Brain maturation depends on a sequence of postnatal events. Brushart et al. found that electrical stimulation at 20 Hz, promote motoneuron regeneration, confirming previous finding of the use of electric field for the orientation and growth of neurite [21].

Neuroblastoma (NB) is the most common malignant solid tumor of childhood and the most common cancer in infancy. It is a neuroendocrine tumor deriving from neural crest of the sympathetic nervous system [24]. A variety of drugs and biological agents have been reported to induce differentiation of NB cells "*in vitro*" and "*in vivo*" [22, 25-29]. This neural crest-derived tumor maintains its capability to differentiate along physiological phenotype and can occasionally undergo "*in vivo*" spontaneous or chemically-induced maturation or regression [30]. A mechanism of tumor regression can involve maturation of neoplastic cells into terminally differentiated, non-proliferating, ganglion-like cells. A second mechanism, strongly

associated with differentiation and reported even for non-neuronal models [29, 31] may involve the induction of programmed cell death, through apoptosis.

Since several papers have shown, that Retinoic Acid is able to reduce the tumorogenicity of these cells through modulation of their neuronal differentiation and cell proliferation [22, 32], we decided to use this well-defined chemical differentiation agent to mimic its effect on Neuroblastoma cell line (LAN-5), even through its electromagnetic information delivery. In this study, we demonstrated that the electro-magnetic signals from Retinoic Acid molecules can be recorded and stored by the aqueous system of the cell culture medium. LAN-5 cells was grown up to 4 days in standard medium (Control = CTR) or in the presence of Retinoic Acid signals (RA-ECM). The cell treated with standard chemical Retinoic Acid solution was also used as a positive control (RA). Cell growth and mortality, analyzed by direct cell count using Trypan Blue dye exclusion assay, showed that treatment with chemical Retinoic Acid dramatically decreased LAN-5 cell growth and increased cell mortality compared to control ones (**Figures 2** and **4**). Interestingly, cells grown in presence of the electro-magnetic signal from RA (RA-ECM), showed a statistical significant decrease of cell growth, similarly to RA treatment, but no changes in cellular mortality (**Figures 2** and **3**). These findings suggest that the electromagnetic information system is able to induce the decrease of cell growth without affecting cell viability.

In order to assess the electromagnetic nature of the conditioned medium effect on cell proliferation, we shielded the Retinoic Acid solution source. The source tube containing RA placed inside the input coil was enrolled with aluminum foil. LAN-5 neuroblastoma cell line was grown up for 4 days in standard medium (CTR) or in the presence of shielded signal from Retinoic Acid (Shielded RA-ECM). The results showed that LAN-5 cultured with the shielded electronically conditioned medium didn't present any changes in the proliferation rate as compared to control (**Figure 3**). Very likely, the shielding procedure prevented the transfer of specific biological activity to the target culture medium. Therefore, these results directly suggest the involvement of the electromagnetic signals in the transmission of bio-mimetic effect.

Conclusions

These "*in vitro*" findings suggest a possible future "*in vivo*" application of electro-magnetic information delivery protocols for the synergic treatment of a wide range of human diseases. By means of biomedical technologies delivering specific informative electromagnetic frequency patterns, through and to aqueous system, could provide an integrative tool in clinical practice. The possibility to induce differentiation elicited by our system through extremely low frequency electromagnetic field represent an effective, minimally manipulating, and safe biotechnological tool to improve neurogenic differentiation in neurodegenerative diseases.

Disclosure of conflict of interest

No conflict of interest.

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