Sys Rev Pharm. 2019;10(1):112-117.
A multifaceted Review journal in the field of Pharmacy

Review article

Deuterium Depleted Water as an Adjuvant in Treatment of Cancer

Anton Syroeshkin*, Olga Levitskaya*, Elena Uspenskaya, Tatiana Pleteneva, Daria Romaykina, Daria Ermakova
Department of Pharmaceutical & Toxicological Chemistry, Medical Institute, Peoples' Friendship University of Russia, 6 Miklukho-Maklaya Street, Moscow, 117198, RUSSIAN FEDERATION.

ABSTRACT

For more than 20 years, since 1993, the Hungarian National Institute of Pharmacy and Nutrition has been conducting a research on the mechanisms of antitumor activity of light water (deuterium depleted water – DDW). Long-term randomized clinical trials in numerous treatment centers in Hungary in compliance with the Good Clinical Practice (GCP) indicate a high sensitivity of tumor cells to lower levels of deuterium (D) in the body. The replacement of the ordinary water (D)/H = 140 ppm) by the "light" one (D/H = 20-105 ppm) is accompanied by a decrease in the size of the tumor, gradual necrosis of tumor cells, an increase in the median survival of cancer patients (prostate cancer, breast cancer, lung cancer, etc.). The content of deuterium in the body at the level of 10-12 mmol is an order of magnitude higher compared to essential trace elements, which makes it possible to predict its key role in biochemical processes. Indeed, mass-spectrometric analysis shows that deuterium bioaccumulation in the human body depends on its phenotype. In contrast, when drinking water is replaced with DDW, the deuterium content in aqueous extracts of biological blood samples, muscle and liver tissues of laboratory animals goes down. The use of DDW as a prophylactic agent and many experimental results obtained *in vitro* and *in vivo* in China, Japan, the USA, Romania, and the Russian Federation make it possible to recommend DDW for use as a safe additional agent in the treatment of cancer.

Key words: DDW, deuterium depleted water, tumor cells.
Correspondences:
Prof. Anton V. Syroeshkin, Ph.D., Dr.Sc.,
Head of the Department of Pharmaceutical & Toxicological Chemistry, People's Friendship University of Russia (PFUR www.rudn.ru), Member of Russian Academy of Natural Sciences, RUSSIAN FEDERATION.
Tel: +7 495 787 3803; Fax: + 7 495 787 2866
E-mail: livmatter@mail.ru, syroeshkin_av@pfur.ru, syroeshkin_av@rudn.university
Olga V. Levitskaya
Assistant of the Department of Pharm. & Toxicological Chemistry, People's Friendship University of Russia, RUSSIAN FEDERATION.
Tel: +7 963 676 12 26
E-mail: levitskayavolha@gmail.com
DOI : 10.5530/srp.2019.1.19

INTRODUCTION

Oncological diseases rank second in terms of incidence and mortality, second only to cardiovascular diseases. According to the WHO estimates[1], about 10 million people die from cancer each year, and according to the World Cancer Research Fund (WCRF) estimates, cancer mortality may exceed 20 million people per year by 2030[2].

Natural water is a mixture of molecules containing stable isotopes ^{16}O, ^{17}O, ^{18}O, ^{1}H, ^{2}H. The ratio of the number of deuterium atoms to protium D/H in fresh and sea waters is 132-156 ppm (μg/g)[3,4]. The content of individual isotopic modifications of water is comparable to the content of the most important trace elements in sea water and human blood plasma[5]. Taking into account that the mass of protium H is two times lower than the mass of deuterium D, the isotopic effect arising from the replacement of isotopes cannot but affect biological systems, for example, the rates of chemical reactions between substances containing them may differ by 5 - 10 times[6-8]. Due to the use of the heavy water (D_2O) in the nuclear industry, the effect of the increased deuterium content on its structure, physical and chemical properties, and physiological processes was studied in detail[9-11]. The replacement of protium with deuterium by 50% leads to the death of mammals[12].

Numerous publications of the last 20 years are devoted to the study of the role of deuterium in natural (12 mmol) or reduced content in the human body[13]. For example, it is known that water depleted of stable heavy isotopes (deuterium and oxygen ^{18}O) reduces the risk of γ-irradiation[14-15] and exposure to stress[16] of the mammals.

It is proved that the light water, i.e. deuterium depleted water (DDW), exhibits antidote properties of individual and combined effects of pharmaceutical substances and excipients of finished dosage forms[17-21]. The mechanisms of such influence are due to the structure, physical and chemical properties of the "light" water and changes in ligand-receptor interactions in biological objects of different hierarchical levels[3]. Tumor cells are extremely sensitive to deuterium depleted water: the use of light water leads to tumor regression and, in some cases, necrosis[22-25]. At the same time, healthy cells are able to adapt to lower deuterium content in water. The light water increases the rate of photosynthesis, promotes the growth of plants and aquatic animals[26-27]. In Europe, the USA, Japan, China and Russia, the deuterium depleted water is used for preventive and curative purposes[28-29].

