The Wedding of Bioelectromagnetic and Biochemistry: Bridging a Molecule and Its Own Electromagnetic Information

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Abstract— Previously we showed an interesting effectiveness of the electromagnetic information delivery of a specific source molecule in mimicking its specific biological effect. Nevertheless the effect was quite lower than the original positive control. Therefore we decide to assess a possible synergism between a reduced dose of a molecule and its own electromagnetic information in order to increase the efficacy of this procedure trying to disclose new possible avenues in translational pharmacology following suggestion from other works reporting synergistic effect between molecules and extremely low frequency electromagnetic fields. A reduced dose of retinoic acid, corresponding to a tenth of the usual, was delivered together with its own electromagnetic information to LAN-5 neuroblatoma cells. The signals from retinoic acid molecules was transferred to the cell culture medium employing as for our previous reports a commercial available electro medical device (Vegaselect 719). The effect on cell differentiation was significantly higher, and statistically significant, than the one obtained by the electromagnetic information delivery procedure when performed alone. A positive and effective synergism between a reduced drug dose and its own electromagnetic information seems to emerge as a promising and useful perspective in translational pharmacology outlining future applications in new drug delivery protocols allowing to reduce the amount of drug's doses especially in the elderly and in the increasing number of patients with multiple comorbidities. Bioelectromagnetic and biochemistry should, therefore, be considered on the way of a promising wedding instead of a permanently independent life. Future researches should either optimize the protocols of preparation of these potentially new drugs, either assess the lifespan of their biological effect in order to translate them into effective clinical application at the bedside through Biolectromagnetic medicine.

1. INTRODUCTION

An increasing amount of works support of the possibility to achieve biological effects by mean of the transfer of specific electromagnetic pattern of signal through aqueous systems both in vitro [1–6] and in clinical applications [7–13]. Previously our group showed an interesting effectiveness of the electromagnetic information delivery of a specific source molecule in mimicking its specific biological effect [14, 15]. The effect was statistically significant and nevertheless it was quite lower than the original positive control. Therefore we decide to assess a possible synergism between a reduced dose of a molecule and its own electromagnetic information in order to increase the efficacy of this procedure trying to disclose new possible avenues in translational pharmacology [16]. We was, also, inspired to follow suggestion from other works reporting synergistic effect between molecules and extremely low frequency electromagnetic fields [17, 18]. Neuroblastoma cell lines are appropriate models "*in vitro*" for investigating the mechanisms of neuronal death and their relation to differentiation [19, 20]. It has been reported that Retinoic Acid is able to reduce the tumorogenicity of these cells through the modulation of their neuronal differentiation and cell proliferation [21].

2. MATERIAL AND METHODS

2.1. Cell Culture

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 constant 95% air and 5% CO_2.

2.2. Growth Curves

Cells were seeded at density of 10×10^3 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 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.

2.3. ELF-electro Magnetic Field Exposure System

The appropriate electromagnetic field (7.0 Hz) and magnetic flux 9.2 mT was generated by a commercial wave generator (Vega Select 719). Signals from the wave generator was driven to a small Helmholtz coils. The coils were 7.5 cm high with a diameter of 15 cm. Each coil constituted of 13 layers of 1 mm copper wires for a total 320 wires.

2.4. Statistical Analysis

Statistics was performed with Student's t-test. A p-value of < 0.05 was considered as being statistically significant.

3. RESULTS

LAN-5 neuroblastoma cell line was grown up for 4 days in 4 different ways: 1- Using standard medium (CTR in Fig. 1) as negative control; 2- using Retinoic Acid at the standard concentration of 1 μM (RA 1 μM in Fig. 1); 3- using Retinoic Acid at the concentration of 0.1 μM (AR 0.1 μM); 4- transferring electronically the signals recorded from RA at its standard concentration to the cell medium culture and then adding a ten times reduced concentration 0.1 μM of RA (RA 0.1 mM + RA-ECM in Fig. 1).

