Original article

Changes of oxidation during use the food diet with deuterium depleted water in laboratory animals with purulent inflammation

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Abstract: *Objective* — To identify quantitative changes of the deuterium content, the intensity of free radical oxidation and antioxidant status of the blood system, as well as the effect of water with a modified isotope composition with low deuterium content on indicators of free radical oxidation of tissues in laboratory animals under physiological conditions and in inflammatory processes.
Materials and methods — The object of the study was blood and homogenates of organs (liver, kidney) of male rats. The basis of the model of oxidative stress was a well-known model of wound healing proposed by L.A. Mamedov based on the surgical treatment of abscess model, and we performed its modification in the course of experimental studies. Determination of deuterium concentration in the plasma was carried out using nuclear magnetic resonance. The method of luminol-dependent H_2O_2-induced chemiluminescence was used for the detection of relatively unstable chemically active radicals in the plasma. Stable radicals detect by the EPR spectrometry.
Results and Conclusion — Thus, it should be noted that blood plasma demonstrates reliable reduction in deuterium concentration when using deuterium depleted water that continues until the values of 90-100 ppm thereafter remaining practically unchanged. At the same time, deuterium depleted water affects prooxidant-antioxidant system of the body reducing the intensity of free radical oxidation and restoring the capacity of the endogenous antioxidant system.

Keywords: antioxidants, oxidative stress, deuterium depleted water, electron paramagnetic resonance

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Introduction

The existence of the human body in permanent contact with adverse environmental factors is impossible without the proper functioning of its non-specific defense systems. The weakening of non-specific resistance is observed in many diseases and specific physiological conditions, which is characterized by the reduction of adaptive responses and contributes to the development of various disorders of homeostasis leading to adverse outcomes, reducing of mental and physical work capacity. Antioxidant system (AOS) is one of the key systems of nonspecific defense, which maintain the ratio of prooxidant and antioxidant factors in the body at the physiological level [1, 2]. Despite the fact that prooxidants - free radicals (superoxide anion radical, nitric oxide, hydroxyl radical, alkyls, alkoxyls and peroxides) and reactive molecules (hydrogen peroxide, hypochlorite anion, hydroperoxides and peroxynitrite) - are actively involved in the regulation of many intracellular processes [3-7] including immune mechanisms, neutralization of xenobiotics, apoptosis, metabolism of bioactive compounds (prostaglandins, biogenic amines), metabolism of bone tissue and the oxidation of hemoglobin in cases of imbalance in prooxidant-antioxidant system of the organism with the prevalence of prooxidant factors. The latter factors start to possess damaging effect at the molecular and cellular level, which is accompanied by a set of typical pathological changes in organs and tissues, called as "oxidative stress" [8, 9].

Taking into account the important role of free radical reactions of oxidation (FRRO) in the regulation of physiological processes and the development of pathological conditions an active search of ways for pharmaceutical and non-pharmacological correction of disorders that are developed in conditions of oxidative stress (OS) is being continued in modern biology, prevention and clinical medicine in order to prevent formation or reduce complications in a variety of diseases (diabetes, atherosclerosis, bronchial asthma, oncopathology, rheumatoid arthritis, neurodegenerative and other diseases) where OS is essential in the pathogenesis [10-14]. The possibility of nutritional correction of oxidative metabolism in the body becomes of considerable interest, which is primarily due to the ability of nutrients to have a significant influence on the health, work capacity and life span, so now, in addition to the optimal balance of nutrients and minerals, an estimation of their effect on the endogenous AOS is carried out [15-17]. One of the most promising nutrients for the correction of the antioxidant capacity of the body is water with a modified isotopic composition (WMIC), for example, water with low content of deuterium [18].