STUDIES ON CELL CULTURES

Preventing the proliferation and migration of tumor cells is a priority of cancer therapy[30]. In the study of nasopharyngeal cancer cell lines (CNE-1, CNE-2, 5-8F, 6-10B, Sune-1), inhibition of tumor cell proliferation was observed as the isotopic D/H ratio (150, 100, 75 and 50 ppm) decreased[22]. At the same time, the proliferation of normal, non-tumor cells, on the contrary, increased (Figure 1).

Identical results were obtained in the study of cell proliferation of human prostate cancer lines PC-3, breast MCF-7, and melanoma M-14[31]. The culture medium was prepared by replacing the ordinary water with DDW with a D/H ratio = 90 ppm. Daily kinetics indicate inhibition of cell division by 10-25%.

Deuterium depleted water affects not only the proliferation of cancer cells but also their structural organization, shape, and size[32].

In environments prepared using deuterium depleted water, there is also observed a decrease in the colony-forming ability of tumor cells and an increase in the number of colonies of normal preosteoblasts (Table 1).

Cell migration required for tumor growth and metastasis is significantly reduced with a decrease in the D/H ratio in water, which was demonstrated in five cell lines of nasopharyngeal cancer (CNE-1, CNE-2, 5-8F, 6-10B, SUNE-1). With a change in the deuterium/protium ratio from 150 to 50 ppm, there is a noticeable decrease in the number of cells in the S phase of the cell cycle (Table 2), in which the DNA replication takes place.

One of the causes of tumor development is DNA damage by free radicals and reactive oxygen species[33]. With a decrease in the relative content of deuterium in the medium, the activity of NADP(H) – quinone oxidoreductase 1 (NQO1), a cytosolic enzyme that protects cells from oxidative stress[22].

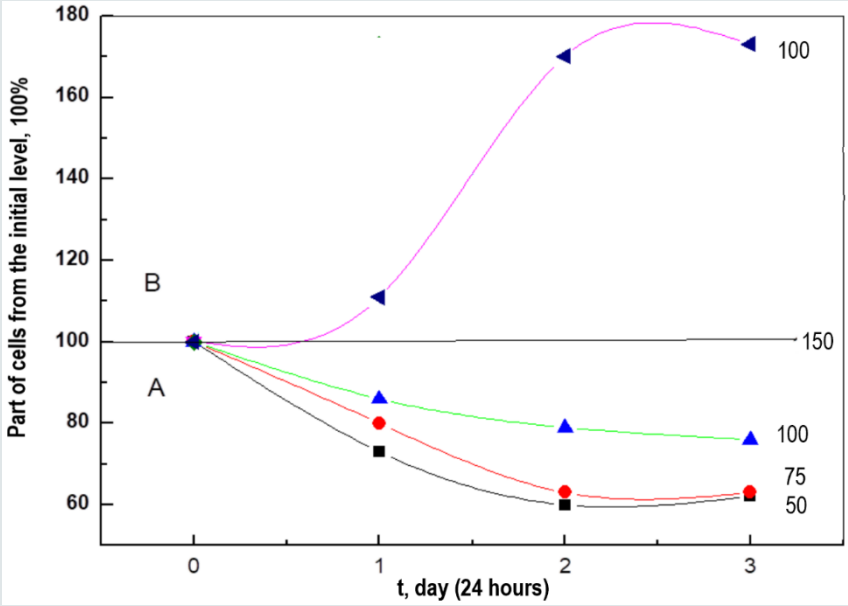

Figure 1: Kinetics of NPC(A) tumor cell death and MC3T3-E1 (B) preosteoblast growth in waters with different isotopic D/H ratios (ppm) (based on results[22]).

Table 1: Dependence of the number of cell colonies on the deuterium content in water according to[22], n=3, M±SD.

Cell cultures (Cancer Research Institute of Southern Medical University)	Number of colonies at different D/H ratios in water		
	150 ppm	75 ppm	50 ppm
CNE-1 (nasopharyngeal carcinoma)	228.0±18.0	23.5±4.5	23.0±4.0
5-8F (nasopharyngeal carcinoma)	137.0±7.0	86.0±6.0	27.0±3.0
MC3T3 - E1 (normal preosteoblasts)	49.5±4.5	83.0±3.0	75.0±4.0

M: mean; **SD:** standard deviation; **ppm:** parts per million.

Table 2: The dependence of the number of cells in the phases of the cell cycle on the D/H ratio in water used for the preparation of the culture medium (according to[22], n=3, M±SD; *p<0.05).

