Anti Tumour Effects Inhibiting Cancer Cells

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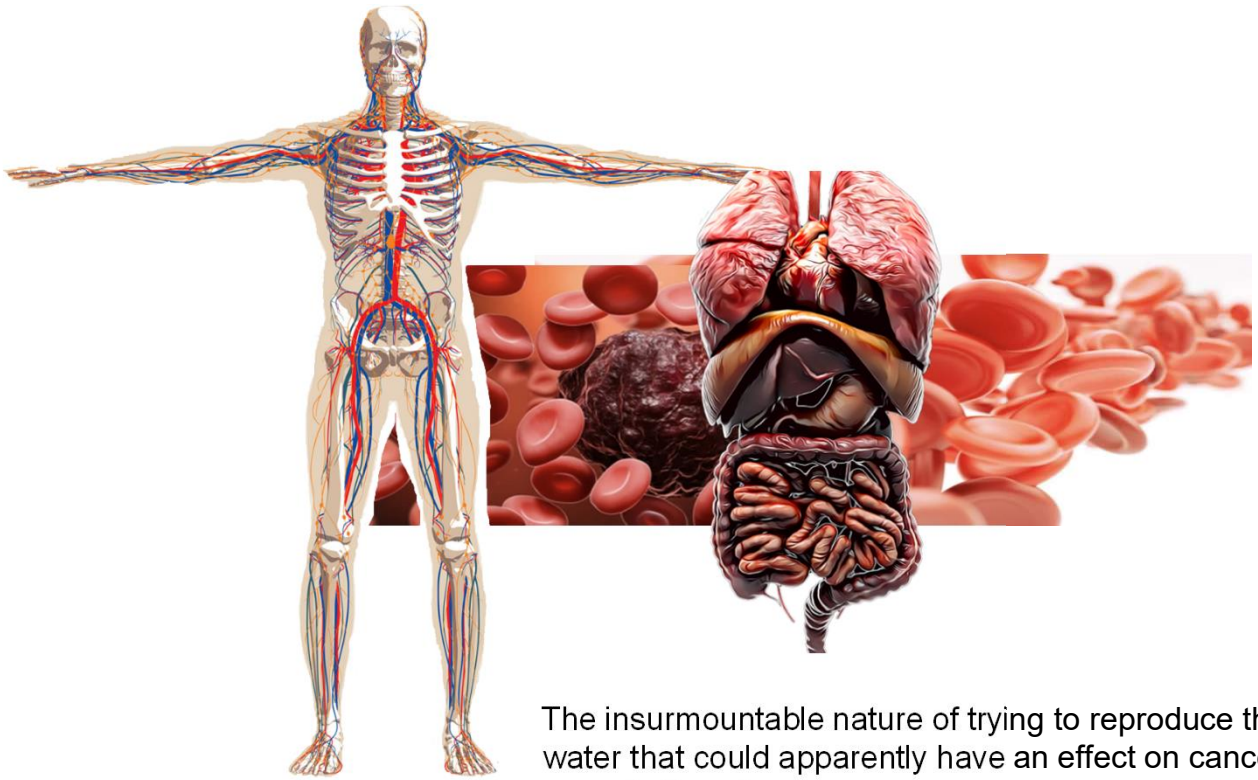

The insurmountable nature of trying to reproduce the water that could apparently have an effect on cancer and other diseases may seem to have been an impossible mission

Dr. Albert Szent Gyorgyi

PEGASUS AQUA

MRET® Technology

US Patented # 6,022,479

Technological revolution for Cancer Patients in the 21st Century

Todd Hobbs January 9, 2023

Through the means of several technological advancements that have resulted in improvements in how we identify, visualize, comprehend, and treat Cancer, what previously appeared unattainable in cancer research is now a reality. Exploring and employing these technologies further may pave the way for accelerating the fight against this illness. The new and innovative technological revolution has made the treatment of cancer patients easy. There are several technological advancements used to cater to cancer patients.

•MRET Technology

According to multiple studies, it is seen that through the MRET technology, cancer risks are solved significantly as consumption of all types of MRET water leads to the significant inhibition of tumor growth and suppression of mutated tumor cells. The daily use of such water solves the cancer problem and is not reasonably complex. Through such simple means, technology has changed the lives of cancer patients worldwide. The unique MRET technology is the creation of the innovative scientist Igor Smirnov. MRET technology has helped improve the lifestyle of millions globally and is a keen technology in improving health. The bacteria-free water can eliminate hazardous chemicals, reduce cancer risk, and help cancer patients. Such iconic inventions are the gift from the mind of Igor Smirnov, and his mind is not limited to science and technology, but the limits of his mind also succeed art. Igor's art and technology craft has helped the world evolve into becoming a better and healthier version of it through his inventions.

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MRET Activated Water and its Succesful Application for Prevention Treatment and Enhanced Tumor Resistance in Oncology

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Abstract

This particular article relates to a fundamental nature on how MRET Activated Water with the modified molecular structure, physical and electrodynamic characteristics may enhance specific molecular mechanisms in living cells. The research regarding the physical parameters of water confirmed that MRET treatment of distilled water leaded to substantial modification of basic physical-molecular properties of water. The anomalous viscosity of MRET water (subject to very low tangent pressure) and electrodynamic characteristics of MRET water (subject to applied electromagnetic field of low frequency range) confirm the high level of long-range dynamic structuring of water molecules in polarized-oriented multilayer formations in activated water produced with the help of MRET activation process. The similarity of molecular formations of cell water and MRET activated water contributes to their compatibility, easy bio-availability and assimilation of MRET activated water in biological systems. The introduction of MRET water to biological systems can contribute to the enhancement of the cellular transduction mechanism and the proper function of cells in biological systems. The significant positive effect of MRET Activated Water regarding the tumor resistance on animals was observed in all groups of mice on different fractions of MRET water in the experiments conducted on 500 mice at Kiev Institute of Experimental Pathology, Oncology and Radiobiology, Ukrainian Academy of Science. The best results were observed in the groups of mice on MRET water activated for 30 minutes (optimal regime of activation). The significant anti-tumor effect of MRET Activated Water on mice was close to the action of the chemotherapy agents and allowed to avoid the side effects that typically follow chemotherapy treatment in oncology.

Keywords: MRET Activated Water, Ehrlich Carcinoma, Sarcoma, Preventive Regime, Enhancement of Cellular Functions, Tumor Resistance, Index of Cytotoxic Activity, Viscosity, Electrical Conductivity, Dielectric Permittivity.

Objectives

MRET Activated Water is produced with the help of patented in the USA Molecular Resonance Effect Technology (MRET). MRET water activator is the stationary source of subtle, low-frequency, resonant electromagnetic field with composite structure. The origin of the low-frequency composite electromagnetic field is the intensive electrical activity inside the nano-circles formed by linear molecular groups of MRET polymer compound (volumetric fractal geometry matrix) when polymeric

body is exposed to the external electromagnetic fields of specific frequency and wavelength [Vysotskii, Smirnov 2005].

This particular article relates to a fundamental nature on how MRET activated water with the modified molecular structure, physical and electrodynamic characteristics may enhance specific molecular mechanisms in living cells. The goal of this investigation was to study on mice the effect of MRET activated water as a potential agent for the prevention and treatment of two kinds of oncology diseases (laboratory models of Ehrlich's ascites tumor and Sarcoma ascites form). The research was conducted under supervision of Prof. V. Vysotskii (Kiev State University, Ukraine), S. Olishevsky, Ph.D., Y. Yanish, Ph.D. (Kiev Institute of Experimental Pathology, Oncology and Radiobiology, Ukrainian Academy of Science), and A. Kornilova, Ph.D. (Moscow State University, Russia) [Vysotskii 2006].

The research regarding the physical parameters of water confirmed that MRET treatment of distilled water leaded to substantial modification of basic physical-molecular properties of distilled water. The level of modification of properties of MRET water depends on the duration of the process of activation. The results also confirmed the ability of MRET activated water to keep its anomalous characteristics for several hours or days at room temperature and especially at low temperature (known in physics as the “long-term water memory” phenomenon [Vysotskii 2005, 2004]).

The experiment conducted on MRET activated water subject to tangent pressure revealed that at very low velocity of motion of water (tangent pressure in the range of 0.004 – 0.005 Pa, temperature 20˚C) viscosity of water activated for 60 minutes decreased about 200 – 250 times compare to non-activated water from the same source. The most significant phenomenon of anomalous low viscosity of activated water, the decrease about 300 – 500 times, was observed for water activated for 30 minutes. These results confirm the hypothesis regarding the alteration of molecular structure in MRET activated water. Particularly, the anomalously low viscosity of MRET activated water in the area of very low tangent pressure confirms the polarized-oriented multilayer molecular structuring of MRET water: the high level of long-range molecular coupling (hydrogen bonding) inside the “layer” and very low level of molecular coupling between the “layers.”