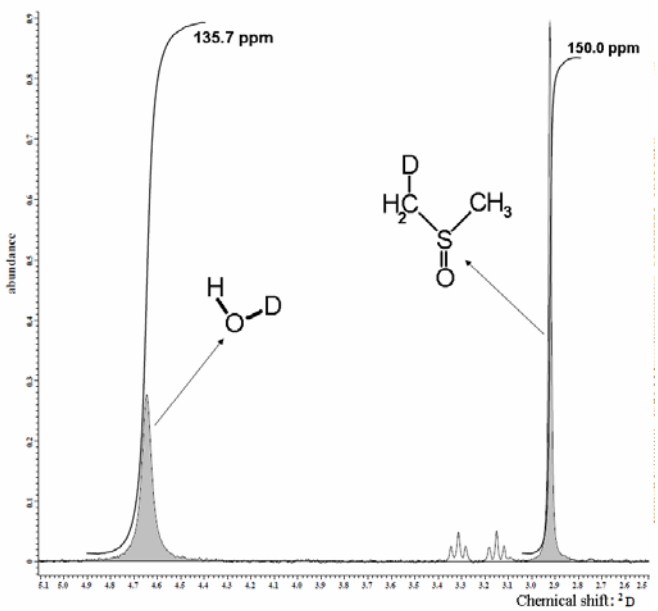

Figure 1. Integral intensity ratio of 2D NMR HDO signal relatively to 2D NMR DMSO-D1 signal

We know that people consume up to 2-3 liters of fluid per day under physiological conditions, so the change in the structure of nutrition caused by WMIC with low deuterium content can affect the values of AOS. Recently all over the world various effects of WMIC with low deuterium content have been actively studied; its main effect on the body is the gradual reduction of deuterium content in biological fluids and tissues due to isotope exchange reactions. The scientific literature often describes biological effects of WMIC, whereas the molecular mechanisms of its action on the body have not been fully examined yet. WMIC with low deuterium content affects the growth of tumor cells of various cultures [19-21], possesses immunomodulating property [22-24] and affects the metabolism of hydrogen peroxide in the liver [25]. All above examples show the great role of isotope composition of water for molecular processes in the body, and therefore the addition of WMIC with low deuterium content to people's diet in states accompanied by the development of the OS may increase the potential of endogenous AOS and provide benefit in the prevention of complications [26]. Thus, the increasing use of WMIC with low deuterium content in people with various pathological conditions, for health improvement (fitness), as well as in professional sports requires further study on the mechanisms of its action, which will allow more efficient use of its effects for prophylaxis and at various clinical conditions.

Objective: To identify quantitative changes of the deuterium content, the intensity of free radical oxidation and antioxidant status of the blood system, as well as the effect of water with a modified isotope composition with low deuterium content on indicators of free radical oxidation of tissues in laboratory animals under physiological conditions and in inflammatory processes.

Material and Methods

The object of the study was blood and homogenates of organs (liver, kidney) of male rats with weight of 90-100 g. The rats were divided into the following groups: group No.1 (which were administered distilled mineralized water (158 ppm) for 30 days, n=40), group No.2 (which were administered distilled mineralized water (158 ppm) for 30 days and suffered from purulent inflammation of soft tissue, n=40), group No.3 (which were administered distilled mineralized water with reduced deuterium content (40 ppm) for 30 days and suffered from purulent inflammation of soft tissue, n=40**)**.

Water with low deuterium content was obtained at the facility developed at Kuban State University [27, 28]. The initial concentration of deuterium in the produced water was 40 ppm.

Two-stage model of oxidative stress was used while modeling purulent wounds in rats. The first stage was a acute stage of oxidative stress, and it was simulated by creating intermuscular abscess in the soft tissues of the long muscles of the back of laboratory animal by using an implanted foreign body. The second stage reflected the chronic stage of oxidative stress, and it was simulated by purulent wound that was formed naturally while draining the abscess and removal of the foreign body.

The basis of the model of oxidative stress was a well-known model of wound healing proposed by L.A. Mamedov based on the surgical treatment of abscess model, and we performed its modification in the course of experimental studies [29].

In order to create a model of abscess, one cut and shaved rat's hair on the middle and lower thirds of the back before the experiment. Then, under local anesthesia with novocaine 0.5% solution - 10 ml syringe needle was applied causing damage of soft tissue (in the area of the long back muscles) at the depth of 3 cm and the width of 2 cm in the intended area of abscess formation. On the day of the experiment a 3 cm length incision of compromised area was made under chloralose-nembutal anesthesia followed by insertion of sterile gauze sponge of 10 mm diameter impregnated with 1 ml of liquid containing the pathogenic strain of *St. aureus* into the soft tissues. Primary sutures of the wound were performed.

After one day animals developed a clinic of wound abscess, and the first (acute) period of oxidative stress simulation started. The sutures were removed in 5 days after infection, corresponding to the transition to the second stage of oxidative stress.

Local treatment of purulent wound under the salve dressings was performed later up to the complete healing by secondary intention.