Cell lines	D/H, ppm	Number of cells in different phases of the cell cycle		
		G1	G2	S
Sune-1	150	73.91±2.18	12.55±0.88	13.55±0.93
	100	65.71±1.98	24.2±1.01	10.08±0.81
	75	60.90±1.90	33.40±1.12	5.70±0.31*
	50	71.45±2.01	24.65±0.68	3.90±0.27*
6-10B	150	13.43±0.65	15.20±0.78	71.37±2.36
	100	30.90±1.50	0.24±0.01	68.86±1.75
	75	38.81±1.64	0.31±0.01	60.88±1.56*
	50	44.15±1.83	15.05±0.75	40.80±1.32*
MC3T3-E1	150	39.70±1.47	12.30±0.91	48.00±1.52
	100	49.80±1.65	9.29±0.85	40.90±1.41*
	75	49.00±1.63	7.33±0.70	43.60±1.49*
	50	46.90±1.55	9.99±0.87	43.10±1.49*

G_1: cell growth; **G_2:** cell growth; **S:** DNA synthesis; **M:** mean; **SD:** standard deviation; **ppm:** parts per million.

DDW has been proven to stimulate cancer cell apoptosis[32] and suppresses gene expression[34-35], which play a key role in tumor formation[36].

RESEARCH ON LABORATORY ANIMALS

The effect of DDW (D/H=94 ppm) on tumor growth was studied in human prostate cell PC-3 transplantation to immunosuppressive mice line CBA/Ca[37]. At a relatively late stage of tumor development (32 days after transplantation), drinking water (D/H=150 ppm) was replaced by DDW (D/H= 94 ppm). In the control group, tumor size increased in all mice, while the experimental group four mice showed complete tumor regression, seven – partial regression, and eight animals showed an increase in tumor size. The life expectancy of animals in the experimental group increased by 40% compared to the control. At an earlier replacement of drinking water with DDW (18 days after tumor cell transplantation), a 40% reduction in tumor size was observed compared to the control.

When drinking water was replaced with DDW in the group of animals subcutaneously injected with tumor cells H460 (human

lung carcinoma), inhibition of growth rate (30%) and tumor weight reduction were found (Table 3).

CBA/Ca mice were transplanted with breast adenocarcinoma tissue MDA-MB-231 and MCF-7[38]. The day before transplantation, drinking water was replaced with DDW (D/H=30 ppm), which led to an increase in animal survival (Table 4). In the case of MDA-MB-231, on 65 days 80% of control animals died against 11% in the experimental group. The MCF-7 xenotransplantation killed 67% and 12% of the control and experimental groups of animals, respectively.

In the experiments on the Wistar line rats with experimental Walker tumors 256 (standard tumors for pre-clinical trials of anticancer agents), DDW with a D/H ratio = 30, 60, 100 ppm was used as drinking water and tap water (D/H=144 ppm) was used for the control group[39]. In addition, 2 ml of water were injected daily subcutaneously. The following parameters were estimated in the experiment: tumor growth; latent period of tumor formation; average life time of animals, as well as anatomical and clinical indicators. The results indicate a positive dynamics in all monitored parameters using DDW, including an increase in animal survival: on the 40th day on average by 75%, and on the 60th day on average by 35% (Table 5).

The prophylactic injection of "light" water (30 days before the transplantation of tumor cells) is also accompanied by an increase in the survival of the experimental animals; at the same time, all animals show inhibition of tumor growth and 25-40% of animals show complete tumor regression[39].

The inhibitory effect of water with low deuterium content in models with transplantable tumors (Lewis lung carcinoma, uterine sarcoma Cm 322 and cervical cancer CC-5) was studied in mice of line BDF_1 and CBA[40]. Animals in both groups started taking light water on the day of tumor transplantation. The inhibitory effect of DDW was judged by tumor volume and animal life expectancy. Water with low deuterium content had a statistically significant inhibitory effect on the growth of tumors studied by 43% on average (p=0.05).

In the experiment on two models of transplantable tumors – Lewis lung carcinoma and cervical cancer CC-5[41], mice began to receive water with low deuterium content a month (4 weeks) before transplantation. Taking into account that the complete renewal of water in the body takes 3 weeks, it was possible to create a "deuterium-free background". The mass spectrometric analysis of the aqueous extracts of biological samples of blood, muscle and liver tissues of Wistar rats indicates bioaccumulation of deuterium in the body[39].

For Lewis lung carcinoma[41], the metastasis inhibition coefficient was estimated. For this purpose, 2 experimental groups of 20 animals and 3 controls (two groups of 20 and one group of 30 animals) were used. The animals of one of the experimental and one control groups were kept until natural death, the animals of the other two were killed on the 17th day after transplantation. The third control group (10 mice) was taken to determine the average lung mass of intact animals. To analyze the inhibitory effect of water with low deuterium content, the criteria used to evaluate anticancer agents were used. In the experimental conditions, it was noted a statistically significant increase in the time of appearance of the first nodules at the site of transplantation of both tumors in the experimental groups. In addition, inhibition of tumor node growth was observed at all periods of measurement of transplanted tumors.

