

Biology

EFFECT OF 8 Hz ELF EMF AND ITS COMBINATION WITH CISPLATIN
ON SARCOMA-180 TISSUE HYDRATION AND OUABAIN BINDING

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The modulation effect of 8 Hz (2.5 mT) extra low frequency electromagnetic fields (ELF EMF) on cisPt induced changes of cell hydration and number of ouabain receptors (Na⁺/K⁺-ATPase molecules) in membrane of Sarcoma-180 tissue was studied. Tissue hydration was measured as wet mass/dry mass and expressed as a water content of g/g in dry mass. The number of ³H-ouabain receptors in membrane was counted by isotope scintillation counter. It was shown, that 8 Hz ELF EMF has dehydration effect on cancer tissue. Besides, 8 Hz ELF EMF is not a modulator of cisPt effect on tumor tissue.

Keywords: Na⁺/K⁺ ATPase, ELF EMF, cisplatin, cancer, hydration, ouabain.

Introduction. It is now well established that cancerous tissue is markedly over-hydrated and can be as much as 90% water and the cancer cells' over-hydration serves as one of the essential diagnostic parameters for cancer [1]. The over-hydration serves as a messenger for activation of abnormal cell proliferation and depressing apoptosis. It is known also that the loss of oxidative capacity by cancerous cell, pointed out by Warburg [2], is accompanied by cell hydration and mitochondrial swelling [3].

Increased cell hydration causes cancer not only by promoting cell division and oncogene expression, but also by inactivating genes inducing cell differentiation and by preventing apoptosis. As intracellular macromolecules are functioning in aqua medium, their activity also depends on cell hydration [4]. Earlier it was shown that the cell hydration is a dynamic cell parameter, through which the metabolic regulation of cell functional activity, such as cell membrane excitability, chemo-sensitivity, enzyme activity and cell proliferation are realized [4–6]. It has a messenger function in the generation of different diseases including cancer, nervous and cardiovascular disorders [6, 7]. There is a close correlation between cell hydration and the number of ouabain receptors (Na⁺/K⁺-ATPase molecules) in cell membrane, that's why ouabain-receptors could be considered as a marker for membrane surface [8]. Cell swelling leads to the increase, while its shrinkage leads to the decrease of the number of functionally active protein molecules in membrane [5].

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The Na/K pump (Na^+/K^+ -ATPase molecules), which has a crucial role in cell volume regulation, is one of extra sensitive sensors for electromagnetic fields (EMF) exposure [9].

Recently, the use of electromagnetic fields has been expanded to therapeutic purposes, because their interactions with living matter produce effects that initiate, accelerate or inhibit biological processes. Extra low frequency electromagnetic fields (ELF EMF) are non-invasive and non-ionizing and even have non-thermal effects on cells and tissues. These properties have led to studies of the influence of ELF EMF on the development of various diseases, including cancer. Some authors have suggested the therapeutic use of ELF EMF for cancer treatment, because in different experimental models ELF EMF have been able to inhibit the growth of cancer cell lines and tumors; however, few *in vivo* experiments have been performed to investigate the molecular mechanisms of ELF EMF in cancer development [10–13] Recent data showed that 4 and 8 Hz (2.5 mT) ELF EMF have pronounced effects on physicochemical properties of water and water solution [14]. It was demonstrated that 4 Hz (2.5 mT) ELF EMF having dehydration effect on tissues have a pronounced antitumor activity on Sarcoma-180 in mice [15, 16].

Since water is a dominant component of environmental medium and biological systems, and its molecular dissociation is extremely sensitive to different environmental factors, it is possible to influence upon tumor cell proliferation using 8 Hz ELF EMF.

There are many drugs applicable for cancer treatment. The platinum (II) compounds represent an important class of antitumor drugs; they are used in 50% of all cancer therapies [17]. One of more effective of them is cis-diammine-dichloroplatinum or cisplatin ($\text{cisPt}(\text{NH}_3)_2\text{Cl}_2$) [18]. Cisplatin is a well known anticancer DNA-damaging agent. But only approximately 1% of the intracellular cisplatin reacts with nuclear DNA [19].

As the cell hydration serves as a main messenger, through which the regulation of functional activity of intracellular macromolecules including DNA activity is realized, it was suggested that by modulating the cell hydration it could be possible to change the tumor sensitivity to cisplatin.

Consequently, the purpose of present work was to check the hypothesis, for which was carried out a study of the modulation effect of 8 Hz (2.5 mT) ELF EMF on cisplatin-induced changes of cell hydration and number of ouabain receptors in cell membrane of Sarcoma-180 tumor tissue.

Materials and Methods. Anticancer activity of cisPt and its probably modulation by 4 Hz EMF was studied on the model of transplantable mice tumor – Sarcoma-180 (Crooker's sarcoma).

