

Electromagnetic Information Transfer of Specific Molecular Signals Mediated through Aqueous Systems: Experimental Findings on Two Human Cellular Models

Alberto Foletti¹, Settimio Grimaldi², Mario Ledda², and Antonella Lisi²

¹Independent Researcher, Lugano, Switzerland

²Institute of Neurobiology and Molecular Medicine, CNR, Rome, Italy

Abstract— Electromagnetic Information Transfer (EMIT) of Specific Molecular Signals according to previous report [3, 5] and ours [1] is having his serious experimental evidence.

The aim of the present work is to understand the possible role of water in mediating the electromagnetic information transfer of biological active molecules [5] such as retinoic acid (RA).

The electromagnetic information signals from the retinoic acid solution (RA-EMIT) was captured and transferred to the target as previously described by a commercially available oscillator (Vegaselect 719). In the present study the retinoic acid signals was first transferred to the cell's culture medium (RA-EMIT conditioned medium) then cells (LAN-5 neuroblastoma and NT2/D1 human teratocarcinoma) cells seeded with RA-EMIT off.

The same experiment was repeated culturing cells under continuous RA-EMIT conditions.

As an overall control the direct differentiating effect of RA solution on cell's culture was reported.

These experimental findings demonstrated that the RA-EMIT conditioned medium behave like retinoic acid thus inducing cell differentiation in both cell's lines.

1. TRANSMISSION APPARATUS

For transmission experiments (Fig. 1) to cells, the input coil coupled to wave generator VEGA select 719 was operated at room temperature, while the output coil was placed in cell incubator. The source tube containing 5 μ M RA and target coil containing LAN-5 cells. The electronic signal corresponding to RA was superimposed to both a 7 Hz sinusoidal frequency carrier modulated at 3 KHz.

The oscillator was then turned on for 12 hrs a day for 5 days. During the experimental procedure, the various parameters such as power, voltage, capacitance and impedance remained constant.

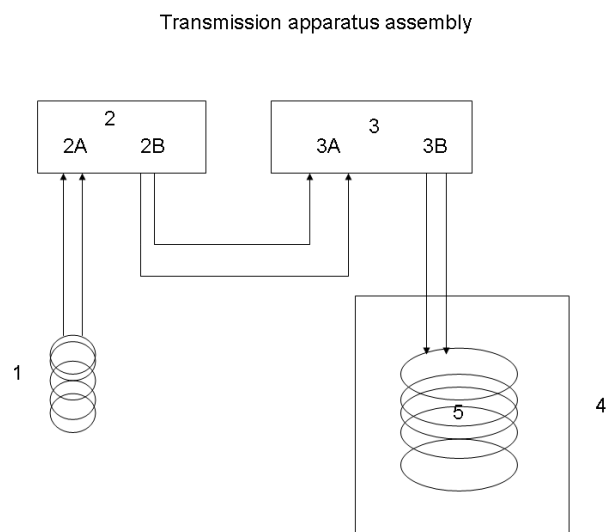


Figure 1: Experimental apparatus and assembly. 1: Source signal coil, 2: Electronic amplifier, 2A: input signal in the electronic amplifier, 2B: output signal from the electronic amplifier, 3: wave generator, 3A: input signal in wave generator, 3B: output signal from wave generator, 4: cell incubator, 5: target coil.

2. CONDITIONED EXPERIMENTS

For conditioned experiments cells medium were first continuously exposed to RA-EMIT for 24 hrs, than RA-EMIT were turned off LAN-5 and NT2/D1cells were seeded and cultured to the end of the experiment.

3. CELLULAR METABOLIC ACTIVITY AND PROLIFERATION BY WST ASSAY

LAN-5 and NT2/D1cells were exposed to the electronically transmitted RA EMS by Vega select 719 and to conditioned medium. For each experiment LAN-5 and NT2/D1 cells were plated into 25 ml 4.2×5.2 cm base Corning flasks (2.0×10^5 /ml cells in a total volume of 5 ml). The flasks were kept in the exposure system continuously for up to 5 days with or without RA-EMS with or without conditioned medium. Cells were then counted and metabolism determined by WST-1 method. The experiment was repeated three times.

