Electromagnetic Biology and Medicine, Early Online: 1–10, 2011

ISSN: 1536-8378 print / 1536-8386 online
DOI: 10.3109/15368378.2011.638029

informa
healthcare

The modulating impact of illumination and background radiation on 8 Hz-induced infrasound effect on physicochemical properties of physiolagical solution

Naira Baghdasaryan, Yerazik Mikayelyan, Sedrak Barseghyan, Erna Dadasyan & Sinerik Ayrapetyan

UNESCO Chair-Life Sciences International Postgraduate Educational Center, Yerevan, Armenia

At present, when the level of background ionizing radiation is increasing in a number of world locations, the problem of the study of biological effect of high background radiation becomes one of the extremely important global problems in modern life sciences. The modern research in biophysics proved that water is a most essential target, through which the biological effects of ionizing and non-ionizing radiations are realized. Therefore, there is no doubt about the strong dependency of non-ionizing radiation-induced effect on the level of background radiation. Findings have shown that illumination and background radiation have a strong modulation effect on infrasound-induced impacts on water physicochemical properties, which could also have appropriate effect on living organisms.

Keywords Infrasound, 8Hz, background radiation, illumination, physiological solution, hydrogen peroxide

INTRODUCTION

Although the biological effect of non ionizing radiation (NIR) can be considered as proven fact (Adey, 1981; Ayrapetyan, 2006; Chaplin, 2011), the detailed mechanisms of its effect is not fully elucidated. Numerous hypotheses of mechanisms have been proposed with regard to the biological effects of NIR on cells and organisms. But none of them have provided a reliable and complete explanation of the experimental findings. One of the most popular and traditional hypotheses is the so-called "Water Hypothesis." Since water is a dominant component of environmental medium and biological systems, and its molecule ionization is extremely sensitive to different environmental factors, it was suggested as a sensor for NIR, through which its biological effects are realized (Chaplin, 2011; Szent-Györgyi, 1968; Klassen, 1982; Ayrapetyan et al., 1994).

At present, it is well documented that NIR could cause changes in water molecule ionization (Gudkova et al., 2005; Domrachev et al., 1992; Ayrapetyan, 2005, 2006a,b, 2008; Ayrapetyan et al., 2006, 2009a,b) whose products could modulate the process

Address correspondence to Sinerik Ayrapetyan, UNESCO Chair-Life Sciences International Postgraduate Educational Center, 31 Acharyan St. 0040 Yerevan, Armenia; E-mail: info@biophys.am

of reactive oxygen species (ROS) formation and change the level of hydrogen peroxide (H_2O_2) in water (Chaplin, 2011). The hydration of water ionization products (H_3O+ and $OH-$) reduces their chemical activity; therefore, it was suggested that NIR-induced dipole vibration, when the hydration shell of ions is removed, can reactivate them and elevate the H_2O_2 formation in aqua medium (Voeikov, 2006; Walczak and Dziuban, 2006). Thus, the NIR could change the level of H_2O_2 in the aqua medium by direct effect on water molecules dissociation and by hydration changes of its ionization products (Chaplin, 2011). Therefore, the H_2O_2 serves as a messenger through which the transduction of NIR-induced water structure changes to intracellular cell metabolism are realized.

Although the light intensity and background radiation are common physical components of the environmental medium, the detailed mechanisms of their influences on water properties are not yet fully clarified. Our previous work in this field has shown that illumination and background radiation have a modulating influence on water physicochemical properties and the degree of manifestation of its effect depends on the initial ionic composition and density of water (Baghdasaryan et al., 2011).

As the water ionization is considered one of the main pathways through which the biological effect of ionizing radiation (IR) is realized, there is no doubt that background ionizing radiation (BGIR) could modulate the NIR effects on water properties in non living and living systems. At the same time, taking into consideration the data from recent scientific literature on the sunlight-induced structural changing of liquid water (Shimokawa et al., 2009) and definite changes in the O—H stretching region (Yokono et al., 2009), it is supposed that illumination and background radiation should be able to modulate different environmental factors-induced effects of on physicochemical properties of water solution (including non ionizing radiation).

Therefore, the purpose of the present work was to study the modulating effect of illumination- and background radiation-(BGR) on NIR (in this case on vibration at infrasound (IS) frequency)-induced changes of physicochemical properties (heat fusion period, contents of H_2O_2 and O_2) of physiological solution.