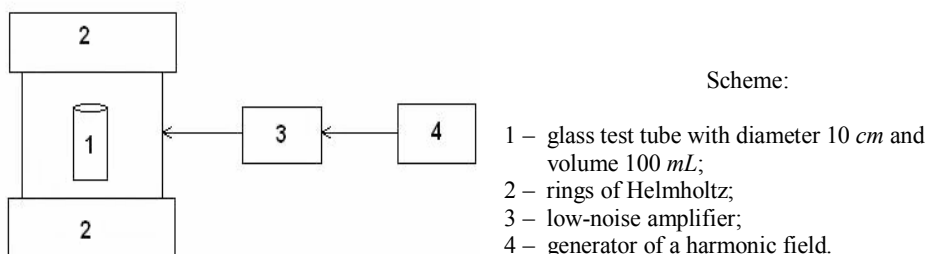
Chemicals. As a physiological solution (PS) was used the Tyrode's solution of following composition (in mM): 137 NaCl; 5.4 KCl; 1.8 CaCl_2 ; 1.05 MgCl_2 ; 5 $\text{C}_6\text{H}_{12}\text{O}_6$; 11.9 NaHCO_3 ; 0.42 NaH_2PO_4 and adjusted to pH 7.4.

For estimation of the number of active pump units in the membrane radioactive [^3H]-ouabain, which had 12 Ci/mM specific activity was used.

ELF EMF Source. The setup for treatment of water by ELF EMF.

The harmonic voltage generated by Generator (4) (see Scheme) after being amplified by low-noise amplifier (3) pass to the Helmholtz rings (2) and due to the

harmonic magnetic field inside of them. The magnetic field created by these rings had high homogeneity.



Animals. Non-linear white male mice with the average weight of 18–20 g were used for the experiments. The animals were purchased from the Animal Resources Centre at the UNESCO Chair-Life Sciences, Republic of Armenia. All procedures were conducted in accordance with the Animal Ethics guidelines.

Experimental Part. Non-linear white 40 male mice with the average weight of 18–20 g were used for the experiments. The mice were placed in special cages that had free-air ventilation. The temperature in the room was 25–27 °C for all control and experiments. The animals had free access to food. The transplantation of the tumor was realized in sterile conditions. Pieces of tumor tissues served as a transplant for the transplantation. Our experimental animals were divided into 4 groups (each group or treatment content 10 mice) correspondingly: 1 – control, 2 – 8 Hz, 3 – cisPt, 4 – 8 Hz+cisPt.

During 6 days the mice in the groups 3 and 4 were supplied by ordinary water and were injected into the peritoneal cavity with (0.1 mg/mL, 0.5 mL per mouse) the testing substance (Cisplatin Ebeve, Austria) after 48 h of the tumor transplantation. The mice of the 2 and 4 groups were supplied by 8 Hz EMF treated water.

Tissue Preparation. 24 hours after the last injections experimental animals were dissected, then the tumors were isolated. In order to avoid the possible effects such as emotional stress and pain sensation (arising at the ordinary technique of animal decapitation-during the forcible immobilization of the awaked animal), the experimental animals were immobilized by dipping their heads into liquid nitrogen (for 3–4 s), which led to the freezing of the heads and death of mice. At the full absence of animals' somatic reflexes upon the extra stimuli tissue samples were removed.

Estimation of Cell Hydration. For the estimation of cell hydration, the tissue slices with similar shape and weight were dried in thermostatically controlled oven for 24 h at 105°C. Tissue hydration was measured as wet mass/dry mass and expressed as water content g/g in dry mass.

Isotop Measurement. For counting the number of ouabain ³H-receptors control and experimental tissue slices with similar shape and weight, the latter were incubated in physiological solution (PS) for 30 min containing radioactive [³H]-ouabain (at 10⁻⁹ M concentration). After that, the slices were washed threefold for 10, 5 and 5 min respectively to remove any radioactive ouabain absorbed by the intracellular spaces and not binded with receptors. Then, the tissue pieces were placed in special vials and each sample was homogenized in 50 µL 68% HNO₃ solution.

Then Bray's scintillation fluid was added (2 mL) and the radioactivity of mixture was counted by Walac 1450 liquid scintillation and luminescent counter (Finland). The results were averaged on the basis of the weight of each individual tissue sample.

Results and Discussion. The series of experiments were held to find out the effects of cisPt and 8 Hz (2.5 mT) ELF EMF treated water, and their combination on tumor tissue hydration and ouabain binding on cancer cell membrane.

As can be seen from the data presented on Fig. 1, ouabain at $10^{-9} M$ concentration has cancer tissue hydrating effect both in case of control and experimental groups. The 8 Hz EMF pretreated water has dehydrating effect on cancer tissue both in ouabain free conditions and in combination with ouabain ($10^{-9} M$). The Fig. 1 shows, that cisPt has dehydrating effect on cancer tissue in presence of ouabain. The opposite effect (statistically not significant) occurs in the absence of ouabain. The Fig. 1 indicates also, that the combinative application of 8 Hz EMF treated water and cisPt, has not demonstrated any significant effect on tumor tissue, both in case of ouabain free process, and with treatment of tumor tissue samples in ouabain containing ($10^{-9} M$) physiological solution.

