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MRET Water Effect in the TgCRND8 Transgenic Amyloid Mice Models

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Abstract - This particular article relates to study *in vivo* regarding the effect of MRET Activated water in Transgenic Amyloid Mice models. It provides some evidence on how MRET Activated water with the modified molecular structure, physical and electrodynamic characteristics may enhance specific molecular mechanisms in living cells. The anomalous proton activity, electrodynamic characteristics and viscosity of MRET Activated water provide some evidence regarding its possible effect on electrical activity and proper function of the cells. Most cells and tissues have electrical properties relevant to their natural function. The living cells and tissues have rather complex structure, consisting of the folding membranes, the specialized connections, and organelles. The localization of electrical properties is particularly important, since each of the complex structures must be expected to have a specific role in the electrical function of the tissue. MRET Activated water with modified proton activity and electrical conductivity can also have ability to enhance a proton pump activity of the cells. As a result it may lead to the restoration of the transduction signaling and the restoration of normal cellular functions. The clinical study *in vivo* in Transgenic Amyloid Mice models conducted at Toronto University proves the validity of the proposed hypothesis.

Keywords - Amyloid, Alzheimer's Model, Viscosity, MRET Water, Molecular Structure

1. Introduction

MRET Activated Water is produced with the help of patented in the USA non-chemical Molecular Resonance Effect Technology (MRET) method. The mechanism that explains the effect of electromagnetic fields on water is related to the existence of defects in molecular structure of water. The stable structural changes in water were detected in experiments by the UV luminescence spectrophotometer. They have been attributed to different water structural defects that include specific centers of luminescence. The nuclear proton spins were considered to be a primary targets of external magnetic fields, since proton lattice of water molecules is unstable and asymmetric. The structural metastability of water was associated with microscopic orbital currents of protons in water-molecular hexagons, and deviation from the stochiometric composition of water. The effects of "memory" of water interacting with electromagnetic fields were supposed to originate from the oscillations of water-molecular hexagons.

Modern research in biophysics provides evidence that even slight change in molecular structure of water could dramatically change physical and physiological properties of water. At the first glance, it appears that water cannot have any long-term memory. It follows from simple estimates. For a long time continuous (quasi crystalline) model of water was the dominant one. Within the framework of this model the spatial structure of potential energy for each one of H_2O molecules is nearly a periodical three-dimensional system of pits and barriers. It is the result of a self-regulating movement of all water molecules, which represents a combination of two independent processes – vibration movement in each one of potential pits and random (fluctuation) leap into a neighboring pit. The average frequency of vibrations in potential pits is approximately the same as the Debye frequency in a solid body (about $\omega_D \approx 10^{13}$ s^{-1}). The average duration of a leap into a neighboring potential pit is equal $\tau_0 \approx 10^{-13}$ s. The average time of staying in one pit is determined by the water temperature T and the energy of activation $\Delta W \approx 0.2$ eV of the diffusion process (the height of the barrier between neighboring pits). Staying within the framework of this model it is easy to make a conclusion that water memory must be preserved for not much longer than the value $<\tau>$, which is by many orders less than data received in numerous experiments. There are two ways out of this logical dead end: either the experiments are not reliable, or the continuous model is incomplete (or wrong). The increasing number of reliable experiments shows that the continuous model is inadequate for the description of water structure. Moreover, continuous non-structural water (according to the data by the Nobel Prize winner Juan Lee) should be not a liquid but a gas because of a relative weakness

