Effects of Electromagnetic Fields over DNA in Tumor Diseases, Biophysical and Biochemical Model Systems

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Abstract
Studies were conducted with model systems of influence of Drossinakis with electromagnetic (e.m.) fields and infrared thermal field (ITF) (Ignatov, Mosin, Niggli, Drossinakis, 2013). The purpose of research is to analyze effects over DNA. In the report is carried out analysis of effects over water and physiological saline. Results are achieved with blood serum of hamsters and physiological processes in hamsters with tumors. The analyses with water are conducted using the methods Nonequilibrium Energy Spectrum (NES) and Differential Nonequilibrium Energy Spectrum (DNES) (Antonov, 1992; Ignatov, 1998). Experiments are carried out with the influence of tumor cells of a mouse in water. It is observed reduction of DNES spectrum according to the control sample of cells in healthy animals. (Antonov, 1992). Reduction is also observed in DNES spectrum in blood serum of people having oncological diseases, compared to the one of healthy people (Ignatov, 2012). Such a reduction is most prevalent in (-0.1387 eV; 8.95 μm; 1117 cm^{-1}). In research of the effects of e.m. fields in water and blood serum from hamsters the range is (-0.08 – -0.14 eV) (8.9 –15.5μm) (645–1129 cm^{-1}). Research is conducted for the effects over Graffi tumor that was implanted in hamsters (Toshkova, Ignatov, Zvetkova, Ignatov, Gluhchev, Drossinakis, 2019). Studies are conducted with pH and oxidation redox potential (ORP) effects of e.m. fields over physiological saline (Gluhchev, Ignatov, Drossinakis, 2019).

Keywords: Infrared thermal field (ITF), electromagnetic fields (e.m. fields), experimental *Graffi* solid tumor, energy spectrum, NES and DNES methods.
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Introduction

In conducted studies with blood serum are analyzed parameters of electromagnetic hydrogen bonds. The hydrogen bonds are electromagnetic among bipolar molecules, where the hydrogen is connected to an atom with large electronegativity, such as nitrogen (N) and oxygen (O). In Fig. 1a are shown hydrogen bonds in DNA. In Fig. 1b is presented donor-acceptor interaction. In Fig. 1c are shown hydrogen bonds in water.

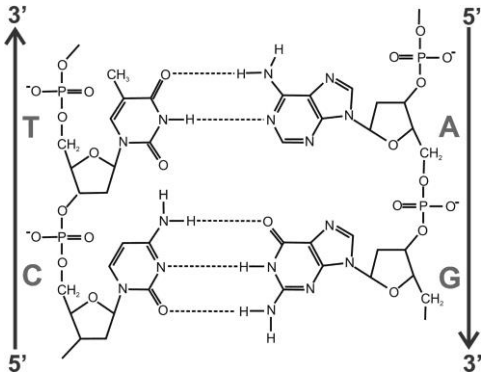

Figure 1a Hydrogen bonds among H and N or O in DNA

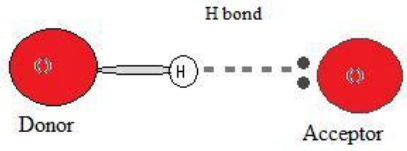

Figure 1b Donor-acceptor interaction of hydrogen bonds among H and N, F or O

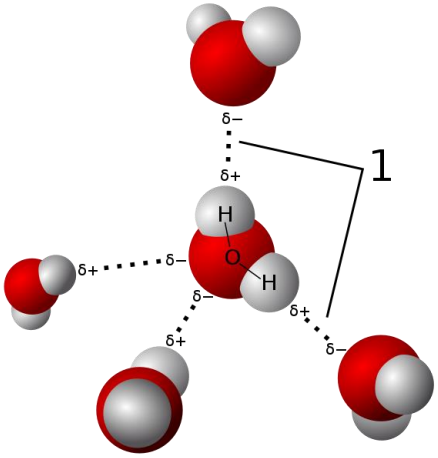

Figure 1c Hydrogen bonds among water molecules

The water is a medium of living processes. The molecules of DNA in the cells are in the form of double helix. The pentose phosphate skeleton of both chains is placed outwards, and the nitrogenous bases are pointed towards the inside of the spiral, and are connected to each other with weak hydrogen bonds.