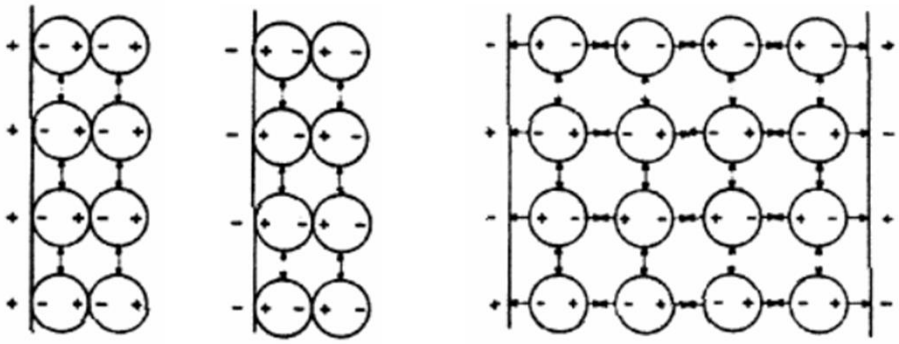
The significant modification of electrodynamic characteristics of distilled water subject to applied electromagnetic field in the range of low frequencies was observed after MRET activation. The electrical conductivity of MRET activated water at 20˚C in the range of frequencies of 0.1 Hz – 100 kHz decreased by 80 – 90% in 30 minutes activated water, and by 66 – 70% in 60 minutes activated water respectively compare to non-activated distilled water. The dielectric permittivity in the very low frequencies range of 0.1 – 1000 Hz decreased by 80 – 90% and in the range of frequencies of 1 – 100 kHz it decreased by 18% in 30 minutes activated water; the decrease by 70 – 85% was observed in the range of 0.1 – 1000 Hz in 60 minutes activated water compare to non-activated water from the same source. It is reasonable to admit that in the range of very low frequencies 0.1 – 1000 Hz the long-range multilayer molecular structures of MRET water (with high level of molecular coupling inside the “layers” and extremely low level of hydrogen bonding between the “layers”) create lower level of resistance of water dipoles to the alignment and, as a result, the dielectric permittivity of MRET water is substantially lower by 70 – 90% compare to non-activated water. This substantial decrease of dielectric permittivity also confirms the direct correlation between viscosity and dielectric permittivity of water in the range of low frequencies of applied EMF [Chaplin 2005].

The investigation regarding the electrodynamic characteristics of MRET water also revealed that the long-term storage of activated water (up to 5 hours at 20˚C) did not substantially affect the modified electrodynamic characteristics of 30 minutes activated water (the reduction of conductivity still kept the level of 66 – 70% and dielectric permittivity kept the level of decrease by 50 – 55% in the range of 0.1 Hz – 1 kHz and by 18% in the range of 1 – 100 kHz respectively). The storage of 60 minutes activated water under the same conditions practically did not affect its electrodynamic characteristics (maximum difference is 2%). These results confirm the ability of MRET activated water to keep its anomalous properties for rather long period of time (known as “long-term water memory”

phenomenon) in case of 30 minutes activation and even higher level of "long-term water memory" phenomenon in case of 60 minutes activation.

The anomalous viscosity of MRET water (subject to very low tangent pressure) and electrodynamic characteristics of MRET water (subject to applied electromagnetic field of low frequency range) confirmed the high level of long-range dynamic structuring of water molecules in polarized-oriented multilayer formations in activated water produced with the help of MRET activation process. The fundamental biophysical theories revealed the scientific paradigm regarding polarized-oriented multilayer (PM) structuring of cell water in biological systems [Ling 2003, 2001, Drost-Hansen 1971, 1991]. According to the PM theory, the assumption of the formation of distinctive dynamic structure by the cell water results from its interaction with some intracellular proteins. More specifically, the dynamic structure of cell water results from its direct or indirect interaction with the positively-charged CO groups (P sites) and negatively-charged NH groups (N sites) on the "backbones" of a pervasive matrix of fully-extended proteins. These P and N-site-bearing proteins and the water molecules with which they interact constitute what is called a NP-NP system. Electrical polarization and directional orientation of multiple layers of water molecules may occur under the influence of one or two (juxtaposed) checkerboard(s) of alternatively positive and negative sites (Fig. 1):

Figure 1: The illustration of the way that individual ions and checkerboards of evenly distributed positively charged P sites alone (a) or negatively charged N sites alone (b) polarize and orient water molecules in immediate contact and further away. Emphasis was, however, on uniformly distanced bipolar surfaces containing alternatively positive (P) and negative (N) sites called an NP surface. When two juxtaposed NP surfaces face one another, the system is called an NP-NP system (c). [Ling 2003].



Parenthetically, water molecules may also be polarized and oriented in "layers" by a NO system or a PO system, in which electrically-neutral O sites replace properly-spaced electrically-charged P or N sites of a classic NP system respectively. The aggregate physical impacts of the NP sites on the bulk-phase water may be somewhat arbitrarily divided into three components: to enhance the average water-to-water interaction of (all) the water molecules in the system (Component 1); to reduce the translational as well as rotational motional freedom of the water molecules (Component 2); and to prolong the stay or *residence time* of each water molecule at a specific preferred location (Component 3). [Ling 2003].

The suggested model of polarized-oriented multilayer structuring of cell water due to the interaction of water dipoles with pervasive matrix of fully-extended proteins constitutes the basis for the cellular transduction mechanism. Based on this scientific approach the similarity of molecular formations of cell water and MRET activated water can contribute to their compatibility, easy bio-availability and assimilation of MRET activated water, as well as to the enhancement of cellular functions in biological systems.

The anomalous electrodynamic characteristics and viscosity of MRET Activated water provide some evidence regarding the possible effect of MRET water on the proper function of cells in biological systems. It is well known that cellular processes in biological systems are driven by the low energy of bio-chemical reactions inside and between the cells and cellular structures. Consequently, such processes create subtle low frequency electromagnetic field and low tangent pressures along water surfaces and the membranes between the cells. The anomalously low viscosity, dielectric permittivity and electrical conductivity of MRET water in the range of very low frequencies that exists in biological systems can contribute to the enhancement of the cellular transduction mechanism and result in improved intracellular/extracellular water exchange and the proper function of cells in biological systems.

The enhancement of the human body hydration was confirmed by Bioelectric Impedance Test conducted on eight human subjects at Global Quantech, Inc., USA with the help of FDA approved Bioelectric Impedance instrumentation, model Imp SFB7 and the following application of multi-frequency bioelectric impedance analysis. The experiment showed 3 times increase of the rate of intracellular/extracellular water exchange: the water exchange took 20 minutes after the ingestion of MRET activated water by human subjects compare to the 60 minutes in case of the ingestion of non-activated water by the same human subjects.

Taking into consideration the ability of MRET water to enhance morphology of human blood cells and to suppress mutated cells *in vitro* [Smirnov 2006-2, 2006-1, 2003], high germicidal activity of MRET water, the inhibition of growth of *kaluss* tissue (mutated cells of botanical origin) in MRET water (Pic 1) already confirmed by previous researches [Vysotskii 2006], the goal of this investigation was to study the effects of different fractions of MRET water on mutated cells in tumors and on the cells of immune system *in vivo*. As a result of this investigation the significant positive effect of MRET Activated water on the development of tumor resistance in animals was observed in the experiments conducted on 500 mice (22 groups with 20 mice in each group and 10 groups with 5 mice in each group).

Pic 1: The effect of MRET water (30 minutes and 60 minutes of activation) on the growth and development of kaluss tissue (mutated cells of botanical origin).

t_{act} = 1 h t_{act} = 0.5 h Control

Methods

In order to find out how different fractions of MRET Activated water affect the tumor resistance of organism the following experimental approaches and techniques were used in this research:

- study of possible anti-tumor efficacy of preventive administration of different fractions of activated water; for this purpose mice received activated water during 2 weeks before tumor cell transplantation and for 3 weeks after transplantation ("preventive treatment" regime);

- study of possible anti-tumor efficacy of therapeutic administration of different fractions of activated water; for this purpose mice received activated water for 3 weeks after tumor cell transplantation ("therapeutic treatment" regime);
- investigation of functional cytotoxic activity of lymphocytes containing natural killer cells (NK-cells) isolated from spleens of mice (without tumors) which received activated water; for this purpose lymphocytes were further incubated with tumor target cells.

Five different fractions of MRET activated water were prepared to elucidate effectiveness of anti-tumor effects of MRET activated water depending on time of its activation. Four water fractions were obtained after water activation for 15, 30, 45 and 60 minutes respectively. Furthermore, before the beginning of investigations a large volume of water was activated for 30 minutes and stored at 4˚C during 45 days. This fraction of activated water was called "old activated water". The inbred adult male BALB/c mice, 11 weeks old, with 23 – 24 g corporal weight were used in the study. These white mice are very susceptible to various oncology diseases. In both experiments (ascitic Ehrlich carcinoma and Sarcoma ascites form) the 5 experimental groups of mice received activated water in "preventive treatment" regime. The other 5 groups were treated with activated water in "therapeutic treatment" regime. One of the 11 groups served as a control and mice from this group received non-activated distilled water. Each group consisted of 20 mice. The cytotoxic activity of NK-cells was studied on 11 groups of mice with 5 mice in each group.

The ascitic Ehrlich carcinoma tumor cells were transplanted to all groups of mice for the first experiment. The first stage of the investigation was finished on the 8th day after tumor cell inoculation, when 10 mice from each group were sacrificed and ascitic fluids containing tumor cells were obtained from peritoneal cavities. The comparison of ascitic fluid volumes and the number of viable tumor cells from "preventive treatment", "therapeutic treatment", and "control" groups of mice allowed to determine the effects of applications of different fractions of activated water on growth and size of tumors in tumor-bearing mice. At the second stage of experiment the life span of tumor-bearing mice was studied.