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of hydrogen links [6]. The presence of a spatial structure in a volume of water was first proved by Bernal (1933). Calculations made on the basis of quantum chemistry have shown that water molecules participate in creation of molecular assemblies and may form different types of associated molecules: "hydrol" H_2O, "dehydrol" $(H_2O)_2$, "trihydrol" $(H_2O)_3$ and so on. Further studies have shown that even much larger associates (clusters) may form in water from water molecules and their structure resembles the form of small pieces of ice [9]. As a rule, these clusters are unstable and appear and disappear spontaneously. The dynamics of such associates lies in the basis of the cluster model of water [3]. More detailed studies have shown that the so-called "clathrate" model is the one closest to reality. In its final form this model was developed by Pauling [4]. The basis of the Pauling's model is the concept that unification of atoms of oxygen and hydrogen can create spatial flexible tetrahedral frames. Formation of a tetrahedral frame was due to the fact that the natural spatial angle between OH-links in a free water molecule H_2O is equal to 104.5°, which is sufficiently close to the exact value of the tetrahedral angle 109.5°.

The Pauling's clathrate model explains very well all features of water including its anomalous condensability. The DNA structure ideally matches the spatial structure of such framed water, given that each macromolecule of DNA regulates water at the distance of up to 300-500 A° away from its surface. Many works address the possibility of unification of the Pauling's model with the cluster model. In that case, separate elements of clathrate frames may, from time to time connect with each other by hydrogen links and form groups with ordered structure (or clusters). The examined features of spatial water structure show that water molecules are always distributed between two loosely connected systems: the quasi amorphous non-structured water and the quasi crystalline structured system of clathrate hydrates. During the process of external influence on water (activation) there is a significant change of its structure and parameters. The hierarchical micro level of the water structure is related to the processes of movement and distribution of separate H_2O molecules between micro cavities of the spatial clathrate water frame and quasi-amorphous non-structured water. That micro level determines non-stationary evolution of H_2O molecules. The process of evolution is determined by two possible directions: molecules can leave the volume of quasi-amorphous water, penetrate the volume of these micro cavities and stay there for a long time in hydrophobic form, or, to the opposite, transfer from micro cavities into the volume of quasi-amorphous water. It is clear that the micro level of water structure is distinguished by a much greater stability with respect to effects of external influences. With all external transformations of the clathrate frame hydrophobic H_2O molecules remain in a stable state in the volume of micro cavities. Such stability makes the micro level of water structure an effective object for organization of a system of long-term water memory. Such principle provides reliable binary system of water memory: occupied clathrates and vacant clathrates of water molecular structure [7], [8]. Another word the presence of a clathrate frame of water may lead to formation of long-term memory and to the recording of information in it.

MRET Activated water with modified proton activity and electrical conductivity can also have ability to enhance a proton pump activity of the cells. A proton pump is an integral membrane protein that is capable of moving protons across the membrane of a cell, mitochondrion, or other subcellular compartments thereby creating a difference or gradient in both pH and electrical charge. As a result it may lead to the restoration of the transduction signaling and restoration of the normal cellular functions.

The clinical test *in vivo* regarding the effect of MRET Activated water in TgCRND8 Transgenic Amyloid Mice Models provides the evidence of the validity of the proposed hypothesis. This research was conducted under the supervision of Paul Fraser, Ph.D., Associate Professor, the Department of Medical Biophysics, Center for Research in Neurodegenerative Diseases, University of Toronto, Canada.

2. Method

3-Week-old transgenic mice (TgCRND8 – amyloid disease model; see manuscript [1] for complete description) were administered MRET-treated water or regular water for 4 months:

- 11 mice were placed on MRET-treated water (mortality – 1 animal lost during course of the investigation, that constitutes 9% mortality rate)
- 6 mice were placed on regular water (2 deaths during course of the investigation that constitutes 33% mortality rate).

MRET water was made by using Millipore water and placing in a special MRET device to activate the water (the water was activated for 30 minutes then stored in sterile glass bottles). Regular water was untreated Millipore water (i.e., filtered through a 0.2 µm filter). The mice on MRET and Regular water used 15ml conical tubes as water bottles. In the cases where wet feed was required, it was made in a plastic drug dose cup using MRET or Regular water and a wooden stir stick, (MRET-treated and regular water did not come in contact with any metal).