Determination of deuterium concentration in the plasma was carried out using nuclear magnetic resonance (NMR) at the pulsed NMR spectrometer JEOL JNM-ECA 400 MHz. Spectra recording was carried out at the corresponding resonance frequency of deuterium nuclei - 61.4 MHz. Recording parameters was as follows: 6.7 s (acquisition time), 20 s (relaxation delay), 5.6 ms (x-pulse), 0.15 Hz (resolution). Recording temperature was -25°C, with the accuracy of stabilization of 0.2 ◦C. Measurements were carried out using 5 mm ampoule with fixed sealed capillary inside containing mixture of deuterated and undeuterated dimethyl sulfoxide (DMSO) according to defined concentration calibrated scale that produce 2D NMR signal at 3.4 ppm (relative to (CD3) 4Si), while 2D NMR HDO signal is in the range of 4.7 ppm (relative to (CD3) 4Si) (*Figure* 1).

Processing of obtained spectra consisted in determining of the ratio of integral intensities of 2D NMR HDO signal of the test sample relative to 2D NMR DMSO-D1 signal, the intensity of which was in turn determined under the same conditions relative to standards - water samples with precisely defined deuterium content (3.7 ppm, 51 ppm, 150 ppm). The measurements of each sample were carried out repeatedly to decrease experimental error. The accuracy of deuterium content determination in biological samples was ±2 ppm.

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Table 1. Indicators of the deuterium content, the intensity of free radical oxidation and state of the antioxidant system in blood and tissues of the rats using water with modified isotope composition in the diet (M±m)

Indicator \ Group	Content of deuterium in the plasma	MFCL of plasma, arbitrary units	AOA of plasma, nA·s	EPR of kidneys, PMC/g	EPR of liver, PMC/g
Group 1 (n=40)	153.3±0.4	1.993±0.024	1246.4±23.7	608.61±44.01	960.23±56.22
Group 2 (n=40)	157.5±0.4	3.058±0.081 *	948.2±21.8 *	747.87±55.13 *	1584.25±112.99 *
Group 3 (n=40)	96.1±0.5 *+	2.716±0.126 *+	1137.1±24.2 *+	720.66±57.07 *	1316.80±66.69 *+

* – P<0.05 in comparison with the indicators of group No. 1;

+ – P<0.05 in comparison with the indicators of group No. 2.

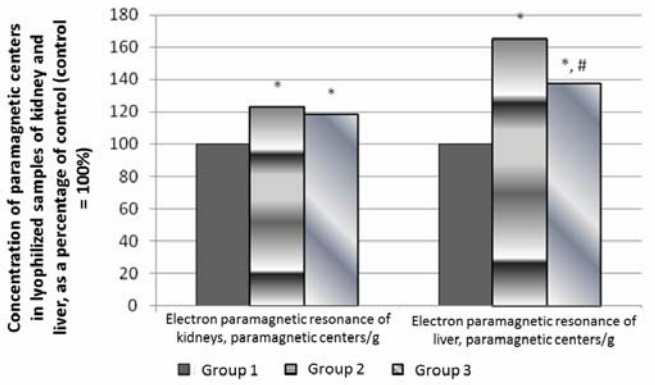

Figure 2. Influence of water with modified composition on values of free radical oxidation in liver and kidneys in laboratory animals with purulent inflammation of soft tissues

* – P <0.05 in comparison with the indicators of group 1;

– P<0.05 in comparison with the indicators of group 2;

PMC/g – the number of paramagnetic centers in 1 gram of lyophilized tissue.

Measurement of electron paramagnetic resonance (EPR) spectra was performed at room temperature at JES Fa 300 spectrometer (JEOL, Japan) in the X-band. The conditions were as following: microwave power was 1 mW, microwave frequency was 9144 MHz and high-frequency amplitude modulation was 0.1 mT. Tissue samples were previously lyophilized (at dehumidification device LS-1000), measured in a quartz ampoule (5 mm); the mass of the sample in the resonator zone was 0.03 g. The concentration of paramagnetic centers (PMC) in the samples was determined by comparison to the signal of a standard sample (TEMPOL). Integrated intensity of EPR signal in the samples was determined by double numerical rectangular integration method [30].

The EPR spectra of liver samples of the laboratory mice contain anisotropic singlet signal which spin-Hamiltonian parameters correspond to those of stable radicals [31-33]. The EPR spectra of kidney samples are similar.