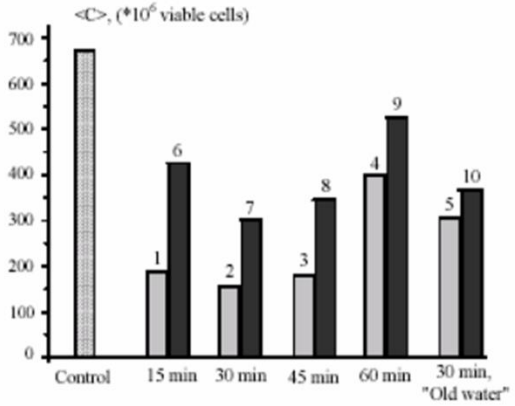
In order to understand the possible mechanism of anti-tumor effects of MRET activated water the study of modification in cytotoxic activity of lymphocytes was conducted on another 11 groups of mice without tumors treated with different fractions of activated water. The lymphocytes contain natural killer cells (NK-cells) which have the natural function of spontaneous cytotoxicity (without prior immunization), very important for proper immune system reactions. Due to such capacity NK-cells are considered to play a critical role in immune surveillance and cancer therapy. NK-cells that infiltrate tumors may protect against tumor spread. They also produce cytokines which can contribute to the elimination of infection. Due to such capacities NK-cells and the methods of enhancement of their functional activity based on the substances of natural origin have great potentials for immune-stimulation.

The purpose of this investigation was to evaluate optimal regimes of MRET water activation and the regime of application of activated water in order to maximize the stimulation of NK-cells cytotoxic activity. In the first stage of the research mice of experimental groups received activated water during different periods of time. Mice of "preventive treatment" groups received different fractions of MRET activated water for 21 days, and mice from "therapeutic treatment" groups received it for 14 days. After the treatment with activated water mononuclear lymphocyte fractions containing NK-cells were isolated from spleens of mice of experimental groups. In the second stage cytotoxic activity of NK-cells incubated with tumor target cells obtained from peritoneal cavities of mice transplanted with ascitic Ehrlich carcinoma was studied. The incubation was conducted for 18 hours at 37˚C in humidified atmosphere with 5% CO_2 and then the micro plates were gently centrifuged during 5 minutes.

Results

The experimental results confirm that consumption of all types of MRET activated water leads to the significant inhibition of tumor growth observed in mice with transplanted tumors. The best results were observed in the groups of mice on MRET water activated for **30 minutes (optimal regime of activation)**. The substantial anti-tumor efficacy was confirmed by very high level of reduction of Total Number of Viable Tumor Cells which is comprised of two processes: diminishing volume of ascitic fluid in peritoneal cavity of tumor-bearing mice (**by 50%** for animals **in "preventive treatment"** group) and decrease of the number of viable tumor cells per unit of tumor tissue (**by 52%** in the same group). The resulting decrease of the Total Number of Viable Tumor Cells was **76%**. The viable tumor cells were determined by Trypan blue exclusion test: the uncolored cells were considered as viable. The test results show the dual mechanism of MRET water effect on tumors: the prevention and reduction of volume of tumors together with the inhibition of viable tumor cells. Approximately the same level of efficacy was observed in other groups (water activated for 15 and 45 minutes) with "preventive treatment" regime of application of activated water. The application of MRET water **in "therapeutic treatment"** regime was less effective with the similar tendency in dependency of the level of anti-tumor efficacy on the activation time. The reduction of Total Number of Viable Tumor Cells **by 55%** was observed for the optimal 30 minutes activated water. It is important to note that the long-term preservation of activated water for 45 days decreased its anti-tumor efficacy but left it on the significantly high level compare to other fractions and non-activated water. Thus, this investigation confirms that MRET activated water is efficient anti-tumor agent. The results of experimental measurements of Average Total Number of Viable Tumor Cells of ascitic *Ehrlich* carcinoma are presented on Fig 2.

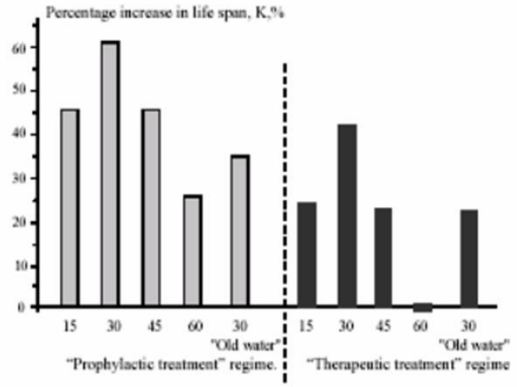
Figure 2: The effect of preventive (1–5) and therapeutic (6–10) application of MRET activated water on average total number of viable cells <C> in an ascetic tumor, obtained from mice inoculated intraperitoneally with tumor cells of Ehrlich carcinoma.



The survival of the investigated animals was daily monitored in order to study the effect of different activated water fractions on dynamic and survival indices of tumor-bearing mice. The data of the dependence of changes of life span of tumor-bearing mice for both application regimes and all types of activated water are presented on Fig 3. It shows that the consumption of MRET Activated water substantially increased survival of tumor-bearing animals. The increase of life span was observed in all groups of mice except "therapeutic treatment" group on water activated for 60 minutes. Water activated for **30 minutes (optimal regime of activation)** produced the most significant effect on survival of mice with transplanted tumors. The life span of mice which received optimal activated water **in "preventive treatment"** regime increased **by 61%.** Significant increase in life span (about

45%) was also observed when mice were treated with MRET activated water (activation time 15 minutes, and 45 minutes) in "preventive treatment" regime. The increase of life span **by 43%** was observed **in "therapeutic treatment"** regime for the optimal 30 minutes activated water.

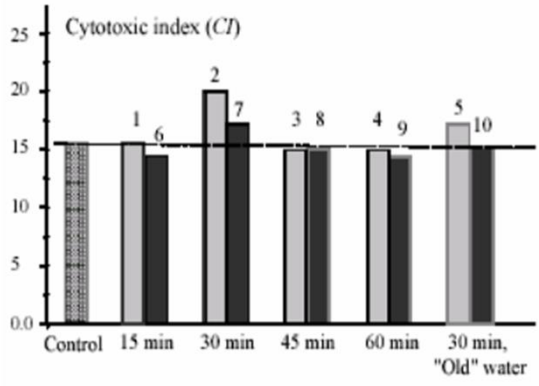
Figure 3: The change of the percentage increase of life span of tumor-bearing mice with ascitic Ehrlich carcinoma which received different types of MRET activated water in "preventive treatment" and "therapeutic treatment" regimes. The digits under the charts correspond with the duration of water activation in minutes.



The similar investigation conducted on mice with transplanted ascitic sarcoma revealed similar tendencies in the efficacy of MRET water as anti-tumor agent with the best results on optimal 30 minutes activated water.

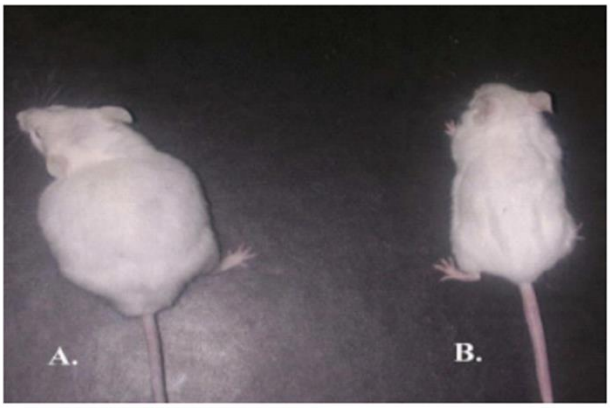
The effect of application of different fractions of MRET activated water on the development of cytotoxic activity of spleen mononuclear lymphocytes with NK-cells is shown on Fig 4. The increase of **cytotoxic index** in both regimes (**21 days and 14 days** of application of activated water for mice without tumors) **by 26% and 10%** respectively was observed only in the groups of mice under MRET water activated for 30 minutes. The cytotoxic index also increased in the group of mice under "old" 30 minutes activated water in "preventive" regime (21 days of MRET water application). No significant changes in cytotoxic index were observed for other water fractions.

Figure 4: The effect of MRET activated water on cytotoxic activity of lymphocytes containing NK-cells. Activated water was applied for mice without tumors in two regimes (for 21 and 14 days), called "preventive" (1–5) and "therapeutic" (6–10).



The comparative picture of tumor-bearing mice on Non-activated water and on MRET activated water (optimal activation time 30 minutes) is presented on Pic 2.

Pic 2: The appearance of mice from "control" (A) and "preventive treatment" groups (optimal activation time 30 minutes) (B) on the 18th day after ascitic Ehrlich carcinoma cell inoculation.