After 4 months of water treatment (i.e. MRET or regular water) the animals were perfused with PBS and tail and brain were taken. One hemisphere of the brain was post fixed serial sectioned, stained with for amyloid plaques (Amyloid-β monoclonal antibody as previously described) and number of plaques counted visually. Plaque density and area covered by the brain were as previously described [2]. The plaques were not counted in the brains of dead animals in both groups and data were not used for statistical calculations. The ½ of the brain was placed in –80°C for further investigation if required.

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3. Results

Table 1. Water Experiment

Mice on MRET-Treated Water

Animal I.D	Sex	Tg	DOB	Date Water Started	Dated Perfused
80139	Male	APPsw+717+	11/25/2004	12/16/2004	3/31/2005
80141	Female	APPsw+717+	11/25/2004	12/16/2004	3/31/2005
80144	Female	APPsw+717+	11/29/2004	12/20/2004	4/4/2005
80147	Male	APPsw+717+	11/29/2004	12/20/2004	4/4/2005
80148	Male	APPsw+717+	11/29/2004	12/20/2004	4/4/2005
80150	Male	APPsw+717+	11/29/2004	12/20/2004	4/4/2005
80152	Male	APPsw+717+	11/29/2004	12/20/2004	4/4/2005
80155	Female	APPsw+717+	11/30/2004	12/20/2004	4/4/2005
80156	Female	APPsw+717+	11/30/2004	12/20/2004	4/4/2005
14	Female	APPsw+717+	11/30/2004	12/20/2004	**dead 2/12/05**
22	Male	APPsw+717+	12/1/2004	12/20/2004	4/4/2005

Mice on Regular Water

Animal I.D	Sex	Tg	DOB	Date Water Started	Date perfused
42	Female	APPsw+717+	12/3/2004	12/24/2004	4/11/2005
46	Female	APPsw+717+	12/6/2004	12/27/2004	4/11/2005
47	Female	APPsw+717+	12/6/2004	12/27/2004	**dead 3/9/05**
51	Female	APPsw+717+	12/6/2004	12/27/2004	4/11/2005
52	Female	APPsw+717+	12/6/2004	12/27/2004	4/11/2005
74	Female	APPsw+717+	12/13/2004	1/3/2005	**dead 2/25/05**

Table 2.

ID #	Treatment	Sex	**Tot Brain Area**		Tot Plq Area		% Area	
80141-432	MRET	Male	**74025820**		11176.4		0.015161	
80139-433	MRET	Female	**65556189**		16866.02		0.025666	
80144-435	MRET	Female	**65458566**		17015.91		0.025971	
80147-436	MRET	Male	**68457147**		8953.497		0.012967	
80148-437	MRET	Male	**58706332**		6403.311		0.010808	
80150-438	MRET	Male	**64526006**		11209.41		0.017419	
80152-439	MRET	Male	**56654022**		5688.929		0.010139	
80155-440	MRET	Female	**62690204**		10490.9		0.01762	
80156-441	MRET	Female	**65557591**		22452.5		0.034328	
22-442	MRET	Male	**60350008**	**Calculated mean:64198189**	14173.5	Calculated mean: 12443.04	0.023542	Calculated mean:0.019362
42-443	Reg	Female	**61295060**	**Standard deviation:1578560**	11231.4	Standard deviation:1649.471	0.018516	Standard deviation: 0.002469
46-444	Reg	Female	**57468975**		10342.38		0.018047	
51-445	Reg	Female	**48103062**		5756.311		0.012288	
52-446	Reg	Female	**56546410**	**Calculated mean: 55853377**	16456.92	Calculated mean: 10946.75	0.029165	Calculated mean: 0.019504
				Standard deviation:2780434		Standard deviation:2193.721		Standard deviation:0.003518
ID #	Treatment	Sex		#plq		Mn plq size		
80141-432	MRET	Male		46		246.0315		