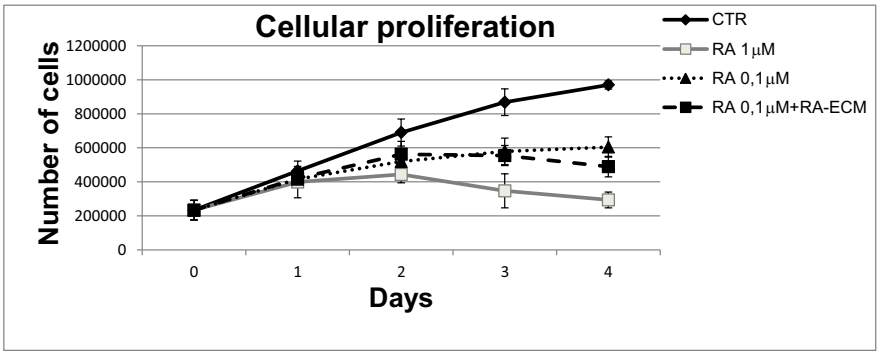

Figure 1: Effect on cellular proliferation detected by direct count for: 1- Standard cell culture medium as negative control (CTR); 2- Retinoic Acid at the standard concentration of 1 μM (RA 1 μM); 3- Retinoic Acid at the concentration of 0.1 μM (AR 0.1 μM); 4- Retinoic Acid at the concentration of 0.1 μM in synergy with electromagnetic information delivery of signals recorded from RA added to the cell medium culture (RA 0.1 mM + RA-ECM).

Cell proliferation was assessed by direct cell count. The results showed that LAN-5 cells treated with 1 μM of RA chemical molecule dramatically decreased their cellular proliferation. Cells proliferation was also decreased of about 40% after treatment with 0.1 μM of RA. It worth to stress that the cells cultured in the medium with the presence of the Retinoic Acid signals, together with 0.1 μM RA chemical molecule, presented a further and statistically significant ($p < 0.05$) reduction of proliferation rate as compared to negative control and to 0.1 μM RA treated cells.

4. DISCUSSION

We analysed the synergistic effect of Retinoic Acid treatment together with the electromagnetic information delivery of its own signals pattern. These data further support the possibility to yield biological effects transferring specific electromagnetic pattern of signal through aqueous systems in vitro [1–6]. It is actually very interesting to confirm that electromagnetic information delivery of a specific source molecule can mimic its specific biological activity [14, 15]. We also support the previous finding of other studies showing a fruitful potential in the combined application of retinoic acid and of direct exposure to extremely low frequency magnetic field on human neuroblastoma cell line [17] but in a water mediated way. It was acknowledged that water could play an essential role in the process of recording, storing and retrieving information through electromagnetic signalling and especially through a resonance effect [22]. A comprehensive explanations of the possible mechanisms by which these effect can occur in aqueous system and in their interactions with living cells has already been reported [16]. In particular a resonance effect can both explain the transfer of the

information pattern from the source input, like retinoic acid samples, to an aqueous system, the culture medium in our experiments, and again from the aqueous system of the cell medium to the target cultured cells [22]. Electromagnetic Information Delivery through Aqueous System has been confirmed to be feasible and effective even when applied in the perspective of enhancing the biological effect of a reduced dose of a standard biochemical drug like retinoic acid. A biophysical namely biolectromagnetic strategy can work in alliance with a biochemical one allowing to reduce the amount of biochemical molecule to be employed to achieve a significant effect.

5. CONCLUSIONS

A positive and effective synergism between a reduced drug dose and its own electromagnetic information delivery seems to emerge as a promising and useful perspective in translational pharmacology outlining future applications in new drug delivery protocols allowing to reduce the amount of drug's doses especially in the elderly and in the increasing number of patients with multiple comorbidities. Bioelectromagnetic and biochemistry should, therefore, be considered on the way of a promising wedding instead of a permanently independent life. Future researches should either optimize the protocols of preparation of these potentially new drugs, either assess the lifespan of their biological effect in order to translate them into effective clinical application at the bedside through Biolectromagnetic medicine [22]. The future for biophysical integrated therapies is opened.

6. DECLARATION OF INTEREST

The authors reports no declarations of interest.

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