Conclusions

The results of investigation of the application of water activated by non-ionizing Molecular Resonance Effect Technology (MRET) process for prevention treatment and enhancement of tumor resistance of animal organisms to two types of oncology diseases *in vivo* on 500 mice are presented. The research conducted on physical parameters of water confirmed that MRET activation process contributed to substantial modification of the basic physical-molecular properties of distilled water (substantial reduction of viscosity as a function of applied tangent pressure, as well as substantial decrease of electrical conductivity and dielectric permittivity as functions of the frequencies of applied electromagnetic field). The significant positive effect of MRET activated water on tumor resistance of biological organisms was observed in the process of this investigation in all groups of mice on different fractions of activated water. The best results were observed in the groups of mice on MRET water activated for 30 minutes (optimal regime of activation). The results were better in "preventive treatment" regime compare to "therapeutic treatment" regime. Additionally, this investigation confirmed that the long-term preservation of activated water at low temperature (around 0°C) for 45 days decreased its anti-tumor efficacy but left it on the significantly high level compare to other fractions. The discussion and detailed description of the mechanism of "long-term memory effect" of MRET activated water was earlier presented and published in [Vysotskii, Smirnov 2005]. The test results show the dual mechanism of MRET water effect on tumors: the prevention and reduction of volume of tumors together with the inhibition of viable tumor cells. The significant anti-tumor effect of MRET activated distilled water on mice was close to the action of the chemotherapy agents and allowed to avoid the side effects that typically follow chemotherapy treatment in oncology.

In the process of investigation of cytotoxic activity of NK-cells the significant increase of lymphocyte cytotoxicity levels was observed when donor mice were treated with MRET water activated for 30 minutes. The results also showed that the extension of the application of MRET water from 14 days to 21 days significantly increased the value of cytotoxicity index. It is possible to admit that the extension of time of application of MRET water will lead to higher levels of enhancement in NK-cells activity. Thus, the application of MRET activated water can be quite promising approach for non-drug stimulation of NK-cells immunization vaccines.

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Investigation of Physical Properties of MRET Activated Water and its Successful Application for Prophylaxis and Treatment of Oncology

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Abstract— **The results of investigation of the effect of water, activated by nonionizing Molecular Resonance Effect Technology (MRET), on prophylaxis and treatment of two kinds of oncology diseases of mice are presented. It was discovered in detailed experiments that under the action of this activator there are very essential modifications of the basic physical-molecular properties of distilled water (decrease of viscosities, electrical conductivity and permittivity by several times, sharp increasing or oscillation of pH exponent etc.).**

The effectiveness of action on water depends on time of irradiation. Potent effect of activated water on total tumor cell content has been observed. In particular, the total tumor cell content in mice of "prophylactic treatment"group which received water activated in the most optimal regime (t_{act} = 30 min) was 4.2-fold decreased in comparison with control mice!

It has been shown that average survival time of mice which received optimal activated water (t_{act} = 30 min) in "prophylactic treatment" regime increased to 61.7% compare to control groups. Very marked increase in life span (about 45%) was observed when mice were treated with activated water (t_{act} = 15 min and 45 min) in "prophylactic treatment" regime. Eeffectiveness of action of such activated distilled water approximately equal action of chemotherapy!

The positive influence (increase by 20%) of optimal fractions of activated water on Cytotoxic activity of mouse lymphocytes *in vitro* at prophylaxis action is also presented.

Keywords— **MRET Activated Water, Ehrlich Carcinoma, Sarcoma, Prophylactic Treatment, Therapeutic Treatment, Idex of Cytotoxic Activity.**

1. PHYSICAL-MOLECULAR PROPERTIES OF MRET ACTIVATED WATER

In the work the results of investigation of the effect of water, activated by nonionizing Molecular Resonance Effect Technology (MRET*), on prophylaxis and treatment of two kinds of oncology diseases of mice (laboratory models of Ehrlich's ascites tumor and Sarcoma ascites form) are presented. Investigated activator of water is the stationary source of low-frequency resonant magnetic field with composite space structure and very weak amplitude (about 1 *Oersted*).

It was discovered in our detailed physical experiments that under the action of this irradiation there are very essential modifications of the basic physical-molecular properties of distilled water.

It was discovered also that influence of MRET activation on properties of distilled water very strongly depends on duration of activation. E.g., at very low velocity of motion of investigated activated water (tension less than 0.01 Pa) viscosity of water, activated during 60 min, decrease by 100 times at 20^0 C compare to nonactivated water. Minimal viscosity at such temperature will be for water activated during 30 min. In this case viscosity decrease by 300 times!

These results are presented on Fig.1

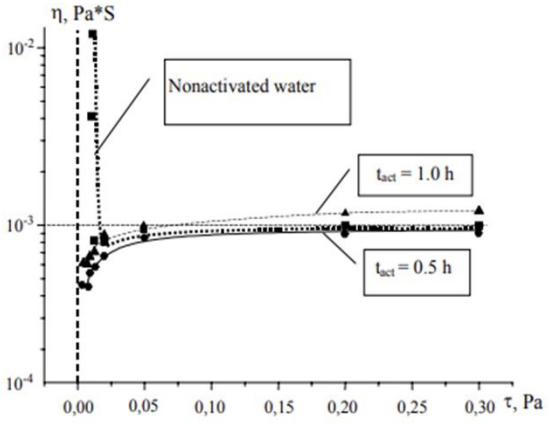

Fig.1. Influence of MRET activation on viscosity of distillated water.

The same strong influence of MRET activation on different electrical characteristics of activated water in the range of low frequencies was observed and presented on Fig.2.

* The US Patent No. 6022479 was granted to US scientist Igor Smirnov in the year 2000 for the invention of the process of MRET activation of liquid substances.

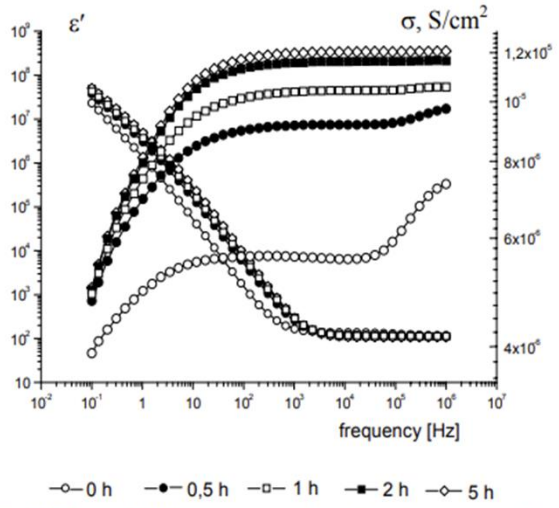

Fig.2. Influence of MRET activation on electrical conductivity (σ) and permittivity (ε′) of distillated water. Water was activated during 30 min and storage at 20 C during 0.5; 1, 2 and 5 hours.

It was discovered also that duration of internal storage of these abnormal characteristics of activated water at low temperature (close to 0^0 C) equals several days or weeks. The problem and concrete mechanisms of long time memory of usual and activated water were studied earlier and was published in [1,2].

11. EXAMINATION OF INFLUENCE OF ACTIVATED WATER ON PROPHYLAXIS AND TREATMENT OF ONCOLOGY

The next important step of investigations was to study the effects of different fractions of activated water on various transformed cells and immune system cells. For research how different fractions of activated water affect on antitumor resistance of organism the following experimental approaches and techniques were used:

- study of possible antitumor effectiveness of prophylactic administration of different fractions of activated water; for that mice received activated water during 2 weeks before tumor cell transplantation and after transplantation ("prophylactic treatment" regime);
- study of possible antitumor effectiveness of therapeutic administration of different fractions of activated water; for that mice received activated water after tumor cell transplantation ("therapeutic treatment" regime);
- investigation of functional activity of lymphocytes with natural killer cell cytotoxicity isolated from spleens of normal (without tumors) mice which received activated water.

A. Research of antitumor effects of different fractions of MRET-activated water in vivo application in regimes of prophylactic and therapeutic treatment tested on tumor model of ascitic Ehrlich carcinoma

Five different fractions of MRET activated water were prepared to elucidate effectiveness of antitumor effects of activated water depending on time of its activation. Four water fractions were obtained after water activation during 15, 30, 45 and 60 min. Moreover, before beginning of investigations a large volume of water was activated during 30 min and stored at 4^0C about 45 days. This fraction of activated water was named as "old activated water". In the study inbred adult male BALB/c mice at 11 weeks of age, with 23 – 24 g corporal weight were used.

The five experimental groups comprised mice which received activated water in "prophylactic treatment" regime. The other 5 groups were treated with activated water in "therapeutic treatment" regime. One of the 11 groups served as a control and comprised mice which received non-activated distilled water. In each group was 20 mice.

The first stage of investigations was finished on the 8th day after tumor cell inoculation, when 10 mice from each group were sacrificed and ascitic fluids containing tumor cells were obtained from peritoneal cavities. Correlation between ascitic fluid volumes and tumor cell number of mice from "prophylactic treatment", "therapeutic treatment", and "control" groups allows to determine the effects of applications of different activated water fractions on growth and size of tumors in tumor-bearing mice. Results of experimental measurements of ascitic fluid average volume and both total and unit of volume average cell number of ascitic *Ehrlich* carcinoma are presented in Fig. 3 - 4.