In all groups of animals treated with low deuterium water, there was an increase in life expectancy compared to the control by 19 % on average.

In the experiment with Lewis lung carcinoma 12% inhibition of metastasis was observed (p<0.05).

Thus, the strengthening of the inhibitory effect of water with a low content of deuterium on the transplanted tumors by creating a "deuterium-free background" was confirmed.

Successful results were obtained in dogs when drinking water was replaced with DDW with natural (non-experimental) breast tumors (7 animals)[37]. The dose of DDW (D/H=90-95 ppm) was in the range

Table 3: Mass of tumors in BALB/c mice two months after transplantation of human lung tumor cells H460 (according to[32] n=8, M±SD; *p<0.05, t-test).

Group of animals	Tumor weight, g
Control (D/H= 150 ppm)	10.64±0.83
Experimental (D/H=50 ppm)	7.36±0.78*

M: mean; **SD:** standard deviation; **ppm:** parts per million.

Table 4: Effect of DDW on CBA/Ca mice survival after transplantation of breast adenocarcinoma tumor cells MDA-MB-231 and MCF-7 (according to [38]).

Days after transplantation	MDA-MB-231		MCF-7	
	ordinary water (control)	DDW (30 ppm)	ordinary water (control)	DDW (30 ppm)
20	5	9	6	8
50	5	9	6	8
65	1	8	2	7
71	0	8	2	7
80	0	7	1	5
87	0	6	1	5

ppm: parts per million.

Table 5. Survival of the Wistar line rats with experimental Walker tumors 256 using water of different isotopic compositions (according to [39]).

Isotopic composition of water, D/H, ppm	Survival at different stages of observation, %		
	30 days	40 days	60 days
144	0	-	-
100	100	70	30
60	100	80	40
30	100	75	35

ppm: parts per million.

of 0.01-0.02 kg per kg of body weight per day. Already in the first 3 weeks, two animals showed a decrease in tumor volume by 60-70%. The complete disappearance of the tumor was recorded in one animal after 8 months of using DDW.

In 2008[42], the use of DDW as an additional component of chemotherapy, which is able to reduce the toxic effects of cytostatics on the liver, kidney, and blood-forming organs of animals, was patented. DDW with ratio D/H = 60 ppm[43] is assumed to be used 60 days before the removal of the tumor and 700 days in the postoperative period. The use of DDW in the indicated dose reduces the toxicity of cytostatic agents (cyclophosphamide, 5-fluorouracil, farmorubicin, vinblastine) by 41% on average. Due to a decrease in the general and specific toxicity of chemotherapy, there is an increase in the median survival of animals. Due to the absence of side effects, DDW can be used as an adjuvant in both animals and humans[37].

DDW APPLICATION IN THE TREATMENT OF ONCOLOGICAL PATIENTS

Currently, DDW is being registered as a therapeutic agent[44]. The Hungarian National Institute of Pharmacy and Nutrition has issued a permission[45] of the compassionate use of DDW by patients with advanced cancers (lung cancer, prostate cancer, breast cancer). The studies were carried out on the basis of 16 medical institutions in Hungary[44-46] in accordance with Good Clinical Practice (GCP). Patients were provided with all available information on the effects of DDW[45]. In a double-blind placebo-controlled study, there was used water obtained by diluting DDW with mineral water containing necessary (essential) trace elements to a deuterium/protium ratio of 105 and 85 ppm. Patients ingested the water. Regardless of the nature of the main therapy, the aim of the study was to investigate the effect of reducing the level of deuterium in the body on the tumor process. The dose of DDW was selected taking into account the daily volume of water consumed and body weight. The method of treatment took into account the need to gradually reduce the deuterium/protium ratio in water – by 10-20 ppm monthly for 6-10 months – and maintain deuterium in the body at the lowest possible level.

Lung cancer

Lung cancer is the most common cause of death in cancer patients[1]. The standard treatment for lung cancer is chemotherapy. The lung cancer has a high metastatic activity, which increases the number and mortality of oncological patients[44]. Taking into account that most cases of the disease are diagnosed at the inoperable or conditionally operable stage of the tumor process, when there are metastases, the main treatment is chemotherapy[47]. The average life expectancy of patients in this group is limited, which leads to the choice of aggressive treatment with serious side effects. According to most clinical studies, the median survival (MS) for patients with brain metastases in case of lung cancer is 4-6 months[44]. In most cases, the blood-brain barrier may prevent conventional chemotherapeutic agents from entering the brain metastasis, so treatment strategies are limited. While deuterium depleted water can affect the central nervous system, easily penetrating the blood-brain barrier[16].