80139-433	MRET	Female		63		263.5271		
80144-435	MRET	Female		54		316.85		
80147-436	MRET	Male		36		250.0812		
80148-437	MRET	Male		28		210.1785		
80150-438	MRET	Male		45		249.3581		
80152-439	MRET	Male		28		205.2654		
80155-440	MRET	Female		39		263.7134		
80156-441	MRET	Female		71		313.8993		
22-442	MRET	Male		60	Calculated mean:47	220.8491	Calculated mean: 253.9754	
42-443	Reg	Female		41	Standard deviation: 4.669047	268.2089	Standard deviation:12.11882	
46-444	Reg	Female		46		229.849		
51-445	Reg	Female		36		154.5907		
52-446	Reg	Female		69	Calculated mean:48	239.5419	Calculated mean:223.0476	
					Standard deviation: 7.291548		Standard deviation:24.22844	

The data analysis reveals that the amyloid pathology associated with these transgenic mice was not significantly affected. This was based upon the analysis of amyloid plaque number, density and average area of the brain occupied by the amyloid deposits (Chart 1, 2, 3):

Chart 1. Amyloid Plaque Number

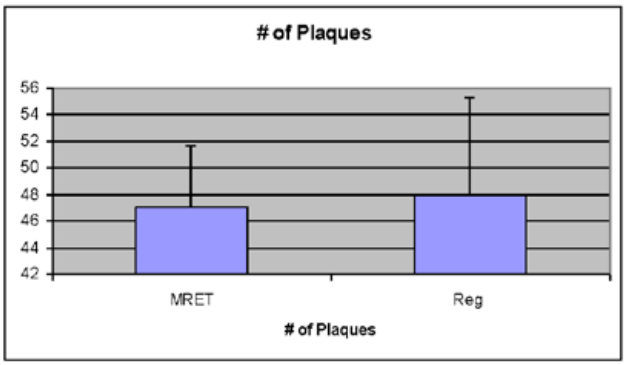


Chart 2. Amyloid Plaque Density

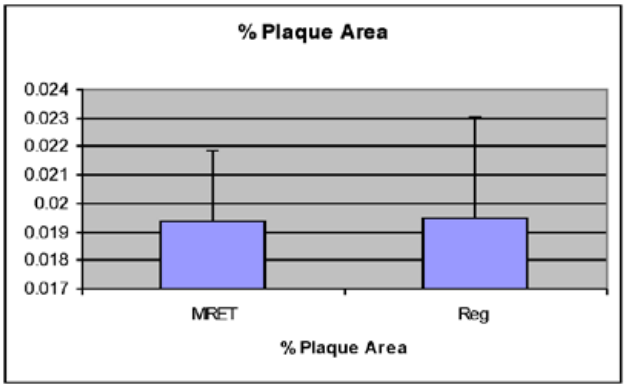
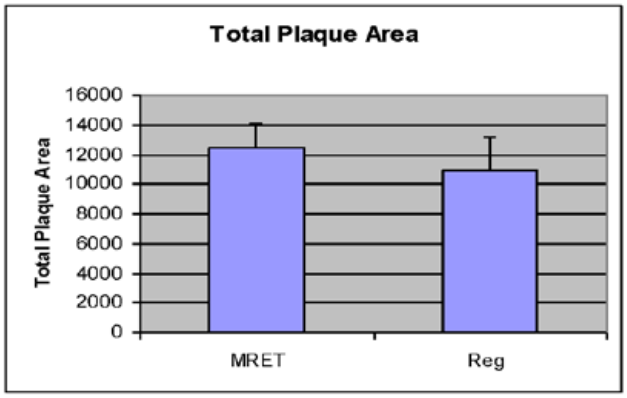
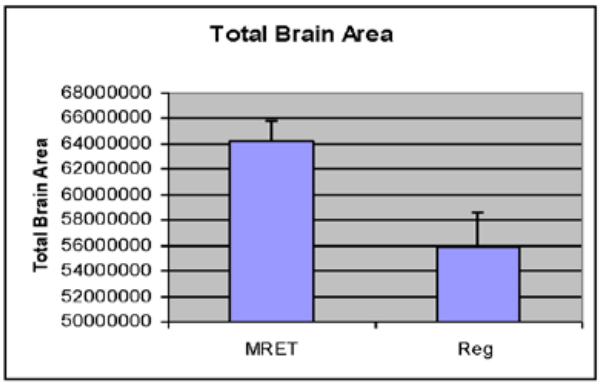