Given that the EPR method can detect mainly stable radicals [34] the method of luminol-dependent H_2O_2-induced chemiluminescence was used for the detection of relatively unstable chemically active radicals in the plasma with hemi lumen tester LT-1 manufactured by Scientific and Production Association Lumin (Rostov-on-Don) in the modification [35-37]. The results obtained in the form of maximum flash chemiluminescence (MFCL) reflected inhibition of FRRO was expressed in arbitrary units (arb. u.) in relation to the flash in control samples without the biological material.

Determination of antioxidant activity (AOA) of blood plasma was carried out additionally to the assessment of the endogenous antioxidant system by amperometric method at antioxidant activity analyzer "Yauza-01-AAA" produced by Scientific and Production Association "Khimavtomatika" OAO (Moscow, Russia) by the method [38]. The method is based on the measurement of electric current, which occurs during the oxidation of biological sample at the surface of the working electrode at the specific potential and comparing the received signal with a standard signal measured under the same conditions. The results were expressed in nanoampere per second (nA*s).

Statistical analysis of the data obtained was carried out by the methods of variation statistics by using Student t-test. Difference was considered as significant at p <0.05. Data in the table are presented as the arithmetic mean (M) and the error of the arithmetic mean (± m).

Results

The study found that lowest deuterium content in biological fluids and tissues was typical for animals of group 3, which were administered WMIC with low deuterium content in their diet. Deuterium content was lower by 37.3% and 38.9% in comparison to the indicators in group 1 and 2 respectively.

This indicates a reliable (p <0.05) change of the deuterium content in the blood in 30 days after the start of the study (*Table* 1). At the same time it should be noted that deuterium plasma content ceased to decrease after reaching a value of 90-100 ppm, and its further decrease didn't happen despite the lower levels of deuterium in WMIC (40 ppm) administered to laboratory animals. This suggests the presence of specific bodily mechanisms regulating an isotope composition of biological fluids in physiological range preventing sudden changes in the quantitative content of hydrogen isotopes in different tissues and organs.

Discussion

When comparing the intensity of free radical formation in lyophilized organs it was found that the liver and kidneys of rats with simulated oxidative stress (Groups 2 and 3) demonstrated a significant increase in the concentration of PMC (*Figure* 2). This indicates a steady prevalence of prooxidant factors on the AOS component at the cellular level. At that, there were more significant changes in liver homogenates from the animals of group 2 where concentration of PMC exceeded the value of a control group 1 by 64.9% (P<0.05).

This fact indicates an active participation of the liver in the elimination of toxic substances accumulated due to the chronic inflammatory processes resulting in increasing synthesis of active oxygen forms in hepatocytes and the development of OS at the tissue and organ levels. It should be noted that the PMC content in liver homogenates of the 3rd group of animals was also significantly higher than in group 1 – by 37.1% (P<0.05), but it was significantly lower than in group 2 – by 16.9% (P<0.05), which

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reflects a less pronounced rate of FRRO in hepatocytes of these animals and probably indicates more active tissue components of endogenous AOS or less toxic load on the liver cells from the focus of inflammation [39-42]. This can be explained by the fact that WMIC with low deuterium content activates other non-specific and protective systems, for example possess immunomodulatory effect accelerating the localization mechanisms of pathological agents by means of cellular immunity.

When studying the processes of FRRO in kidney homogenates less pronounced changes were obtained in rats with simulated OS though they were significantly increased in group 2 (by 22.9%, P<0.05) and in group 3 (by 18.4%, P<0.05) in comparison with values of the control group 1 [43-45]. There were not any significant differences in the both experimental groups 2 and 3, which may be caused due to lower specific influence of WMIC with low deuterium content on the endogenous AOS of kidneys or less capacity of low and medium weight hydrophilic toxic substances to activate the free radical processes in the kidney tissue. It is known that some of them (e.g. urea, oligopeptides, and uric acid) may exhibit antioxidant effect by participating in the neutralization of free radicals that reduces the amount of free radicals in the organs of the excretory system [46, 47].

Changes in the blood were more significant in nature due to the integrating function of the blood as a biological fluid reflecting the whole spectrum of changes in the body. A significant decrease of AOA in rats in group 2 (by 23.9%, P<0.05) was revealed during the study of plasma, while rates in group 3 had a much smaller decrease in AOA, and its values significantly exceeded those of the group 3 (by 19.9%, P<0.05). Similar changes characterized the reduction of endogenous AOS capacity especially its low molecular component throughout the body. This can lead to various repeated pathological processes and complications. In turn, the blood level of FRRO in rats from group 2 and group 3 was significantly increased (P<0.05) compared to controls by 53.7% and 36.7% respectively (*Figure* 3).

