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Full Length Research Paper

The synergistic effects of sodium valproate and extremely low frequency electromagnetic field on angiogenesis

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Angiogenesis is a complex activity needed for physiological process. However, it is required for many pathological conditions as solid tumor progression and metastasis. So, today, researchers study on angiogenesis to find new clinical methods. In this research, Ross fertilized eggs were used and were divided in 5 random groups of 70 which consisted of : control, sham-exposed, treated with 5 µl sodium valproate solution, treated with 0.04 T electromagnetic field and treated with both sodium valproate and 0.04 T electromagnetic field. On day 12, chorioallantoic membrane (CAM) was examined and photographed. Then the numbers and lengths of vessels in special area on CAM were measured and compared with each other by t-test ($p < 0.05$). Comparison between average number and length of vessels in controls and sham-exposed did not show any significant difference. In groups 3 and 4, a significant decrease was shown in comparison with controls. Comparison between group 3 and 5 showed a significant decrease in the average number and length of vessels in group 5. As a result, we found that 0.04 T magnetic field could enhance the effect of sodium valproate as an anti-angiogenic drug on CAM.

Key words: Angiogenesis, chorioalantoic membrane, sodium valproate, magnetic field.

INTRODUCTION

Genetic analyses carried out over the last years have yielded important insights into the molecular and cellular mechanisms underlying the formation of vessels. Blood vessel formation is orchestrated by a plenitude of different proteins, including cell adhesion molecules, extracellular matrix, proteases, etc. The term angiogenesis historically refers to the sprouting of capillaries from pre-existing venules and is now used in embryology to describe the complex growth and remodeling processes that transform the primary vascular plexus into the mature vascular tree. Following embryonic and postnatal development, blood vessel endothelial cells proliferate and may remain quiescent for several years. In the adult organism, new blood vessel formation is tightly controlled and occurs only under certain physiological and pathological conditions such as pregnancy, wound healing, diabetic retinopathy or solid tumor growth. Resent studies have suggested that in the adult organism, there are circulating endothelial progenitor cells (EPC) which are derived from bone marrow. Circulating EPC has been reported to contribute to angiogenesis in ischemic, inflamed or malignant tissue. EPC recruitment is stimulated by hypoxia, vascular-endothelial growth factor (VEGF), angiopoietins and other cytokines (Breier, 2006). Angiogenesis is a key pathway crucial to the patho-physiology of both vascular disease and solid cancer. In physiological conditions, a fine balance of pro- and anti-angiogenic factors is maintained as part of normal homeostatic mechanisms. Balasubramanian and Reed (2003) reported that pro-angiogenic phenotypes would have increased

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susceptibility to solid cancers and decreased pre-disposition to cardiovascular diseases and vice versa with the anti-angiogenic phenotypes.

Angiogenesis, which is important in cancer therapy, prompts improvements in researches on tumor vasculature. Jekunan (2003) reported that study on endothelial cells proliferation can make a new strategy in tumors treatment, because each endothelial cell has 2000 - 20000 different receptors and there are hundreds secondary messengers in these cells; inhibitors which are active in endothelial cells are in three main groups: endothelial cells activator blockers, endothelial cells direct inhibitors and the special inhibitors of endothelial signaling pathways; so several treatment assays can be used (Lioyd et al., 2003; Jekunen and Kairemo, 2003). Valproic acid (VPA) (2-propylpentanoic acid) is an eight carbon branched chain fatty acid that has considerable structural similarities to butyric acid, a four carbon fatty acid. Its sodium salt, valproate, is a commonly prescribed mood stabilizing and broad spectrum anti-seizure substance that has proven efficient in controlling partial and generalized epileptic seizures, bipolar disorders, schizophrenia and neuropathic pain (Shawa et al., 2000). It is slightly soluble in water and highly soluble in organic solvents, and can be easily delivered to organisms in the form of sodium or magnesium salts. In the human brain, valporic acid affects the function of the neurotransmitter, GABA by potentiating the inhibitory activity of GABA through several ways including the inhibition of GABA degradation, increased synthesis of GABA and decreased GABA turnover, so block voltage-dependent Na+ channels and modulate the firing frequency of neurons (Kostrouchova et al., 2007). Valporic acid is a histone deacetylases, a chromatin rearrangement modulator and inhibitor. The teratogenic and anticancer effects of VPA have been suggested to be at least in part attributable to histone deacetylases inhibition. Histone deacetylases induces apoptosis in different cell types. VPA induces apoptosis in a limited number of cell types while being nontoxic or even cytoprotective in other cells (Michaelis et al., 2006; Shayannejad et al., 2006).