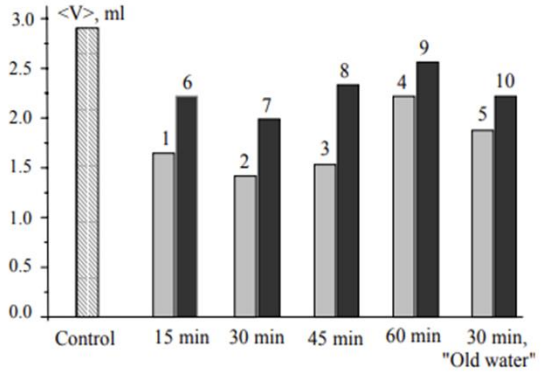

Fig. 3. Effect of prophylactic (1–5) and therapeutic (6–10) application of MRET activated water on average volume <V> of ascitic fluid obtained from mice inoculated intraperitoneally with tumor cells of Ehrlich carcinoma

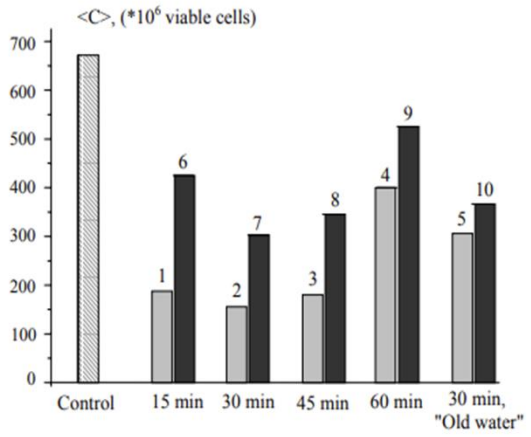

Fig. 4. Effect of prophylactic (1–5) and therapeutic (6–10) application of MRET activated water on average number of viable cells <C> in an ascitic tumor, obtained from mice inoculated intraperitoneally with tumor cells of Ehrlich carcinoma.

Results obtained of experimental investigations suggest that applications of optimal types of activated water in optimal regime show substantial positive effects resulted in tumor growth inhibition observed in mice with transplanted tumor. Positive antitumor effect was displayed in decrease of both the volume of ascitic fluid in peritoneal cavity of tumor-bearing mice by 2 times in animals of "prophylactic treatment" group (t_{act} = 30 min) and content of viable tumor cells (by 4.2 times in the same group). Similar tendency and approximately the same efficacy were observed in other groups with prophylactic application of activated water. It is important to note that although long-term storage of activated water decreased its antitumor activity but did not abrogate it. Such water is as usual sufficiently effective antitumor substance.

Other set of mice (10 in each group) continued to receive the same activated water fraction. The survival of these animals were daily monitored in order to study the effects of certain activated water fractions on dynamic and survival indices of tumor-bearing mice.

Data of dependence of changes in percentage increase life span of tumor-bearing mice and both application regime and type of activated water are presented on Fig. 5.

Application of activated water has a substantially influence on survival of tumor-bearing animals. When activated water was applied in "prophylactic treatment" regime, the increase in life span was observed in all groups of mice. Water activated during 30 min has the most potent effect on survival mice with transplanted tumors. It has been shown that average survival time of mice which received optimal activated water (t_{act} = 30 min) in "prophylactic treatment" regime increased to 61.7%. Very marked increase in life span (about 45%) was observed when mice were treated with activated water (t_{act} = 15 min and 45 min) in "prophylactic treatment" regime.

When mice received activated water in "therapeutic treatment" regime, significant positive effects on increase in life span were also observed. However, values of estimated index were 30–50% lower than in "prophylactic treatment" regime.

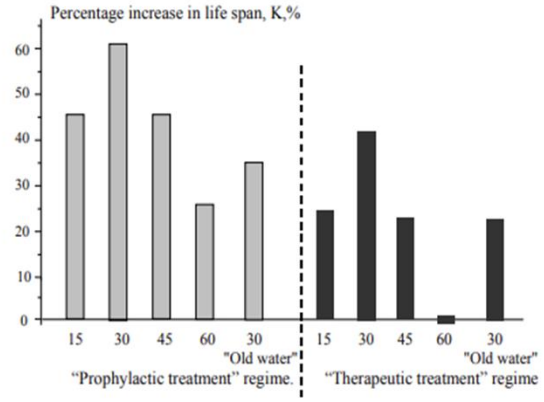

Fig. 5. Change of percentage increase in life span of tumor-bearing mice with ascitic Ehrlich carcinoma which received different types of MRET activated water in "prophylactic treatment" and "therapeutic treatment" regimes The digits near the charts correspond to duration of water activation in minutes.

Efficacy of prophylactic application of activated water is very close to the same of potent antitumor chemotherapeutic drugs!

Effect of activated water on tumor and survival of mice with sarcoma 37 was similar to the same of ascitic Ehrlich carcinoma model but however was less expressed.

B. Research of activated water effects on cytotoxic activity of murine lymphocytes

In order to understand the possible mechanism of antitumor effects of MRET activated water the studies of changes in cytotoxic activity of lymphocytes of mice treated with different fractions of activated water were carried. Natural killer (NK) cells are important cells of immune system. Based on their defining function of spontaneous cytotoxicity without prior immunization, NK cells have been thought to play a critical role in immune surveillance and cancer therapy. Results of investigations of effects of different activated water fractions on NK cell activity are presented below. The study was aimed to evaluate optimal regimes of water activation and regime of activated water application for maximal stimulation of NK cell cytotoxic activity.

In the first stage of investigation, mice of experimental and control groups received activated water during different time. Mice of "prophylactic treatment" groups received different fractions of MRET activated water during 21 days, whether "therapeutic treatment" groups – during 14 days. When treatment with activated water was finished,

mononuclear lymphocyte fractions enriched with NK cells were isolated from spleens of mice of experimental groups.

In the second stage, cytotoxic activity of NK cells incubated with tumor target cells obtained from peritoneal cavities mice transplanted with ascitic Ehrlich carcinoma was studied.

Effects of prophylactic and therapeutic application of different fractions of MRET activated water on levels of cytotoxic activity of splenic mononuclear lymphocytes with NK-activity are showed on Fig. 6 and Fig. 7.

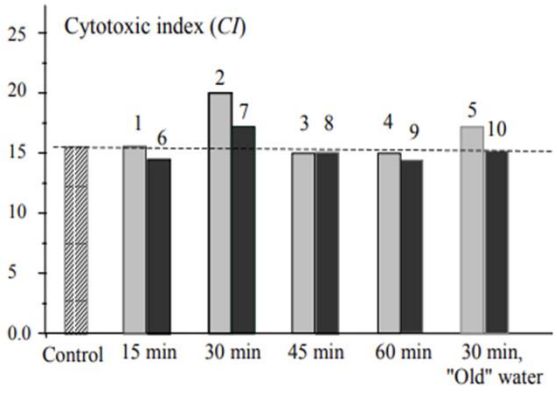

Fig. 6. Effects of MRET activated water on cytotoxic activity of lymphocytes with natural killer cell activity. Activated water was applied in prophylactic (1–5) and therapeutic (6–10) regimes.

Fig. 7 shows the changes of cytotoxic activity of murine mononuclear lymphocytes stimulated during activated water treatment in comparison with the same activity values of lymphocytes isolated from spleens of non-activated water treated mice.

The data obtained demonstrate that immunostimulatory potential of activated water is dependent of both duration of activation and duration of storage of activated water before treatment of mice. Our results clearly demonstrate the changes of cytotoxic activity of NK cells of mice after period of treatment with activated water was prolonged. In particular, when water activated during 30 min was applied in prophylactic regime significant increase of cytotoxic activity of lymphocytes was observed.

Application of this water in "prophylactic treatment" regime resulted in increase of cytotoxicity index on 20 % as compared to control values obtained after application of the same but non-activated water.

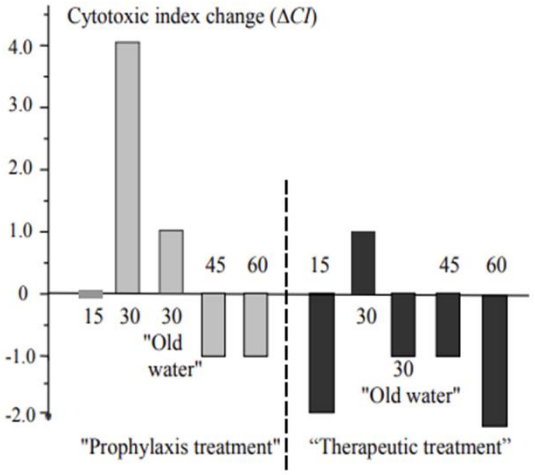

Fig. 7. Changes of cytotoxic activity of mononuclear lymphocytes of mice received different types of MRET activated water in comparison to results of non-activated water application. Figures at respective diagrams corresponds to duration of water activation expressed in minutes.

In conclusion, application of activated water can induce significant activation of NK cell cytotoxic potential. In this scenario, it is apparent that application of activated water in tumor-bearing immunocompetent hosts would result in cytotoxic activation of NK cells to destroy tumor cells. Thus, obtained results may be important for future therapeutic approaches that implicate activated water.

Acknowledgment

The present work was conducted through a support from MRET Technology Inc. The authors would like to thank Mrs. Diana Suk.

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The Effect of MRET (Molecular Resonance Effect Technology) Activated Water on Enhanced Tumor Resistance in Oncology

Igor V. Smirnov, Ph.D.

ABSTRACT

This article relates to a fundamental nature on how MRET (molecular resonance effect technology) Activated Water and its modified molecular structure, physical, and electrodynamic characteristics, may enhance specific molecular mechanisms in living cells. The research regarding the physical parameters of water confirmed that MRET treatment of distilled water leads to a substantial modification of the basic physical-molecular properties of water. The anomalous viscosity of MRET water (subject to very low tangent pressure) and electrodynamic characteristics of MRET water (subject to applied electromagnetic field of low frequency range) confirm the high level of long-range dynamic structuring of water molecules in polarized-oriented multilayer formations in activated water that are produced with the help of MRET activation process. This paper is concerned with the potential of MRET Activated Water as a possible agent for the prevention and treatment of cancer.