The life expectancy of patients in the experimental group was much higher than the average values for patients with brain metastases and amounted to 10-55 months. During the treatment, complete regression of metastases and primary tumor was observed in two patients, one of the patients did not have a relapse of lung carcinoma within the next two years after the treatment, the progression of multiple brain tumors was stopped in some patients, and the volume of the primary tumor significantly decreased. In addition, increased intracranial pressure and symptoms of thrombophlebitis decreased. Thus, in the absence of side effects, the quality of life of oncological patients increased.

Breast cancer

The antitumor effect of DDW was found during clinical studies in patients with breast cancer (n=232)[44,48]. The average time from the diagnosis to initiation of DDW taking was 36 months, the average duration of the treatment was 25 months. During 90 days, the ordinary drinking water was replaced with DDW, keeping the standard treatment regimen. After 2-3 months, DDW was temporarily discontinued. The regimen was repeated after 4-6 months from the beginning of the treatment. A retrospective study of life expectancy shows an increase in patient survival with DDW compared to other clinical groups. Thus, the average life expectancy of patients was: 148 months (12.3 years) from diagnosis. It should be taken into account that 56% of patients had stage 4 cancer. The survival of patients at an early stage of the disease was 217 months (18.1 years) and 52 months (4.3 years) for patients in later stages.

In addition, a subgroup of 74 late-stage patients was selected to assess the effectiveness of DDW. The average time from the diagnosis to initiation of DDW treatment was 181 days (5.9 months); the average duration of DDW treatment was 402 days (13.2 months). As a result of the use of DDW in addition to conventional treatment, 16 patients (21%) had a complete remission and 27 patients (36.5%) had a partial remission; 12 patients (16.2%) showed the stop of the tumor progression; in 19 patients (25.7%) it was recorded a progression of the disease.

It was found that the effectiveness and result of the treatment depend on the dose of DDW. The majority of patients, whose condition did not change or who had a progression of the disease, took DDW in a lower dose and/or irregularly. The complete tumor regression was recorded when DDW was consumed in higher doses. For them, the median survival (52 months) was 2-3 times higher than in the group receiving the standard treatment only (12-31 months).

Prostate cancer

Prostate cancer ranks third among the causes of death of men with cancer[49]. Clinical trials indicate that the median survival with this disease is in the range of 15 to 21 months[45]. The treatment regimens were consistent with the treatment standards. The criteria for evaluating the effectiveness of the adjuvant therapy were: changes in the condition of the tumor, complaints about urination, PSA level. Patients' health was monitored for a year after the completion of DDW taking.

A homogeneous group of 32 people with bone metastases was selected for the statistical analysis. Due to the short duration of treatment (4 months), none of the patients had complete tumor regression. However, seven of 22 patients had a partial regression. The disease progressed in four patients. The prostate volume decreased in 18 patients.

In the first year after the treatment, 2 people died (9%) in the group of patients who consumed DDW, and 9 people (41%) in the control group. The inverse correlation between PSA level and DDW dose was found.

Thus, the consumption of DDW at early stages of prostate cancer can slow down the progression of the disease. At the same time, almost two decades of research have not found any adverse effects of light water on the body, in particular, no significant changes in blood tests, even when taking maximum doses of DDW.

Preclinical Toxicity Studies[50], prospective and retrospective clinical studies indicate the absence of adverse body reactions when taking DDW with a deuterium/protium ratio of 25 to 105 ppm. Inclusion of DDW in the existing treatment regimens at any stage of the disease[51] increases survival, delays tumor progression, improves the quality of life of oncological patients.

In recent years, attempts have been made to substantiate the molecular mechanisms of the action of "light" water on biological objects[52].

In particular, the results were obtained on reducing the amount of deuterium in individual organs and tissues of mammals when replacing drinking water with DDW[39,43].

The results of our research show that the processes described in the review can find an explanation not only by the kinetic isotope effect, which is manifested in the acceleration of native reactions in aqueous media with low deuterium content[53-55] but also by changing the chiral properties of biological media while reducing the ratio of hydrogen isotopologues D/H[56,57]. One of the possible mechanisms of DDW influence on tumor processes is associated with a decrease in the migratory activity of cancer cells, as shown by human lines A549 and HT29[58]. It should be particularly emphasized that the mechanisms of deuterium action on biological systems *in vivo* and *in vitro* have not been fully understood.

CONCLUSION

The positive effects of using deuterium depleted water make it possible to consider it as a promising adjuvant antitumor agent. Despite the increasing number of scientific publications related to the use of deuterium depleted water, its mechanisms of action have not been completely clarified. The inclusion of deuterium depleted water in the treatment of cancer and other diseases requires randomized studies of its safety according to the approved Protocol for the bioequivalence evaluation of generic and patented therapeutic agents. To control the content of deuterium in the human body and its influence on the biochemical processes, it is necessary to develop complex techniques based on modern analytical methods: NMR, chromatography mass-spectrometry, AES-ICP.