Several mechanisms, both thermal and nonthermal, are well established, by which electromagnetic fields can interact with biological systems. Thermal mechanisms are related to heating of tissue and generate when electrical energy changes to heat. Nonthermal mechanisms directly depend on electromagnetic field itself, and low frequency electric fields interfere with cell membrane stimulation and decrease it (Litvak et al., 2002). A report was published in 2004, which reported on the effect of 0.2 T electromagnetic field on angiogenesis in chick chorioallantoic membrane (Ruggerio et al., 2004). So, in this research, we studied synergistic effects of valporic acid and 0.04 T electromagnetic field on angiogenesis with this hypothesis that if 0.04 T electromagnetic field can increase inhibitory effect of valporic acid on angiogenesis, it can be a safe complementary treatment, and the effective dose of valpoic acid could be reduced.

MATERIALS AND METHODS

This research was conducted in the Research Laboratory in Biology Department of Mashhad Islamic Azad University. We used 70 fertilized ross eggs held in an incubator with 38°C temperature and 65% moisture. In day 2 of incubation, windows were opened for eggs under sterile condition (17) (Laminair flow, Teslar AV-100, Spain) and eggs were divided into 5 random groups including:

1) Control that were held in normal condition.
2) Sham-exposed that were placed in electromagnetic field, but in turn of position.
3) Treated by sodium valproate.
4) Treated with electromagnetic field
5) Treated with both sodium valproate and electromagnetic field.

In day 8, a gelatine sponge with 1 × 4 × 4 diameter was placed on chorioalantoic membrane (CAM) and soaked with five microliter of sodium valproate solution in members of groups 3 and 5 on this sponge (sodium valproate tablets are 250 mg of the solution in DMSO, the dosage is based on weight of 10 days embryo compared with human dosage 25 mg per Kg). In day 10, members of group 4 and 5 were placed in 0.04 T electromagnetic field (made in Biology Research Laboratory of Islamic Azad University Of Mashhad, Iran) for 4 h (Figure 1). Chorioalantoic membrane were examined daily and photographed (by photo-stereomicroscope, Zeiss, Germany) at day 12 in 0.65 × 10 × 4 magnification (Figure 2). Data were analysed for the number and length of angiogenic blood vessels by software programs and t-test.

RESULT

As shown in Table 1, there is no difference between members of control (C) and sham-exposed (Sh) groups. In the group that was treated with sodium valproate (val), a significant decrease was shown in the average number and length of vessels when compared with the average in control group (Chart 1). Groups treated with 0.04 T electromagnetic field (MF) also showed a significant decrease in average number and length of vessels in comparison with controls (Chart 2). Finally, comparison between group val and group treated with both sodium valproate and electromagnetic field (MF + val) showed a significant decrease in the average number and length of vessels in group MF + R.

DISCUSSION

In 2007, Isenberg and his co-workers showed that sodium valproate significantly inhibit pro-angiogenic responses of vascular cells under both wound healing and tumor growth conditions; vascular cell migration through extracellular matrix was significantly blocked by and sodium valproate too. They found that phosphorylation of heat shoke protein 27 (hsp27) was