INTRODUCTION

Molecular Resonance Effect Technology (MRET) Activated Water is produced by a Water Activator patented in the USA (MRET, US Patent # 6022479). The MRET Water Activator is the stationary source of subtle, low-frequency, resonant electromagnetic field of the composite structure. The origin of the low-frequency composite electromagnetic field is the intensive electrical activity inside the nano-circles, which is formed by linear molecular groups of MRET polymer compound (volumetric fractal geometry matrix) when a polymeric body is exposed to the external electromagnetic fields of specific frequency and wavelength.[1]

The goal of this investigation was to study (in mice) the effect of MRET activated water as a potential agent for the prevention and treatment of two kinds of cancer (laboratory models of Ehrlich's ascites tumor and Sarcoma ascites form). The research was conducted under supervision of Professor V Vysotskii (Kiev State University, Ukraine), S Olishevsky, Ph.D., Y Yanish, Ph.D. (Kiev Institute of Experimental Pathology, Oncology, and Radiobiology, Ukrainian Academy of Science), and A Kornilova, Ph.D. (Moscow State University, Russia).

The anomalous viscosity of MRET water (subject to very low tangent pressure) and electrodynamic characteristics of MRET water (subject to applied electromagnetic field of low frequency range) confirmed the high level of long-range dynamic structuring of water molecules in polarized-oriented multilayer formations that are present in activated water produced with the help of the MRET activation process.[2] The fundamental biophysical theories revealed the scientific paradigm regarding the polarized-oriented multilayer structuring of cell water in biological systems. The interaction of water dipoles with a pervasive matrix of fully-extended proteins constitutes the basis for the cellular transduction mechanism.[3] Based on this scientific approach, the similarity of molecular formations of cell water and MRET activated water can contribute to their compatibility, easy bio-availability, and assimilation of MRET activated water, as well as to the enhancement of cellular functions in biological systems.

METHODS

The following experimental approaches and techniques were used in this research in order to find out how different fractions of MRET Activated water affect the tumor resistance of an organism:

- Study of possible anti-tumor efficacy of preventive administration of different fractions of activated water; mice received activated water during 2 weeks before tumor cell transplantation and for 3 weeks after transplantation (preventive treatment regime).
- Study of possible anti-tumor efficacy of therapeutic administration of different fractions of activated water; mice received activated water for 3 weeks after tumor cell transplantation (therapeutic treatment regime).
- Investigation of functional cytotoxic activity of lymphocytes containing natural killer cells (NK-cells) isolated from spleens of mice (without tumors) which received activated water; for this purpose lymphocytes were further incubated with tumor target cells.

Five different fractions of MRET activated water were prepared to elucidate the effectiveness of the anti-tumor effects of MRET activated water depending upon the time of its activation. Four water fractions were obtained after water activation for 15, 30, 45, and 60 minutes respectively. Furthermore, before the beginning of the investigation a large volume of water was activated for 30 minutes and stored at 4°C during 45 days. This fraction of activated water was called "old activated water".

Inbred adult male BALB/c mice, 11 weeks old, with 23 – 24 g corporal weight were used in the study. These white mice are very susceptible to various oncological diseases. In total, 11 groups of mice were used for the preventive and therapeutic study, and each group consisted of 20 mice. Of the 11 groups, 5 were used in the preventive arm of the study and another five were used in the therapeutic arm of the study, these ten groups of mice all received MRET Activated Water. The remaining group served as a control, and mice from this group received non-activated distilled water.

The ascitic Ehrlich carcinoma tumor cells were transplanted to all groups of mice for the first experiment. The first stage of the investigation was finished on the 8th day after tumor cell inoculation, when 10 mice from each group were sacrificed and ascitic fluids containing tumor cells were obtained from peritoneal cavities. The comparison of ascitic fluid volumes and the number of viable tumor cells from “preventive treatment”, ”therapeutic treatment”, and ”control” groups of mice allowed for the study of the effects of application of different fractions of activated water on growth and size of tumors in tumor-bearing mice. In the second stage of the experiment the lifespan of tumor-bearing mice was studied.

In order to understand the possible mechanism behind the anti-tumor effects of MRET activated water an investigation into the modification of the cytotoxic activity of lymphocytes by MRET Activated Water was conducted on another 11 groups of tumor-free mice (each containing 5 mice) that had been treated with different fractions of activated water. The lymphocytes contain natural killer cells (NK-cells), which have the natural function of spontaneous cytotoxicity (without prior immunization), a function that is very important for proper immune system reactions. Due to this ability NK-cells are considered to play a critical role in immune surveillance and cancer therapy. NK-cells that infiltrate tumors may protect against tumor spread. They also produce cytokines that can contribute to the elimination of infection. Therefore substances that are capable of enhancing the functional activity of NK-cells are of great interest.

The purpose of this investigation was to evaluate optimal regimes of MRET water activation and the regime of application of activated water in order to maximize the stimulation of the cytotoxic activity of NK-cells. In the first stage of the research mice of experimental groups received activated water during different periods of time. Mice of “preventive treatment” groups received different fractions of MRET Activated Water for 21 days, and mice from “therapeutic treatment” groups received MRET Activated Water for 14 days. After treatment with MRET Activated Water, mononuclear lymphocyte fractions containing NK-cells were isolated from the spleens of mice in the experimental groups. In the second stage, the cytotoxic activity of NK-cells incubated with tumor target cells obtained from peritoneal cavities of mice transplanted with ascitic Ehrlich carcinoma was studied. The incubation was conducted for 18 hours at 37°C in a humidified atmosphere with 5% CO_2, and then the micro plates were gently centrifuged for 5 minutes.

RESULTS

The experimental results showed that consumption of all types of MRET activated water lead to a significant inhibition of tumor growth in mice with transplanted tumors. The best results were observed in the groups of mice treated with MRET water activated for 30 minutes (optimal regime of activation). The substantial anti-tumor efficacy was confirmed by the very high level of reduction of the total number of viable tumor cells, which is comprised of two processes: diminishing volume of ascitic fluid in peritoneal cavity of tumor-bearing mice (50% for animals in “preventive treatment” group) and a decrease of the number of viable tumor cells per unit of tumor tissue (52% in the same group). The resulting decrease of the total number of viable tumor cells was 76%. The viable tumor cells were determined by the Trypan Blue Exclusion Test: the uncolored cells were considered as viable.

The test results show the dual mechanism of MRET Activated Water’s effect on tumors: the prevention and reduction of the volume of the tumor together with the inhibition of viable tumor cells. An approximate level of efficacy was observed in other groups (water activated for 15 and 45 minutes) within the “preventive treatment” regime of application of MRET Activated Water. The application of MRET Activated Water in the “therapeutic treatment” regime was less effective. The reduction of the total number of viable tumor cells by 55% was observed for the optimal 30 minutes MRET Activated Water. It

is important to note that the long-term preservation of MRET Activated Water for 45 days decreased its anti-tumor efficacy; however its effects were still significant in comparison with other fractions and non-activated water. Thus, this investigation confirms that MRET Activated Water is an effective anti-tumor agent. The results of the experimental measurements of the average total number of viable tumor cells of ascitic Ehrlich carcinoma are presented on Fig. 1.

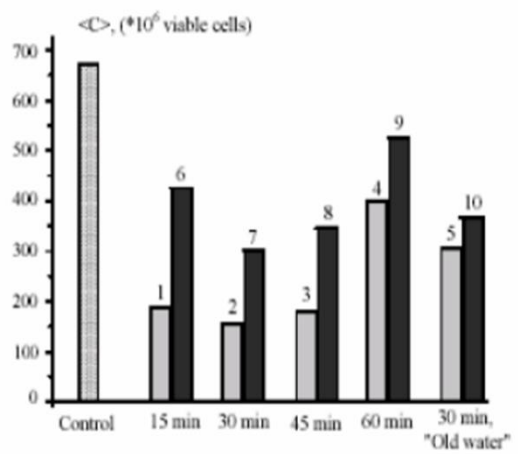

Fig. 1 The effect of preventive (1–5) and therapeutic (6–10) application of MRET Activated Water on the average total number of viable cells <C> in an ascetic tumor, obtained from mice inoculated intra-peritoneal with tumor cells of Ehrlich carcinoma.

The survival of the investigated animals was monitored daily in order to study the effect of different MRET Activated Water fractions on dynamic and survival indices of tumor-bearing mice. The data of the dependence of changes of lifespan of tumor-bearing mice for both application regimes and all types of activated water are presented in Fig. 2. The results confirmed that the consumption of MRET Activated Water substantially increased the survival of tumor-bearing animals. The increase of lifespan was observed in all groups of mice except those in the “therapeutic treatment” group on water activated for 60 minutes. Water activated for 30 minutes (optimal regime of activation) produced the most significant effect on survival of mice with transplanted tumors. The lifespan of mice that received optimal MRET Activated Water in the “preventive treatment” regime increased by 61%. Significant increase in lifespan (approximately 45%) were also observed when mice were treated with MRET Activated Water (activation time 15 minutes, and 45 minutes) in the “preventive treatment” regime. The increase of life span by 43% was observed in the “therapeutic treatment” regime group administered the optimal 30 minutes MRET Activated Water.