CONTRIBUTIONS OF AUTHORS

All the author has contributed equally.

ACKNOWLEDGEMENT

The publication has been prepared with the support of the «RUDN University Program 5-100».

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

ABBREVIATIONS

DDW: Deuterium Depleted Water; **GCP**: Good Clinical Practice; **MS**: Median Survival; **PSA**: Prostate-Specific Antigen; **WCRF**: World Cancer Research Fund.

REFERENCES

1. who.int [homepage on the Internet]. World Health Organization [updated 2018 Sep 12; cited 2019 Apr 8]. Available from: https://www.who.int/ru/news-room/fact-sheets/detail/cancer/.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018 Nov;68(6): 394-424.
3. Goncharuk VV, Lapshin VB, Burdeinaya TN, Pleteneva TV, Chernopyatko AS, Atamanenko ID, *et al.* Physicochemical Properties and Biological Activity of the Water Depleted of Heavy Isotopes. Journal of Water Chemistry and Technology. 2011;33(1):15-25.
4. Bowen GJ, Winter DA, Spero HJ, Zierenberg RA, Reeder MD, Cerling TE, *et al.* Stable hydrogen and oxygen isotope ratios of bottled waters of the world. Rapid Commun Mass Spectrom. 2005;19:3442-50.
5. Timakov AA. Main effects of light water. 8th all-Rus. Conf. "Physicochemical processes in selection of atoms and molecules. 2003 Nov 6-10; Zvenigorod, Russia.
6. Lobyshev VN, Kalinichenko LP. Isotopic effects in biological systems. M. Nauka. 1978.
7. Cleland WW. The Use of Isotope Effects to Determine Enzyme Mechanisms. JBC. 2003; 278 (52):51975-51984.
8. Lewis GN. Biology of heavy water. Nature. 1934; 133:620-620.
9. Harvey EN. Biological effects of heavy water. Biol Bull. 1934; 66(2):91-96.
10. Mosin O, Ignatov I. Biological Influence of Deuterium on Prokaryotic and Eukaryotic cells. Journal of Medicine, Physiology and Biophysics. 2014; 1:52-72.
11. Cisanova ES, Syroeshkin AV, Uspenskaya EV, Ul'yantsev AS, Pleteneva TV, Klimova, EV,. Berseneva EA. The study of biological activity and the ratio of deuterium/protium (D/H) in water using a cellular biosensor S. ambiguum. Studied in Russia. 2010. 2010; 46:558-93. Russian.
12. Medvedev ZA. To drink or not to drink? Science and life. 2008; 6:114-21.
13. Ignatov II, Mosin OV. Water: hygiene and ecology. 2013; 1(3):22-32.
14. Rakov DV. Alleviation of gamma-radiation damage by water with reduced deuterium and 18O content. Aerospace and environmental medicine. 2007; 41(3):36-39.
15. Kulikova EI, Kriuchkova DM, Severiukhin IS, Gaevskiĭ VN, Ivanov AA. Radiomodifying properties of deuterium-depleted water with poor content of heavier isotopes of oxygen. Aerospace and environmental medicine. 2012; 46(6):45-50.
16. Strekalova T, Evansa M, Chernopiatko A, Coucha Yv, Costa-Nunes J, Cespugliof R *et al.* Deuterium content of water increases depression susceptibility: The potential role of a serotonin-related mechanism. Behav Brain Res. 2015; 277: 237-244.
17. Cărpinişan L, Petcu MD, Petrovici S, Chiş C, Ghişe A, Zehan R. The Influence of Deuterium Depleted Water on the Hematocrit and the Leukocyte Formula in Rats Intoxicated With Chromium. Scientific Papers: Animal Science and Biotechnologies. 2010; 43(1):464-468.
18. Avila DS, Somlyai G, Somlyai I, Aschner M. Anti-aging effects of deuterium depletion on Mn-induced toxicity in a C. elegans model. Toxicol Lett. 2012; 211:319-324.
19. Olariu L, Petcu M, Cuna S, Scurtu M, Tulcan C, Brudiu I. The role of deuterium depleted water (DDW) administration in blood deuterium concentration in Cr (VI) intoxicated rats, Lucrări Stiinlifice Medicină Veterinară. 2010; 43(2):193-196.
20. Doina PM, Olariu V, Scurtu M, Tulcan C, Brudiu I, Muntean D, *et al.* The Effect of Deuterium Depleted Water on some Hepatic Enzimes' Activity in Rats Intoxicated with Chromium (VI). Fascicula: Ecotoxicologie, Zootehnie şi Tehnologii de Industrie Alimentară. 2012; XI/B(11): 521-526.
21. Burdeinaya TN, Zrelov OY, Maksimova TV, Morozova MA, Mtei GI, Uspenskaya EV, *et al.* Combined toxicity of Na_2EDTA and D_2O. Vestnik RUDN, Medicine. 2013; 2:5-9.
22. Wang H, Zhu B, He Zu, Daia Zh, Huanga G, Lia B, *et al.* Deuterium-depleted water (DDW) inhibits the proliferation and migration of nasopharyngeal carcinoma cells *in vitro.* Biomedicine and Pharmacotherapy. 2013; 67:489-496.
23. Roxana EM. Deuterium - Depleted Water in Cancer Therapy. Environmental Engineering and Management Journal. 2010; 9(11):1543-1545.
24. Krempels K, Somlyai I, Somlyai G. Molecular and Clinical Effects of Deuterium Depleted Water in Treatment and Prevention of Cancer. Positive Health Online [serial on the Internet]. 2013 Feb [cited 2019 Mar 13] 203: [about 3 p.]. Available from: http://www.positivehealth.com/article/cancer/molecular-and-clinical-effects-of-deuterium-depleted-water-in-treatment-and-prevention-of-cancer
25. Krempels K, Abonyi O, Balog K, Somlya I. Deuterium Depletion in Cancer Treatment and Prevention. Positive Health Online [serial on the Internet]. 2013 Oct [cited 2019 Mar 18] 209: [about 3 p.]. Available from: http://www.positivehealth.com/article/cancer/deuterium-depletion-in-cancer-treatment-and-prevention-practical-application-advice
26. Seki K, Usui T. Process for promoting growth of agricultural products and aquatic animals, and for treating pancreatic disease, involves using deuterium-depleted water having specific deuterium concentration. Patent JP2005328812-A 2005.
27. Lobyshev VI, Kirkin AA. Influence of variations in the isotopic composition of water on its biological activity. Scientific proceedings of the VI International Congress "Weak and super weak fields and radiations in biology and medicine. 2012 Jul 2 - 6; Saint-Petersburg, Russia.
28. Feng H, Changgong M. Method for Production of Deuterium Depleted Potable Water. Ind. Eng. Chem. Res. 2011; 50:378-381.
29. Langvey.ru/en/ [homepage on the Internet]. Moscow: Langvey light water [updated 2017 Feb 23; cited 2002 Apr 19]. Available from: https://www.langvey.ru/en/light-water.html.