The quantification of LAN-5 and NT2/D1 metabolic activity, as an index of cellular proliferation, was performed by a colorimetric assay based on oxidation of tetrazolium salts (Cell Proliferation Reagent water-soluble tetrazolium salt (WST)-1; Roche Diagnostics, Basel, Switzerland). Cells were cultured for up to 5 days in a normal humidified incubator (control) or in the presence of the RA-EMS (exposed), and they were analysed by means of the formazan dye every 24 h. WST reagent diluted to 1 : 10 was added in the wells at 4 h, 1, 2, 3 and 6 days after plating, and then incubated for 2 h in humidified atmosphere (37 °C, 5% CO₂). Quantification of the formazan dye produced was performed by absorbance measurement at 450 nm with a scanning multiwell spectrophotometer (Biotrack II; Amersham Biosciences, Little Chalfont, UK).

4. STATISTICAL ANALYSIS

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

5. RESULTS

5.1. Electronically Transmitted RA-EMIT and Conditioned Medium Effect on LAN-5 and NT2/D1cell Metabolism

The cell growth rate was analyzed by the WST-1 both in LAN-5 and NT2/D1cells as control (not exposed electronically transmitted RA) or exposed to the field with or without conditioned medium. An inhibition in the cell metabolism in the electronically transmitted RA (RA-EMIT) exposed and conditioned medium exposed was statistically ($p < 0.01$) significant after 5 days exposure (Table 1).

LAN-5 and NT2/D1 metabolic activity by WST-1 analysis in presence of RA-EMS and conditioned medium compared with RA as control.

6. DISCUSSION

Low frequency electromagnetic fields at 50 or 60 Hz indeed are reported to stimulate nerve regeneration, alter gene transcription [4] and they may also play a synergistic role in cellular processes that are already activated, such as cell proliferation. Despite an increasing number of publications demonstrate 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.

A possible mechanisms evoked to explain the mechanism of EM field action to biological system is involving Ca²⁺ transport across cell membrane, to trigger the signal transduction cascade.

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

Table 1: Cellular metabolic activity and proliferation by WST assay.

LAN-5 and NT2/D1 metabolic activity by WST-1			
Cells line	Control	RA-EMIT	conditioned medium
Lan-5	1	0.7	0.5
NT2/D1	1	0.66	0.51
±S.D			

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. Brain maturation depends on a sequence of postnatal events. The electromagnetic information signals from the retinoic acid solution (RA-EMIT) was captured and transferred to the target as previously described by a commercially available oscillator (Vegaselect 719). The retinoic acid signals was first transferred to the cell's culture medium (RA-EMIT conditioned medium) then cells (LAN-5 neuroblastoma and NT2/D1 human teratocarcinoma) cells seeded with RA-EMIT off.

As an overall control the direct differentiating effect of RA solution on cell's culture was reported.

These experimental findings demonstrated that the RA-EMIT conditioned medium behave like retinoic acid thus inducing cell differentiation in both cell's lines demonstrating a significant effects on cells proliferation leading to a 30% inhibition of cell metabolism.

Taken together all these data support an evident effect of the electronically transmitted retinoic acid (RA-EMS) electromagnetic field of driving LAN-5 and NT2/D1 cells toward a neuronal differentiation, which resembles the effect determined by morphogens, such as retinoic acid in its chemical form. 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.

The physical nature of the nanostructures which support the EMS resonance remains to be determined. Several speculation at this time can proposed as a model to explain the effect of RA-EMIT conditioned medium on cells differentiation ranging from self maintained water structure due to RA-EMIT conditioned medium exposure as well as an alteration between free and bound calcium in RA-EMIT conditioned medium.

REFERENCES

1. Foletti, A. and S. Grimaldi, "Differentiation of human LAN-5 neuroblastoma cells by electronically transmitted retinoic acid (RA)," *PIERS Online*, Vol. 6, No. 6, 518–522, 2010.
2. Lo, S. Y., X. Geng, and D. Gann, "Evidence for the existence of stable-water-cluster at room temperature and normal pressure," *Physics Letters A*, Vol. 373, 3872–3876, 2009.
3. Montagnier, L., J. Aissa, S. Ferris, J.-L. Montagnier, and C. Lavallée, "Electromagnetic signals are produced by aqueous nanostructures derived from bacterial DNA sequences," *Interdisciplinary Sciences Computational Life Sciences*, Vol. 1, 245–253, 2009.
4. Preparata, G., "QED and medicine," *Rivista di Biologia*, Vol. 93, 467–512, 2000.
5. Thomas, Y., M. Schiff, L. Belkadi, P. Jurgens, L. Kahhak, and J. Benveniste, "Activation of human neutrophils by electronically transmitted phorbol-myristate acetate," *Medical Hypotheses*, Vol. 54, 33–39, 2000.