Chart 3. Amyloid Plaque Average Area



This research shows very interesting fact regarding the enhanced development of the brain tissue in the group of mice on MRET water. The mean value of total brain area for mice on MRET water was by 15% higher comparing with the mean value of total brain area for mice on regular water (Chart 4):

Chart 4. Measurement of the Total Brain Area



The increase of total brain area by **15%** was observed in the group of mice on MRET-treated water comparing to the

group of mice on regular water. The difference in mean values is statistically significant with ***p=0.012***.

Statistical calculation of the p-level:

Statistical calculation of *p*-level is based on Student's t-distribution concept:

$$\mathbf{M_{mret} - t_{p,9}\sigma_{mret}/\sqrt{9} \geq M_{reg} + t_{p,3}\sigma_{reg}/\sqrt{3}}$$

$$\mathbf{M_{mret} - M_{reg} \geq t_{p,9}\sigma_{mret}/\sqrt{9} + t_{p,3}\sigma_{reg}/\sqrt{3}}$$

According to this research data:

$$\mathbf{M_{mret} = 64.20\ 10^6;\ M_{reg} = 55.85\ 10^6;\ \sigma_{mret} = 1.58\ 10^6;\ \sigma_{reg} = 2.78\ 10^6}$$

Thus, $\mathbf{8.34 \geq t_{p,9}\ 0.53 + t_{p,3}\ 1.61}$

With $\mathbf{p = 0.012}$ it makes $\mathbf{8.34 \geq 2.69x0.53 + 4.18x1.61 = 1.43 + 6.72 = 8.15;}$

This calculation proves that the difference in mean values of total brain area for the group of mice on MRET water and the group of mice on regular water is statistically significant with *p*-level ***p = 0.012.***

Another potentially interesting observation made during the course of this investigation was the decreased mortality of the MRET-treated animals as compared to the controls. We typically see a 25-40% mortality in these mice as is evidenced by the animals on the regular water administration. In contrast, a significant decrease in this mortality rate was seen in the group receiving the MRET-activated water (Chart 5):

Chart 5. Mortality Rate as Observed in Mice on MRET-Treated Water and on Regular Water

The mortality rate of **33%** was observed in the group of mice on regular water. This mortality rate corresponds to the typical mortality rate of **25-40%** for transgenic amyloid mice model. The mortality rate in the group of mice on MRET water significantly reduced to **9%**. This fact confirms the reduction of typical mortality rate for transgenic amyloid mice model by **73%** for the group of mice on MRET water. The difference in mean values is statistically significant with ***p=0.05.***

Statistical Calculation of *p*-level:

In compliance with Student's t-distribution concept:

$$\mathbf{M_{mret} + t_{p,10}\,\sigma_{mret}/\sqrt{10} \leq 25;\ \ t_{p,10} \leq (25 - M_{mret})\ \sqrt{10}/\ \sigma_{mret};}$$

According to the research data $\mathbf{M_{mret} = 9\%,\ \sigma_{mret} = \sqrt{(91^2 + 10x9^2)/11} = 28.7;}$