30. Somlyai G. Defeating Cancer! The Biological Effects of Deuterium Depletion. Bloomington: Author House; 2002.

31. Somlyai G, Laskay G, Berkényi T, Jákli Gy, Jancsó G. Naturally occurring deuterium may have a central role in cell signaling. Synthesis and Applications of Isotopically Labelled Compounds. 1997; 24:137-141.

32. Feng-Song C, Ya-Ru Zh, Hong-Cai Sh, Zong-Hua A, Su-Yi Zh, Ju-Yong W. Deuterium-depleted water inhibits human lung carcinoma cell growth by apoptosis. Experimental and Therapeutic Medicine. 2010; 1(2):277-283.

33. Bakirov BA, Karimov DO, Viktorova TV. Polymorphism of genes tumor necrosis factor α, the main regulator of the tumor suppressor p53 and NADP(H) quinoneoxido reductases in patients with chronic lymphocytic leukemia. Cytokines and inflammation. 2011; 4:21-6.

34. Somlyai G, Gyöngyi Z. Deuterium Depletion Can Decrease the Expression of c-myc, Ha-Ras and p53 Gene in Carcinogen-Treated Mice. International Journal of *In Vivo* Research. 2000; 14: 437-440.

35. Gyongyi Z, Budarn F, Szabor I, Ember I, Kiss I, Krempels K, *et al.* Deuterium Depleted Water Effects on Survival of Lung Cancer Patients and Expression of Kras, Bcl2, and Myc Genes in Mouse Lung. Nutrition and Cancer. 2013; 65(2):240-46.

36. Soucek L, Whitfield J, Martins CP, Finch AJ, Murphy DJ, Sodir NM, *et al.* Modelling Myc inhibition as a cancer therapy. Nature. 2008; 455:679-683.

37. Somlyai G, Laskay G, Berkényi T, Galbács Z, Galbács G, Kiss SA, *et al.* The Biological Effects of Deuterium-Depleted Water, a Possible New Tool in Cancer Therapy. Z. Onkologie/Journal of Oncology. 1998; 30(4):91-94.

38. Somlyai G, Jancsó G, Jákli G, Vass K, Barna B, Lakics V, *et al.* Naturally occurring deuterium is essential for the normal growth rate of cells. FEBS Letters. 1993; 317 (1):1-4.

39. Stefănescu I, Manolescu N, Comişel V, Tamaian R, Titescu G. Deuterium Depleted Water Effects on Walker Tumours. Bulletin UASVM. Veterinary Medicine. 2008; 65(1):443-450.