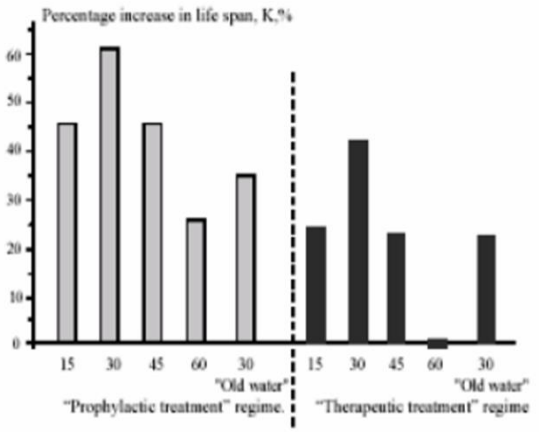

Fig. 2 The change of the percentage increase of lifespan of tumor-bearing mice with ascitic Ehrlich carcinoma that received different types of MRET Activated Water in “preventive treatment” and “therapeutic treatment” regimes. The digits under the charts correspond with the duration of water activation in minutes.

The similar investigation conducted on mice with transplanted ascitic sarcoma revealed similar tendencies in the efficacy of MRET Activated Water as an anti-tumor agent with the best results achieved with water activated for 30 minutes.

The effect of application of different fractions of MRET Activated Water on the cytotoxic activity of spleen mononuclear lymphocytes with NK-cells is shown in Fig 3. The increase in the cytotoxic index in both regimes (21 days and 14 days of application of MRET Activated Water for mice without tumors) by 26% and 10% respectively was observed only in the groups of mice receiving MRET Activated Water that had been activated for 30 minutes. The cytotoxic index also increased in the group of mice treated with "old" 30 minutes MRET Activated Water in the "preventive" regime (21 days of MRET Activated Water application). No significant changes in cytotoxic index were observed for the other water fractions.

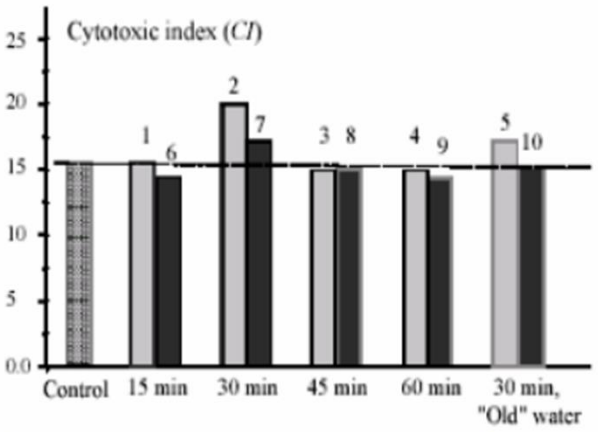

Fig. 3 The effect of MRET Activated Water on the cytotoxic activity of lymphocytes containing NK-cells. MRET Activated Water was given to mice without tumors in two regimes (for 21 and 14 days), called "preventive" (1–5) and "therapeutic" (6–10).

CONCLUDING REMARKS

The research conducted confirmed that the MRET activation process contributed to substantial modification of the basic physical-molecular properties of distilled water (substantial reduction of viscosity as a function of applied tangent pressure, as well as a substantial decrease of electrical conductivity and dielectric permittivity as functions of the frequencies of applied electromagnetic field).

The significant positive effect of MRET Activated Water on the tumor resistance of biological organisms was observed in the process of this investigation in all groups of mice receiving different fractions of water. The best results were observed in the groups of mice treated with MRET Activated Water which had been activated for 30 minutes (optimal regime of activation). Better results were obtained from the "preventive treatment" regime than with the "therapeutic treatment" regime. Additionally, this investigation confirmed that the long-term preservation of activated water at low temperature (around 0˚C) for 45 days decreased its anti-tumor efficacy; however it still displayed significant anti-tumor properties. The test results show the dual mechanism by which MRET Activated Water exerts its effect upon tumors: the prevention and inhibition of tumor growth together with the reduction of quantity of viable tumor cells. The significant anti-tumor effect of MRET Activated Water on mice was close to the action of chemotherapy agents, however unlike chemotherapy agents MRET Activated Water does not cause unpleasant adverse side effects.

In the process of investigation of the cytotoxic activity of NK-cells the significant increase of lymphocyte cytotoxicity levels was observed when donor mice were treated with MRET Activated Water that had been activated for 30 minutes. The results also showed that the extension of the administration of MRET Activated Water from 14 days to 21 days significantly increased the value of its cytotoxicity index. It is possible to admit that the extension of time of application of MRET Activated Water will lead to a greater enhancement in NK-cell activity. Thus, the application of MRET Activated Water may offer a promising approach for non-drug stimulation of NK-cells.

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Dr. Igor Smirnov graduated from St. Petersburg Naval Academy, Russia, obtaining M.S. in Mechanical Engineering in 1975. In 1986 he graduated from St. Petersburg State University obtaining his Ph.D. in Clinical Psychology. In the 1980's he was actively involved in advanced research regarding the influence of low frequency electromagnetic oscillations on human cellular structure conducted at St. Petersburg University. Based on this research program he developed Molecular Resonance Effect technology (MRET) which was patented in the USA in 2000. He was also involved in advanced research and study of the psychosomatic development of children swimming in infancy. This research program was conducted at St. Petersburg Children Hospital. The results of this scientific research program was published and disseminated through the office of World Health Organization in Munich, Germany [http://www.dissertation.com/book.php?method=ISBN&book=1581122845]. He is a President of Global Quantech, Inc., biotech research company.

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The comparative analysis of the effect of MRET treatment on morphology of HeLa cancer cells and PBMC normal cells

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ABSTRACT

This particular article relates to a fundamental nature on how MRET Activated Water with the modified molecular structure, physical and electrodynamic characteristics may affect molecular mechanisms in living cells. The research regarding the physical parameters of water confirmed that MRET treatment of distilled water leaded to substantial modification of basic physical-molecular properties of water. The anomalous viscosity of MRET water (subject to very low tangent pressure) and electrodynamic characteristics of MRET water (subject to applied electromagnetic field of low frequency range) confirm the high level of long-range dynamic structuring of water molecules in polarized-oriented multilayer formations in activated water produced with the help of MRET activation process. The similarity of molecular formations of cell water and MRET activated water contributes to compatibility and easy assimilation of MRET activated water in living cells. The introduction of MRET water to biological systems may contribute to the enhancement of the cellular transduction mechanism and proper function of cells in biological systems. The *in vitro* study conducted at AltheaDx Technology confirmed that MRET activated water based medium did not affect the living cells on genetic level; it affected the morphology of normal PBMC cells in a positive way increasing their viability and on the other hand promoted significant inhibition of growth of HeLa cancer cells.

Keywords: MRET Activated Water, HeLa cells, PBMC cells, Viability, Viscosity, Electrical Conductivity, Dielectric Permittivity.

INTRODUCTION

This particular article relates to a fundamental nature on how MRET activated water with the modified molecular structure, physical and electrodynamic characteristics may affect specific molecular mechanisms in living cells. The goal of this investigation was to study *in vitro* the effect of MRET activated water based medium on growth and viability of HeLa cancer cells and normal PBMC cells.

MRET Activated Water is produced with the help of patented in the USA Molecular Resonance Effect Technology (MRET). MRET water activator is the stationary source of subtle, low-frequency, resonant electromagnetic field with composite structure. The origin of the low-frequency composite electromagnetic field is the intensive electrical activity inside the nano-circles formed by linear molecular groups of MRET polymer compound (volumetric fractal geometry matrix) when polymeric body is exposed to the external electromagnetic fields of specific frequency and wavelength (Vysotskii *et al.*2005). The research regarding the physical parameters of water confirmed that MRET treatment of distilled water leaded to substantial modification of basic physical-molecular properties of distilled water. The level of modification of properties of MRET water depends on the duration of the process of activation. The results also confirmed the ability of MRET activated water to keep its anomalous characteristics for several hours or days at room temperature and especially at low temperature (known in physics as the "long-term water memory" phenomenon (Vysotskii *et al.*2004).

The experiment conducted on MRET activated water subjected to the tangential pressure revealed that at very low velocity of motion of water (tangential pressure in the range of 0.004 – 0.005 Pa, temperature 20˚C) the viscosity of water activated for 60 minutes decreased about 200 – 250 times compare to non-activated water from the same source. The most significant phenomenon of anomalous low viscosity of activated water, the decrease about 300 – 500 times, was observed for water activated for 30 minutes. These results confirm the hypothesis regarding the modification of molecular structure in MRET activated water. Particularly, the anomalously low viscosity of MRET activated water in the area of very low tangent pressure confirms the polarized-oriented multilayer molecular structuring of MRET water: the high level

of long-range molecular coupling (hydrogen bonding) inside the "layer" and very low level of molecular coupling between the "layers."