The multiple, although weak hydrogen bonds, provide stability to the molecule of DNA. The oxygen and nitrogen are electronegative atoms in the nitrogenous bases. Each separate nucleotide contains phosphate, deoxyribose saccharide, and one of the four nitrogenous bases separated into two categories – purine and pyrimidine. The purine bases adenine (A) and guanine (G) are larger and contain two aromatic rings. The pyrimidine bases cytosine (C) and thymine (T). Research shows that the range of e.m. interaction of hydrogen bonds in DNA varies from 1 to 10 THz (10^{12}Hz)(30 –300 μm)(33.4 – 333.6 cm^{-1}) (Tang et al., 2018). In such

a range are conducted studies of the DNA changes in cancer diseases (Calvin et al., 2012). The authors investigate within the range 21 до 37 THz (8.9 –15.5μm) (645–1129 cm^{-1}) of effects over cancer cells, as the radiation of e.m. waves is in such a range (Ignatov, Mosin, Niggli, Drossinakis, 2013).
During research with physiological saline is studied the change of pH and ORP.
During the influence with e.m. waves is researched the survival rate of hamsters with tumors, as well as the change in size of tumor. The DNA damage contributes to ageing and cancer, as the result depends on the type and number of lesions (injury) in DNA. The cancer related diseases are one of the main reasons for changes in DNA (Hoeijmakers, 2009). During the influence with e.m. fields is observed change of erythrocytes and the animal hair of hamsters. (Toshkova, Ignatov, Zvetkova, Gluhchev, Drossinakis, 2019).

2. Materials and methods

2.1. Experimental animals

Hamsters, breed “Golden Syrian”, aged 2-4 months with weight around 90-100 g were used in the trials. The animals were grown in standard conditions in individual plastic cages with free access to food and water.

2. 2. Experimental tumor model

Tumor cells ($1\text{-}2.10^6$) from the experimental *Graffi* solid tumor are transplanted subcutaneously in the back of hamsters. Between days 7 and 15 after the transplantation tumor appears, grows progressively and the hamsters die around 30-35 days. In this tumor model 100% tumor transplantation and 100% mortality are observed. No spontaneous tumor’s regression takes place. (Toshkova, 1995).

2.3. Influence by electromagnetic and infrared thermal fields

This type of influence is delivered by Christos Drossinakis who holds the hamsters in the hands for a couple of minutes (Fig. 2).

Figure 2. Bioinfluence with Infrared Thermal Field and e.m. waves on hamsters with experimental tumor

2.4. Hematology examination

Blood smears from experimental Graffi tumour-bearing and control hamsters are prepared, stained by May-Gruenwald Giemsa method and examined light-microscopically.

2.5. Ethical aspects

All experiments were conducted in accordance with the European convention for protection of vertebrate animals, used for experimental and other scientific purposes (OJ L 222) and approved from the National Veterinary Medical Office.

2.6. NES and DNES Spectral Analyses

The device for DNES spectral analysis based on an optical principle was designed by A. Antonov. For this, a hermetic camera for evaporation of water drops under stable temperature (+22–24 ^{0}C) conditions was used. The water drops were placed on a water-proof transparent pad, which consisted of thin maylar folio and a glass plate. The light was monochromatic with filter for yellow color with wavelength at λ = 580±7 nm. The device measures the angle of evaporation of water drops from 72.3^0 to 0^0. The DNES-spectrum was measured in the range of -0.08– -0.1387 eV or λ = 8.9–13.8 μm using a specially designed computer program. The main estimation criterion in these studies was the average energy ($\Delta E_{H...O}$) of hydrogen O...H-bonds between H_2O molecules in water samples and hamster serum blood.

3. Results

3.1. Influence of e.m. waves over hydrogen bonds in water medium. Hydrogen bonds in DNA

In the Fig. 3 are presented the four monomers building DNA - deoxyadenosine, deoxycytidine, deoxyguanosine, deoxythymidine. With the arrows are marked the hydrogen bonds. DNA is located in water medium, and effects of e.m. fields over hydrogen bonds oxygen-hydrogen are being investigated.