40. Turusov, VS, Sinyak YY, Grigoriev AI, Zaridze DG, Antoshina YY, Trukhanova LS, Gor'kova TG. Low-deuterium water effect on transplantable tumors. Oncology. 2005; 51(1): 99-102.

41. Turusov, VS, Sinyak YY, Antoshina YY, Trukhanova LS, Gorkova TG. Influence of preliminary administration of water with reduced deuterium content on the growth of transplanted tumors in mice. Oncology. 2006; 52(1):59-62.

42. Anghel R, Balanescu I, Balint E, Begu D, Cinca S, Comisel V. *et al.* Deuterium depleted water (DDW) using as adjuvant in cancer therapy for cytostatics toxicity reduction. Patent WO 2006019327 B1; 2008.

43. Manolescu N, Balanescu I, Valeca S, Traicu R, Marculescu D, Niculita P. *et al.* Method for *in vivo* determination of efficient concentration of deuterium depleted water for cancer therapy, by administering deuterium depleted water to animals before and after tumor grafting, and monitoring immunological conditions in animals. PatentWO2005017522-A2; 2005.

44. Krempels K, Somlyai I, Somlyai G. A Retrospective Evaluation of the Effects of Deuterium Depleted Water Consumption on 4 Patients with Brain Metastases from Lung Cancer. Integrative Cancer Therapies. 2008; 7(3):172-181.

45. Kovocs A, Guller I, Krempels K, Somlyai I, Jonosi I, Gyongyi Z. *et al.* Deuterium Depletion May Delay the Progression of Prostate Cancer. J. Cancer Therapy. 2011; 2:548-56.

46. Krempels K, Somlyai I, Gyöngyi Z, Ember I, Balog K, *et al.* A retrospective study of survival in breast cancer patients undergoing deuterium depletion in addition to conventional therapies. J Cancer Res Ther. 2013; 1(8): 194-200.

47. Gorbunova VA. Chemotherapy of lung cancer. Independent publication for medical practitioners. 2001; 5:186-189.

48. Yamamoto N, Katsumata N, Watanabe T, Omuro Y, Ando M, Narabayashi M, *et al.* Clinical Characteristics of Patients with Metastatic Breast Cancer with Complete Remission Following Systemic Treatment. Jpn J Clin Oncol. 1998; 28(6):368-373.

49. cancerresearchuk.org [homepage on the Internet]. London: Cancer Research UK [updated 2017 Oct 23; cited May 2019]. Available from: https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/prostate-cancer/mortality#source24.

50. Torok G, Csik M, Pinter A. Effects of Different Deuterium Concentrations of the Media on the Bacterial Growth and Mutagenesis. Egészségtudomány/Health Science. 2000; 44:331-338.

51. Somlyai G, Kovarcs A, Guller I. Deuterium has a key role in tumour development – new target in anticancer drug development. Ejc supplements. 2010; 8(5):155-225.

52. Boros LG, Agostino DP, Katz HE, Roth JP, Meuillet EJ, Somlyai G. Submolecular regulation of cell transformation by deuterium depleting water exchange reactions in the tricarboxylic acid substrate cycle. Med Hypotheses. 2016; 87:69-74.

53. Syroeshkina A, Pleteneva T, Uspenskaya E, Zlatskiya I, Antipova N, Grebennikova T, *et al.* D/H control of chemical kinetics in water solutions under low deuterium concentrations. Chemical Engineering Journal [In Press].

54. Zrelov OY, Syroeshkin AV, Uspenskaya EV, Titorovich OV, Pleteneva TV. Effect of water isotopic composition on galactose mutarotation kinetics. Pharmaceutical Chemistry Journal. 2015; 49(6):413-416.

55. Levitskaya OV, Syroeshkin AV, Pleteneva TV. Arrhenius kinetics as a bioactivity assessment criterion for drug substances and excipients. Pharmaceutical Chemistry Journal. 2016; 49(11): 779-781.

56. Goncharuk VV, Syroeshkin AV, Pleteneva TV, Uspenskaya EV, Levitskaya OV, Tverdislov VA. On the possibility of chiral structure density submillimeter inhomogeneities existing in water. Journal of Water Chemistry and Technology. 2017; 39(6):319-324.

57. Syroeshkin AV, Pleteneva TV, Uspenskaya EV, Levitskaya OV, Tribot-Laspiere MA, Zlatsky IA, *et al.* Polarimetric research of pharmaceutical substances in aqueous solutions with different water isotopologues ratio. International Journal of Applied Pharmaceutics. 2018; 10(5);243-248.

58. Goncharuk VV, Pleteneva TV, Grebennikova TV, Syroeshkin AV, Uspenskaya EV, Antipova NV, *et al.* Determination of Biological Activity of Water Having a Different Isotope Ratio of Protium and Deuterium Journal of Water Chemistry and Technology. 2018; 40(1);27-34.