The significant modification of electrodynamic characteristics of distilled water subjected to applied electromagnetic field in the range of low frequencies was observed after MRET activation. The electrical conductivity of MRET activated water at 20°C in the range of frequencies of 0.1 Hz – 100 kHz decreased by 80 – 90% in 30 minutes activated water, and by 66 – 70% in 60 minutes activated water respectively compare to non-activated distilled water. The dielectric permittivity in the very low frequency range of 0.1 – 1000 Hz decreased by 80 – 90% and in the range of frequencies of 1 – 100 kHz it decreased by 18% in 30 minutes activated water; the decrease by 70 – 85% was observed in the range of 0.1 – 1000 Hz in 60 minutes activated water compare to non-activated water from the same source. It is reasonable to admit that in the range of very low frequencies 0.1 – 1000 Hz the long-range multilayer molecular structures of MRET water (with high level of molecular coupling inside the "layers" and extremely low level of hydrogen bonding between the "layers") create lower level of resistance of water dipoles to the alignment and, as a result, the dielectric permittivity of MRET water is substantially lower by 70 – 90% compare to non-activated water. This substantial decrease of dielectric permittivity also confirms the direct correlation between viscosity and dielectric permittivity of water in the range of low frequencies of applied EMF (Drost, 1971).

The investigation regarding the electrodynamic characteristics of MRET water also revealed that the long-term storage of activated water (up to 5 hours at 20°C) did not substantially affect the modified electrodynamic characteristics of 30 minutes activated water (the reduction of conductivity still kept the level of 66 – 70% and dielectric permittivity kept the level of decrease by 50 – 55% in the range of 0.1 Hz – 1 kHz and by 18% in the range of 1 – 100 kHz respectively). The storage of 60 minutes activated water under the same conditions practically did not affect its electrodynamic characteristics (maximum difference is 2%). These results confirm the ability of MRET activated water to keep its anomalous properties for rather long period of time (known as "long-term water memory" phenomenon) in case of 30 minutes activation and even higher level of "long-term water memory" phenomenon in case of 60 minutes activation.

Thus, the anomalous viscosity of MRET water (subject to very low tangent pressure) and electrodynamic characteristics of MRET water (subject to applied electromagnetic field of low frequency range) confirmed the high level of long-range dynamic structuring of water molecules in polarized-oriented multilayer formations in activated water produced with the help of MRET activation process. The fundamental biophysical theories revealed the scientific paradigm regarding polarized-oriented multilayer (PM) structuring of cell water in biological systems [4]. According to the PM theory, the assumption of the formation of distinctive dynamic structure by the cell water results from its interaction with some intracellular proteins. More specifically, the dynamic structure of cell water results from its direct or indirect interaction with the positively-charged CO groups (P sites) and negatively-charged NH groups (N sites) on the "backbones" of a pervasive matrix of fully-extended proteins. These P and N-site-bearing proteins and the water molecules with which they interact constitute what is called a NP-NP system. Electrical polarization and directional orientation of multiple layers of water molecules may occur under the influence of one or two (juxtaposed) checkerboard(s) of alternatively positive and negative sites. Parenthetically, water molecules may also be polarized and oriented in "layers" by a NO system or a PO system, in which electrically-neutral O sites replace properly-spaced electrically-charged P or N sites of a classic NP system respectively. The aggregate physical impacts of the NP sites on the bulk-phase water may be somewhat arbitrarily divided into three components: to enhance the average water-to-water interaction of (all) the water molecules in the system (Component 1); to reduce the translational as well as rotational motional freedom of the water molecules (Component 2); and to prolong the stay or *residence time* of each water molecule at a specific preferred location (Component 3). (Ling, 2005).

The interaction of water dipoles with pervasive matrix of fully-extended proteins constitutes the basis for the cellular transduction mechanism. Based on this scientific approach the similarity of molecular formations of cell water and MRET activated water can contribute to their compatibility, easy bio-availability and assimilation of MRET activated water, as well as to the enhancement of cellular functions in biological systems.

The anomalous electrodynamic characteristics and viscosity of MRET Activated water provide some evidence regarding the possible effect of MRET water on the proper function of cells in biological systems. It is well known that cellular processes in biological systems are driven by the low energy of bio-chemical reactions inside and between the cells and cellular structures. Consequently, such processes create subtle low frequency electromagnetic field and low tangent pressures along water surfaces and the membranes between the cells. The anomalously low viscosity, dielectric permittivity and electrical conductivity of MRET water in the range of very low frequencies that exists in biological systems can contribute to the enhancement of the cellular transduction mechanism and result in improved intracellular/extracellular water exchange and the proper function of cells in biological systems.

MATERIALS AND METHODS

The *in vitro* investigations on normal PMBC cells (peripheral blood mononuclear cell) and on HeLa cancer cells (cell line ATCC # CCL-2 cervical adenocarcinoma) were conducted under the supervision of Patrick Pezzoli, Ph.D. at AltheaDx Technologies, San Diego, USA. The experiments analyzed: cells lysed at 0 hour, cells cultured for 24 hours in untreated medium and cells cultured for 24 hours in medium treated with MRET activator for 30 minutes. DNA samples from each batch were processed and the resultant data was analyzed using Affymetrix Genotyping Console 3.0 to obtain genotype calls and copy number calls. Cell counts and % viability were obtained using the Trypan Blue exclusion technique.

RESULTS

The Affymetrix Genotyping technique data revealed no difference between the zero hour (control), MRET treated and untreated samples in term of genotypes and copy number calls. Thus, MRET activation of water based medium did not induce any changes in cells on genetic level.

The studies showed that in MRET activated water based medium the viability of normal cells (PBMC) was higher (Table 1), and the viability of cancer cells (HeLa) was lower (Table 2) compared to the viability in untreated water based medium.

Table 1: PBMC cell counts and % viability

Sample	Cell Count	% Viability	Viable cells
0 hour	$3.27x10^6$	91.5	$2.99x10^6$
Untreated	$0.27x10^6$	83.4	$0.73x10^6$
Treated	$0.77x10^6$	88.8	$0.69x10^6$

Table 2: HeLa cell counts and % viability

Sample	Cell Count	% Viability	Viable cells
0 hour	$3x10^6$	92	$3.27x10^6$
Untreated	$7x10^6$	97	$6.79x10^6$
Treated	$5x10^6$	92	$4.60x10^6$

For normal cells (PBMC) the changes in cell counts were similar for untreated and MRET treated medium (Fig 1). Thus, MRET treatment did not affect the growth of normal cells. For cancer cells (HeLa) the experimental data revealed significant inhibition of cancer cells growth in MRET treated medium. The growth of viable cancer cells was inhibited by 54% in MRET treated medium compared to untreated medium (Fig 2).

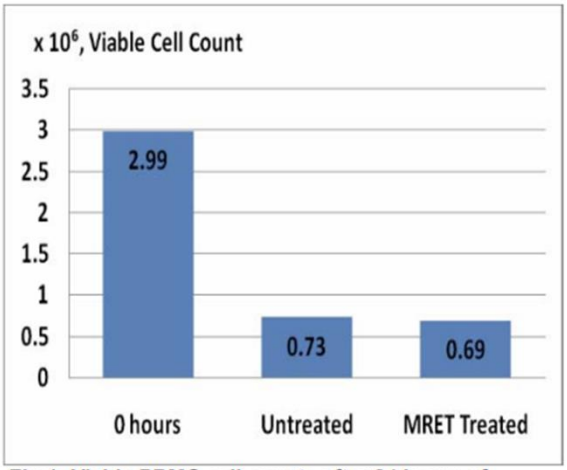

Fig 1: Viable PBMC cell counts after 24 hours of incubation.

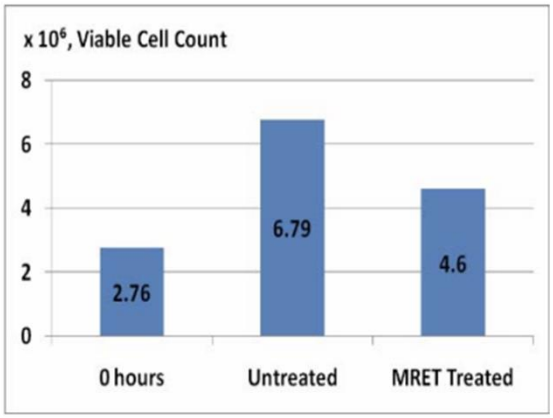

Fig 2: Viable HeLa cell counts after 24 Hours of incubation

DISCUSSION

The results of AltheaDx research on HeLa cancer cells *in vitro* support the results obtained earlier in the investigation regarding the effects of MRET water on tumor resistance in animal model. The study on 500 mice was conducted under the supervision of Professor V. Vysotskii, S. Olishevsky, Ph.D. and Y. Yanish, Ph.D. at Kyiv Institute of Experimental Pathology, Oncology and Radiobiology of Ukrainian Academy of Science. It showed substantial inhibition of growth of viable tumor cells following the consumption of MRET water. In the course of this investigation the groups of mice in "preventive regime" ingested MRET water for 2 weeks prior to the inoculation of Ehrlich carcinoma cancer cells and for 3 weeks after the inoculation. The groups of mice in "therapeutic regime" ingested MRET water only during 3 weeks after the inoculation of Ehrlich carcinoma cancer cells. Following the consumption of MRET water activated for 30 minutes (the optimal time of activation) the growth of viable tumor cells was inhibited by 76% in "preventive regime" and by 55% in "therapeutic regime."[6]

CONCLUSION

It is possible to conclude that the studies conducted at AltheaDx Technology confirm that MRET activated water based medium did not affect the cells on genetic level; it affected the morphology of normal PBMC cells in a positive way increasing their viability and promoted significant inhibition of growth of HeLa cancer cells.

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