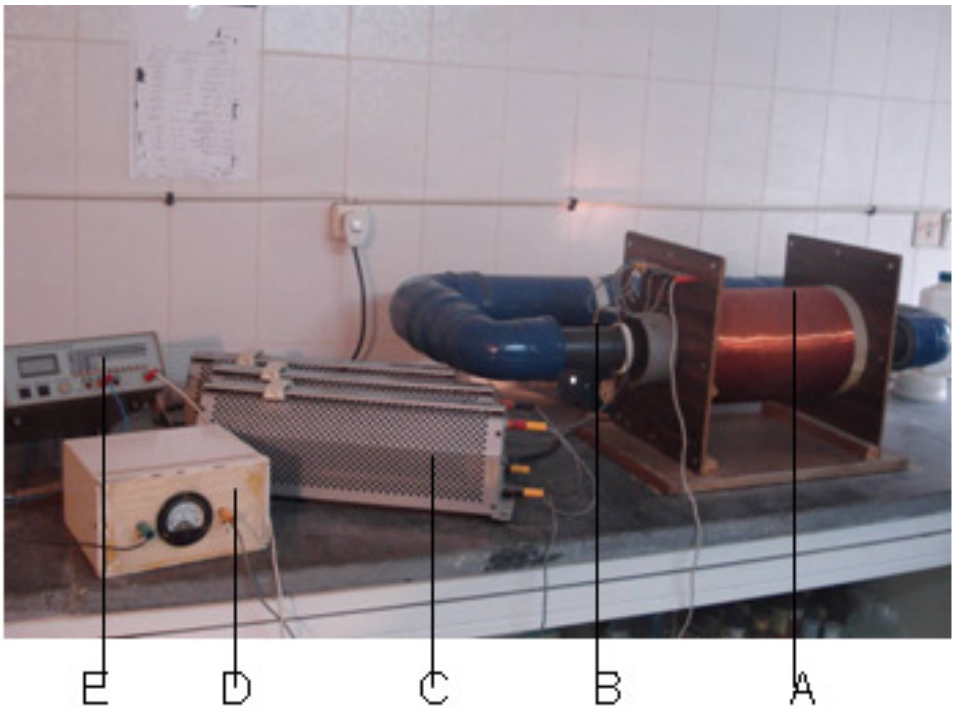

Figure 1. The electromagnetic system with incubator (A) electrical bubin, (B) incubator, (C) resistant, (D) condenser and (E) ammeter.

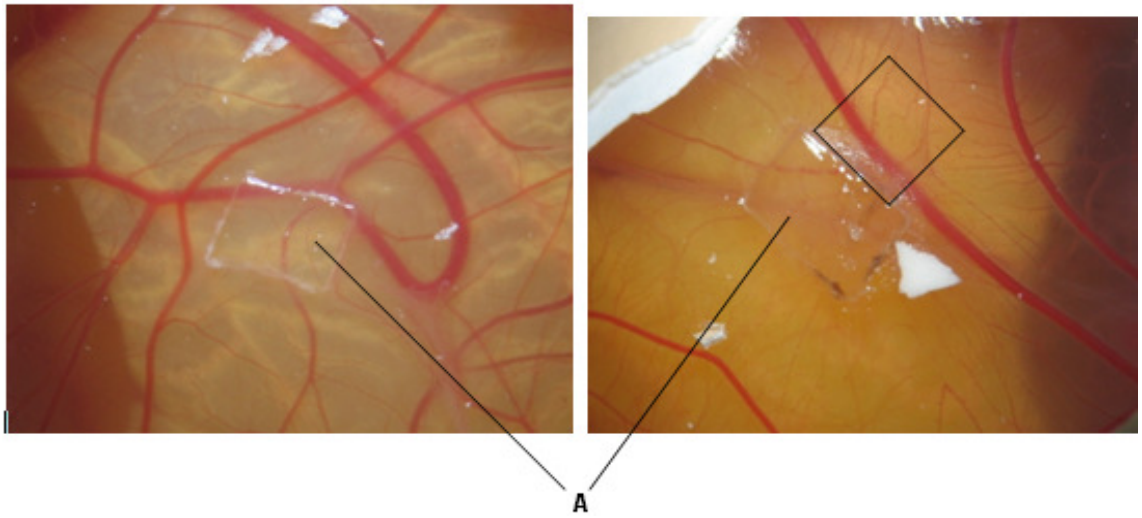

Figure 2. Chorioalantoic membrane with gelatin sponges on it. The right one is related to MF + val case (treated with sodium valproate and 0.04 T electromagnetic field) and the left is related to val case (treated with sodium valproate). (A) Gelatin sponge. Black quadrant show one of the measured area.

Table 1. Comparison between average number (N) and length (L) of vessels in experimental groups (±SD).

Experimental groups/parameter	C	Sh	MF	val	Val + MF
N	42.00±7.26	42.93±6.73	26.69±7.88	33.57±5.09	23.23±4.50
L	57.25±5.05	5.40±58.22	9.88±44.41	5.77±45.73	9.95±36.83

C: Control, Sh: sham-exposed, MF: treated with 0.04 T electromagnetic field, val: treated with sodium valproate, val + MF: treated with both sodium valproate and electromagnetic field.

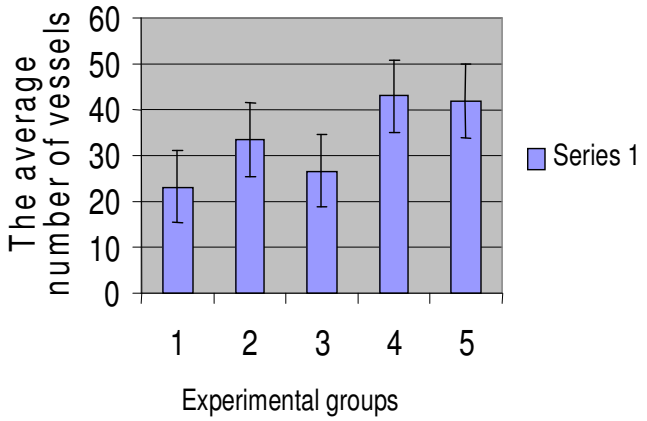

Chart 1. Comparison between the numbers of vessels. (1) Treated with both sodium valproate and 0.04 T electromagnetic field, (2) treated with sodium valproate, (3) treated with 0.04 T electromagnetic field, (4) control and (5) sham-exposed. $P < 0.05$.