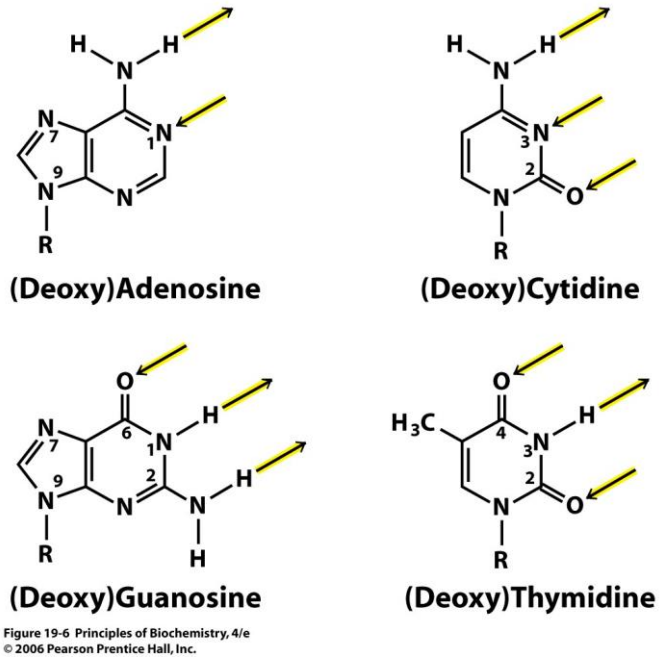

Figure 3. Four monomers, which are created DNA – deoxyadenosine, deoxycytidine, deoxyguanosine, deoxythymidine

3.2. Results with pH and ORP

In order to assess the bio-influence over blood is used physiological saline, compatible with the human blood, consisting of 0.9% solution of NaCl. Before the experiment are measured 50 ml from a bottle containing 0.5 l from the solution that perform the role of a control sample.The bottle with the remaining 450 ml are held from the operator for a time period of 20 minutes.For the control sample and the solution with bioinfluence are measured the parameters pH, ORP (Oxidation Reduction Potential) and electric conductivity in milliamperes. The achieved results are presented in the following Table 1

Table 1.

	pH	ORP (mV)
Control Sample	6.96	150
Sample with e.m. waves	6.25	150

They show that:

1. There is a decrease of 0.71 pH of the sample compared to the control sample.
2. No difference is reported in the values of ORP.

Findings

1. During the study of physiological saline is observed 5-times increase of hydrogen ions and a change in conductivity. It is a proof for recovery of the ion balance. In the healthy cells the potential for transmission of hydrogen ions H+ through the membrane is (-140 mV), and in the cancer cells it is (-70 mV) (Alberts et al., 1994). The tendency is recovery of potential when influencing with ITF and e.m. waves.
2. The achieved results in hamsters with experimental tumor and physiological saline present biophysical, biochemical, biological effects of influence with ITF and e.m. waves. The processes of improvement of ion exchange and electrical potential in the cells, of the anaemic syndrome, and the synthesis of basic proteins, support the body protection and the immune status, and they hold up the tumor process.

Conclusion

1. It can be considered as established the effect of bioinfluence of Christos Drossinakis over the values of pH of physiological saline.
2. Due to the 65% of water content in the human body, it can be considered that its parameters irrespective of its consistency in the blood and internal organs, will be influenced from an external bioinfluence, which will affect their functioning, and hence the health condition of the body.
3. The potential of hydrogen ions in a healthy cell is -140mV, and in a cancer cell -70 mV. The increase of the number of hydrogen ions reveals a process of recovery in the potential of a cancer cell to a healthy condition.

3.2. Electromagnetic parameters with DNES method during influence with electromagnetic fields over blood serum of hamsters with tumor

Table 2 shows the results from the influence of e.m. fields over distribution by energies of hydrogen bonds in 1% solution of blood serum.

Table 1 DNES spectrum is measured in eV for (-E) of 1% solution of blood serum of hamsters with tumors with influence of e.m. fields, and control group of hamsters with tumors. The function of distribution by energies is df(E) in eV^{-1} for DNES spectrum. The results show that at 8.95 μm the difference in the function of distribution by energies e f(E) in eV^{-1} for blood serum of hamsters with tumor compared to blood serum of healthy hamsters is highest. At 8.95 μm is observed decrease of the energy of hydrogen bonds in tumor diseases. With efficient influence, as well as with e.m. fields, the value in DNES is the highest, and the local extreme value is the highest and it is positive.