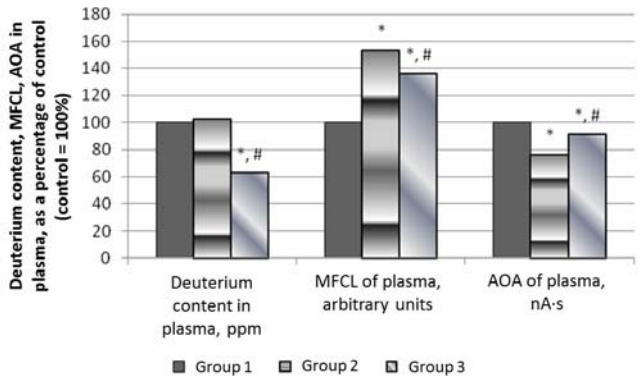

Figure 3. Influence of water with modified composition on the values of deuterium content, MFCH and AOA of blood plasma in laboratory animals with purulent inflammation of soft tissues

* – P<0.05 in comparison with the indicators of group 1;

– P<0.05 in comparison with the indicators of group 2;

MFCL - maximal flash chemiluminescence, arbitrary units; AOA - antioxidant activity, nA per second.

This fact indicates an expressed activation of pro-oxidant component which in turn leads to the depletion of low-molecular antioxidant factors and the development of OS. Less pronounced changes of prooxidant indicators were observed in the animals from group 3, which can be explained by lower toxic load on their non-specific defense system, more rapid neutralization of endogenous toxic substances in the liver, as well as immunomodulatory effects of WMIC with low deuterium content that reduces inflammatory changes in the animals.

All these diverse effects associated with influence of WMIC with low deuterium content to the rats can be explained by a number of mechanisms that are realized at the molecular and cellular levels in vivo. Thus, the consumption of WMIC with low deuterium content leads to the chemical exchange reactions of H_2O to D_2O and NDO in cells and to the faster H±D exchange in hydroxyl, sulfhydryl and amino groups of all organic compounds including proteins, nucleic acids, lipids, sugars that can have an influence on low-molecular component of AOS, which have thiol (-SH) and hydroxyl (-OH) groups as one of the main factors. In addition, the presence of deuterium in biological systems leads to changes in the structure and properties of nucleic acids and proteins during the formation of dynamic short-hydrogen (deuterium) bonds, one of the most important for the structure of macromolecules, which can reduce the activity of anti-radical protection enzymes (catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase), thereby reducing the body's antioxidant capacity. In turn, WMIC with low deuterium content restores the enzyme component of AOS while reducing tissue deuterium content both through direct interaction with the -OH or -SH groups in the active centers and by the activation of transcription due to changes in oscillating moments in the chains of nucleic acid molecules and facilitating their energetic interaction with enzymes providing the genetic code reading.

Also there is violation of ion transport in the cell membrane against the background of high content of deuterium as well as an increase of biomembrane resistance which has particularly negative effect on its excitability [48]. Therefore the use of WMIC with low deuterium content eliminates these negative factors, reduces the viscosity of the membrane, increases its permeability for ions and improves signal transduction of primary and secondary messengers that in turn restores adequate energy exchange in the tissues and reduces prooxidant load at cell structures. In this case, the effects of WMIC will be similar to other indirect antioxidants of our body (e.g., to hormones).

Conclusion

Thus, it should be noted that blood plasma demonstrates reliable reduction in deuterium concentration when using WMIC with low deuterium content that continues until the values of 90-100 ppm thereafter remaining practically unchanged. At the same time, WMIC with low deuterium content affects prooxidant-antioxidant system of the body reducing the intensity of FRRO and restoring the capacity of the endogenous AOS. The largest direct and indirect antioxidant WMIC effect is observed in blood plasma and in hepatocytes, whereas the intensity of free radical processes of the excretory system varies less substantially when giving WMIC to the diet. At the same time the positive effect of WMIC with low deuterium content on inflammatory processes is explained by its possible immunomodulatory effect that reduces the negative effects of bacterial endotoxin substances on the body. All of this allows us to consider WMIC as a promising agent for the

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correction of nutritional imbalance of prooxidant-antioxidant system of the body.

Conflict of interest

This study was supported by Grant of the Russian Federation President for state support of young Russian scientists (1568.2014.4), the state task of the Ministry of Education and Science of the Russian Federation, the project No. 1269.

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