$\mathbf{t_{p,10} \leq 1.76,}$ the corresponding *p-level* ***p=0.05.***

4. Discussion

The expanding interest in dementia has nurtured a worldwide initiative exploring a wide array of disease mechanisms and approaches to treatment. The divergent perspective on the exact cause of this destruction have fostered different theories about what initiates the process and how best to intervene. The theory with the largest following remains the "amyloid hypothesis," which assigns a central role to abnormal processing of amyloid precursor protein (APP), a protein found widely throughout the body. This abnormal processing yields a fragment called beta-amyloid (Aβ), which aggregates by stages into the amyloid plaques that is a hallmark of Alzheimer pathology. Proponents of the amyloid hypothesis see production and aggregation of Aβ as the key event in nerve cell disruption and destruction. Neuropathology studies are inconclusive as to the pathogenic role of amyloid in Alzheimer's disease. Quantitative radio-immunoassays show that equal amounts of soluble APP are found in the brains of people with Alzheimer's disease and age-matched individuals without Alzheimer's disease, casting doubt on the role of APP. This makes determining the relationship of amyloid to Alzheimer's disease more difficult: dense plaques accumulate with age even in individuals who have no cognitive impairment.

The second most prominent Alzheimer theory assigns a causative role to *tau*, a protein that normally helps organize and stabilize a cell's internal "skeleton." In Alzheimer's, *tau* deforms and loses its ability to support the cell, eventually aggregating into neurofibrillary tangles - the other hallmark of Alzheimer's brain lesion. Although no therapies targeting *tau* have reached clinical trials, many experts remain convinced that understanding *tau* will reveal crucial clues about Alzheimer's devastating effects on nerve cells as well as chemical steps vulnerable to intervention. However, there is no conclusive evidence indicating that amyloid plaques and neurofibrillary tangles are the cause and not a product of Alzheimer's disease. Plaques and tangles can be observed in the brains of individuals without any detectable form of dementia [5].

Another promising direction of therapies for Alzheimer's disease is development and human clinical testing of the drugs that stimulate production of ***nerve growth factors*** (NGFs), proteins that regulate nerve cell maturation, survival, and repair. Nerve growth factors are an active area of research in stroke, spinal cord injury, and other nerve-damaging conditions as well as in Alzheimer's disease. NGFs not only protect the cholinergic nerve cells of the brain that are most sensitive to damage by Alzheimer's disease but also increase the amount of the enzyme that makes acetylcholine, the critical messenger for memory, that is lost in Alzheimer's disease.

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The theory of ***nerve growth factors*** correlates with the mechanism of MRET Water effect in animals with Alzheimer's disease model. Such mechanism may explain the anomalous longevity of Transgenic Amyloid Mice Models treated with MRET Activated Water. The results of this investigation provide evidence that MRET water stimulates and enhances the development of the brain tissues and consequently may regulate nerve cell maturation, survival, and repair. It is unlikely that the increase of total brain area leads to the development of hydrocephalus or "simple swell" of brain tissue in this experiment because no tumors or swelling were observed during histological analysis. The behavioral and cognitive ability of mice on MRET water was normal and they did not show any abnormal behavior during the experiment. Dr. Fraser actually had comment that mice on MRET water were physiologically more active to compare with mice on regular water. The observation of mice on MRET water also suggests that their brain to body ratio was normal.

5. Conclusion

From analysis, the amyloid pathology associated with these transgenic mice was not significantly affected. This conclusion is based upon the investigation of amyloid plaque number, density and average area of the brain occupied by the amyloid deposits.

One potentially interesting observation made during the course of the investigation was the decreased mortality of the MRET-treated animals compared to the control ones. We typically see a 25-40% mortality in these mice as is evidenced by the animals on the regular water administration. In contrast, a significant decrease in this mortality rate was observed in the group receiving MRET-activated water. The 33% mortality rate was observed for mice on regular water, and 9% mortality rate for mice on MRET- treated water in this experiment.

This research also showed very interesting fact regarding the enhanced development of the brain tissue in the group of mice on MRET water. The mean value of total brain area for mice on MRET water was by 15% higher comparing with the mean value of total brain area for mice on regular water.

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