3.3. Energy of hydrogen bonds

The average energy ($E_{H...O}$) of hydrogen H…O-bonds among individual H_2O molecules in 1% solution of Sample of blood serum of hamsters with cancer after influence of Drossinakis is measured at E=-0.1285 eV. The result for the Control sample in 1% solution of blood serum of hamsters with cancer is E=-0.1214 eV. The results obtained with the NES method are recalculated with the DNES method as a difference of the NES (Sample) minus the NES (Control Sample) equalled the DNES spectrum of 1% solution of blood serum from hamsters with cancer.

Δf(E) = f (sample 1) – f (control sample 3)

Thus, the result for 1% solution of blood serum from hamster recalculated with the DNES method is ΔE=-0.0071±0.0011 eV. The result shows the increasing of the values of the energy of hydrogen bonds in 1% solution of blood serum of hamsters with tumoreafter influence of Drossinakis regarding control sample blood serum of hamsters with tumors.

Fig. 4 displays DNES spectrum of 1% solution of blood serum of hamsters with tumors with influence from e.m. fields, and control group of hamsters with tumors. At the x-axis are reported the values of energy (-E) of hydrogen bonds. A portion of these bonds are in DNA. At the y-axis is given the function of distribution by energies for DNES df (E) в eV^{-1}. The positive values of the spectrum indicate effects over the tumor cells. Biological studies reveal improvement of the life status of tumor cells compared to the condition of healthy ones. It comes as a result of the improved replication of DNA.

DNES spectrum*										
-E (eV) x-axis	0.0962	0.0987	0.1012	0.1037	0.1062	0.1087	0.1087	0.1112	0.1137	0.1162
df(eV^{-1}) y-axis	**0**	**-42.1**	**0**	**0**	**0**	**0**	**0**	**0**	**-15.2**	**0**
-E (eV) x-axis	0.1187	0.1212	0.1237	0.1262	0.1287	0.1312	0.1337	0.1362	0.1387	
df(eV^{-1}) y-axis	**0**	**43.6**	**-42.1**	**0**	**28.6**	**-13.5**	**-13.5**	**-13.5**	**72.2**	.

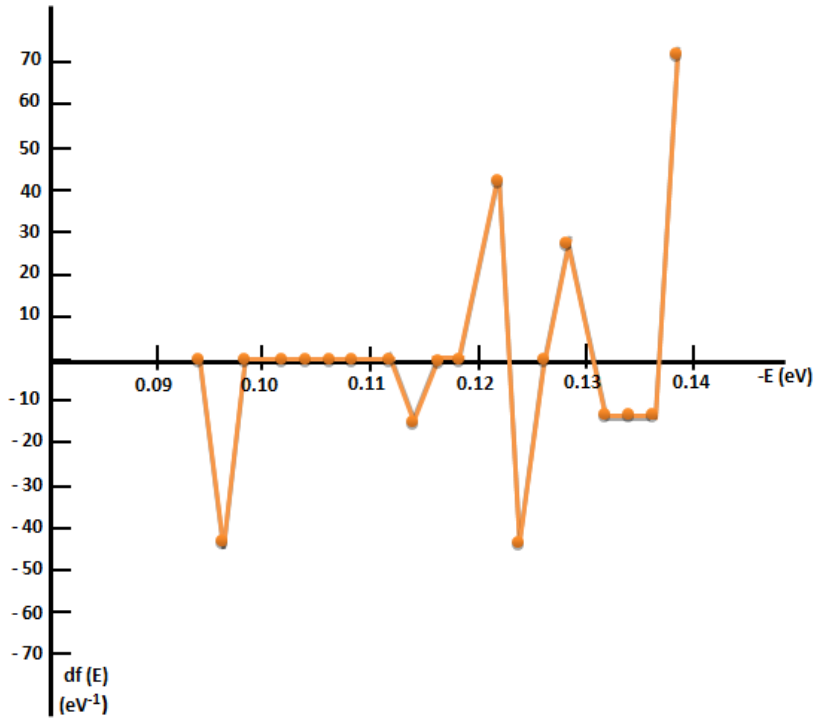

Figure 4. DNES of 1% solution of blood serum of hamsters with tumors with influence of e.m. fields as difference of control group of hamsters with tumors

3.4. Biological parameters

Following biometric parameters have been measured for the evaluation of bioinfluence on the tumor: transplantability, tumor size (mm), lethality/mortality rate and survival rate. Their measurement revealed significant differences between the experimental and control group.