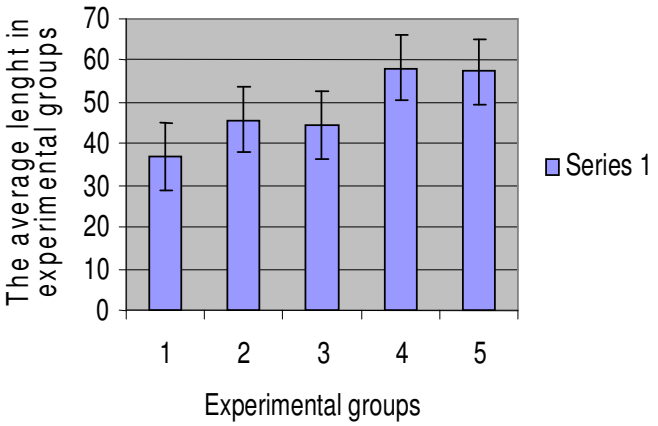

Chart 2. Comparison between the length of vessels. (1) Treated with both sodium valproate and 0.04 T electromagnetic field, (2) treated with sodium valproate, (3) treated with 0.04 T electromagnetic field, (4) control and (5) sham-exposed. $P < 0.05$.

induced by sodium valproate, suggesting that this agents exerts some of their antiangiogenic effects by regulating signaling pathways downstream of Hsp27. They suggested that sodium valproate is an angiogenesis inhibitory agent in Zebra fish, so it can be used in cancer therapy (Isenberg et al., 2007). Valporic acid was capable of blocking the proliferation of neuroblastoma cells and inducing neuroblastoma cell apoptosis *in vitro* too (Qiwei et al., 2007). In this research, we saw a significant decrease in the number and length of vessels in chick chorioallantoic membrane treated with 5 µl sodium valproate, as that reported earlier. In extremely low frequency (ELF, < 300 Hz), part of the electromagnetic Williams and his co-workers spectrum in experimental therapies, have been emerging for a variety of medical conditions, such as non-union bone fractures, skin ulcers, migraines and degenerative nerves (Shupak, 2003). Tsai and his co-workers found that pulse electromagnetic fields stimulation with specific parameters had an effect on regulating the osteoblast proliferation and differentiation *in vitro* (Tsai et al., 2007). Several investigators reported that weak magnetic fields increased the rate of morphological abnormalities in chick embryo, and others have reported that these fields do not appear to affect embryo morphology at all, so there are several factors that cause different effects of electromagnetic fields: the type of intensity and the time of exposure of electromagnetic field, and the genetics of the treated cases too (Farrel et al., 1997; Lahijani and Sajadi, 2004). reported reduction of angiogenesis in breast adenocarcinoma cells treated with 10, 15 and 20 µT electromagnetic fields. In 2003, Okana showed that 10 and 15 mT static electromagnetic fields reduce angiogenesis in rats with experimental hypertension (Mckay et al., 2007). Okana demonstrated that 120 mT static magnetic field (SMF) significantly reversed the inhibitory effects of TGF-β1 on *in vitro* arteriogenesis, he suggested that SMF could have the potential to modify tubular formation, depending on the origin of the cells and the experimental conditions, including angiogenesis inhibitors or stimulators in the medium used for incubation, field intensity, localization of exposure, exposure duration and heterogeneous or homogeneous magnetic fields (Okana et al., 2007).

Delle showed that some important functions of human microvascular endothelial cells (*in vitro*), like proliferation, migration and tube formation are increased under the influence of a sinusoidal electromagnetic field (1 mT, 50 Hz), and the organization of the actin and focal adhesion inside the cell, the state of activation and the distribution of VEGF receptors were also affected (Delle et al., 2008). In this research, we saw inhibitory effect of 0.04 T electromagnetic field on angiogenesis and that it can depend on differences between intensity of electromagnetic fields and the genetics of experimental groups. In 2004, Bare suggested that by creating a synergism of biochemical, electrochemical and electronic principles, the practitioner should be able to achieve a superior treatment outcome; he reported that present chemotherapy regimens can cause permanent damage to various vital organs including the heart, lung and kidneys; even without such permanent damage, it can cause short term toxicity manifested as repression of the hematopoietic system and other types of physical unpleasantness (Bare, 2004). In this research, we used 0.04 T electromagnetic field and sodium valproate as a synergism and saw increased inhibitory effect of sodium valproate on angiogenesis.

Conclusion

Based on this research, we reported that electromagnetic

field with 0.04 T intensity can enhance the inhibitory effect of sodium valproate on angiogenesis in chick chorioalantoic membrane. Decreasing dosage of sodium valproate and different intensities of electromagnetic fields can be used in next researches.

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