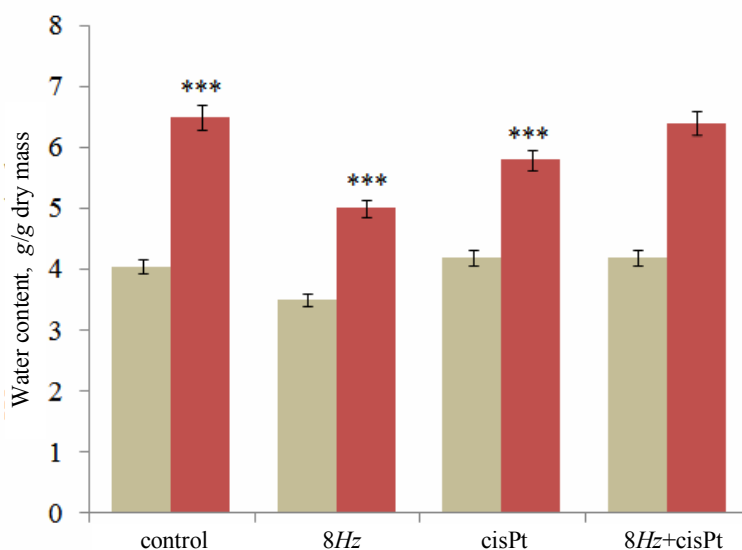


Fig. 1. The effect of 8 Hz EMF and cisPt, their combination and ouabain on the cancer (Sarcoma-180) tissue hydration. The experimental animals were supplied by 8 Hz EMF treated water, injected with cisPt correspondingly. Half part of tissue samples were incubated in physiological solution containing radioactive [3H]-ouabain ($10^{-9} M$) (■) and the other part – ouabain free (□).

The Fig. 2 indicates, that the ouabain binding of cell membrane has significantly changed. 8 Hz has dehydration effect on cell membrane (Fig. 1), which is accompanied with increased ouabain binding with cell membrane. This could be explained by increased affinity of ouabain receptors (Na^+/K^+ -ATPase).

As can be seen from Fig. 2, the positive correlation between tissue hydration and ouabain binding of cancer cell membrane-dehydration is accompanied with decrease of ouabain binding. The Fig. 2 shows also, that 8 Hz ELF EMF with cisPt causes diminution of ouabain binding.

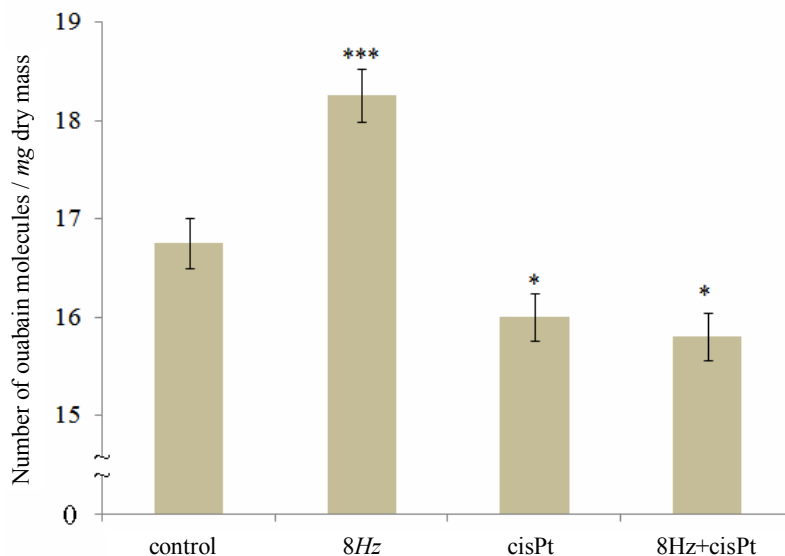


Fig. 2. Binding of radioactive [^3H]-ouabain on the cancer cell membrane. The experimental animals were supplied by 8 Hz EMF treated water, injected with cisPt, and were used their combination correspondingly. Tissue samples were incubated in physiological solution, containing radioactive [^3H]-ouabain (10^{-9}M).

Conclusion.

- 8 Hz ELF EMF (2.5 mT) pretreated water has dehydrating effect on cancer tissue and ouabain affinity modulating effect on cancer cell membrane;
- CisPt has depressing effect on cancer tissue hydration;
- combination of 8 Hz ELF with cisPt affect on the correlation between cell hydration and ouabain binding on cell membrane;
- 8 Hz ELF EMF don't stimulates the cisPt induced tumor depressing effect;
- 8 Hz ELF EMF can be a possible tool in cancer therapy;
- 8 Hz ELF EMF combination with cisPt is not a useful tool for stimulation of cisPt antitumor effect.

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