3.4.1. Life span of hamsters

Hamsters that have undergone bioinfluence have had low mortality. Until the 35th day, mortality was 0, at day 40- 40%, at day 45 was 80%, and at day 50 it was 100%. At the same time in the control group the lethality was 50% on the 30th day and reached 100% on the 38th day. Thus the average survival rate of hamsters with therapy was 43.6±5.8 days and for control group-without therapy – 31.75±6.8 days, which was 12 days longer than the controls.

Also, some delay in tumor transplantability and growth was registered. The conclusion we can draw from the obtained results is that bioinfluence therapy (in this scheme of application and duration) in hamsters with developed tumor doesn't stop the tumor growth, but delayed its progression, decreased lethality and prolonged average survival time.

3.4.2. *Hematological research*

Cytological differences in the erythrocyte /RBCs-/ morphology and differentiation were noticed in the blood smears of hamsters from control group vs bio-influenced hamsters with implanted myeloid tumours of *Graffi*. The observed differences probably indicate positive effects of the near infrared bio-influence on the

erythropoiesis of *Graffi* tumour-bearing hamsters, that may lead to improvement of the anemia-syndrome - obligatory developed in this and/or in other experimental models of myeloid malignancies.

Conclusion

The results achieved from the tests with 5 days course of ITF and e.m. waves treatment of hamsters with experimental subcutaneous tumor are positive. Prolonged survival rate and decreased mortality between the experimental and control group, as well as lowered transplantability and slowed tumor growth were observed. The present results are the base for conducting further tests that aim to establish the optimum regimen of bioinfluence with regards to frequency and duration of the therapeutic procedures,

The mathematical model of blood serum solution of hamsters with cancer after the Drossinakis' influence gives significant information about the possible number of hydrogen bonds as a percent of H_2O molecules with different distribution of energy relative to the same number in the two control groups.

As a result of different energies of hydrogen bonds, the surface tension of the blood serum solution of cancer hamsters is increased after the treatment relative to the control samples. This effect is connected with the preservation and increase in the energy of the biochemical processes between water molecules and biomolecules. The achieved results of hamsters from experimental bio-influence of Christos Drossinakis reveal their biological efficiency and can be subject of future studies. Extending the life of the hamsters is an indicator of improving immune status. The obtained results correspond to recent data from the medical scientific literature for the positive effect of the near infrared irradiation on the structure and function of erythrocyte membrane in normal and pathological conditions. *The mitochondrial polarity in cancer cells was found to be lower than* that of normal cells. Drossinakis is increasing the *mitochondrial polarity.*

The basic conclusion is that Drossinakis is able to increase the average energy of hydrogen bonds among water molecules in the blood of hamsters with cancer after treatment compared to the average energy of hydrogen bonds among water molecules in the blood of non-treated hamsters hamsters as control group.

In the report there are results with DNA. DNES spectrum of 1% solution of blood serum of hamsters with tumors with influence from e.m. fields, and control group of hamsters with tumors. At the x-axis of Fig. 4 are reported the values of energy (-E) of hydrogen bonds. A portion of these bonds are in DNA. At the y-axis is given the function of distribution by energies for DNES df (E) в eV^{-1}. The positive values of the spectrum indicate effects over the tumor cells. Biological studies reveal improvement of the life status of tumor cells compared to the condition of healthy ones. It comes as a result of the improved replication of DNA. After the influence the hydrogen bonds among oxygen (O) and hydrogen (H) in DNA helix have bigger energy.

During the study of physiological saline is observed 5-times increase of hydrogen ions and a change in conductivity. It is a proof for recovery of the ion balance. In the healthy cells the potential for transmission of hydrogen ions H+ through the membrane is (-140 mV), and in the cancer cells it is (-70 mV). The tendency is recovery of potential when influencing with ITF and e.m. waves. The potential of hydrogen ions in a healthy cell is -140mV, and in a cancer cell -70 mV. The increase of the number of hydrogen ions reveals a process of recovery in the potential of a cancer cell to a healthy condition.

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