MATERIALS AND METHODS

Physiological Solution

The experiments were performed on physiological solution (PS) for snail *Helix pomatia*, having the following composition (in mM): *NaCl*-80, *KCl*-4, $CaCl_2$-7, $MgCl_2$-5, *Tris*–*HCl*-5, $pH = 7.5$, and $G = 95.56 \pm 3 \times 10^{-2}$Siemens. PS with mentioned content was chosen because in following biological in vitro studies the isolated single neuron and heart muscle of a snail will be taken as experimental models. Before the start of experiments fresh prepared PS was stored in a plastic container in a lead box (E$v = 0$ lux illumination, R $< 1\mu$Rongen/h background radiation at $T = 18 \pm 0.5^oC$ temperature) for 24 h.

Experimental Medium

The experiments were carried out in the following media:

(1) in light ($E_v = 500 - 550$ lux) and normal BGR which we have conditionally named as ***NBGR in Light;***
(2) in dark (in wooden box, $E_v = 0$ lux) and normal BGR – ***NBGR in Dark***;
(3) in dark and low BGR (in lead box $E_v = 0$ lux, R $< 1\mu$Rongen/hour) – ***LBGR in Dark***.

The illumination intensity was measured by lux meter L-0A (PPUH SONOPAN, Poland) and expressed in lux. All the experiments were performed in the same area of the laboratory. The background radiation was measured by RM-60 Micro-Roentgen Radiation Monitor (Aware Electronics Corp, Wilmington DE, USA) interfaced with personal computer (PC).

PS samples incubated in ***NBGR in Light*** ($E_v = 500 - 550$ lux and $R = 17$–18 μRoentgen/hour) at 18°C conditions were taken as controls (100%). Dark- and low BGR-induced effects on PS properties were expressed in % of controls.

The wooden box was used for ***NBGR in Dark*** exposition of PS samples, while the ***LBGR in Dark*** exposition were performed in the lead box (DS-OOO, Russia), having cylindrical form: height: 32 cm; outside diameter: 17.5 cm; walls thickness: 4 cm; weight: 50 kg.

Treatment of PS Samples by Infrasound

A special device was assembled, presented in Fig. 1, which allows the investigation of the treatment of samples by infrasonic frequency. A glass test tube (1) with 10 mm diameter and 10 ml volume was fixed in the holder of the vibrator (2). The vibrator was driven by the sine-wave generator (4) (GZ-118, Russia) through the power amplifier (3). To obtain stable amplitude for vertical vibration a feedback coil was used. The vibrator was constructed in the department of engineering at LSIEC on the basis of the IVCh-01 device (Russia). For matching the output power of the sine-wave generator with the driving power vibration a special power amplifier (3, IRPhEA NAS, Armenia) was used. The frequency of vibration was measured by a frequency meter – Ch 3–47 (Russia, not shown in Fig. 1).

The Study of Heat Fusion Period (HFP) of Frozen PS Samples

The *HFP* of frozen *PS* samples was estimated by the following method: a hermetic plastic tube (volume 1 ml) that had a thermo-sensor at the bottom was filled with 10 min sham pre-exposed or IS-pretreated (experimental) samples (per 0.5 ml). Then the tube was inserted into the Dewar vessel containing liquid N_2 for deep freezing (up to −75°C). After withdrawing from the liquid N_2 the tube was left to melt in the thermostat at 22 ± 0.5°C condition. The temperature of samples was recorded during the melting process by extra-sensitive thermometer Biophys-TT (IRPhEA NAS, Armenia) connected to PC through Digidata 1322A data acquisition system (Molecular Devices, Sunnyvale, CA). The data were analyzed by dataTrax 2-computer software.

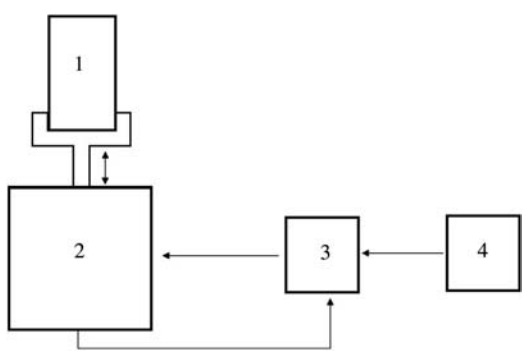

FIGURE 1 The schematic diagram of a device for investigation of infrasonic-treated physiological solution: glass test tube, vibrator with the coil of feedback coupling, power amplifier, the generator of sine-wave voltage.

Determination of H_2O_2 Content in PS Samples

The concentration of H_2O_2 was measured in samples (per 10 ml) pre-exposed (sham) and pre-treated by 8 Hz 30 dB IS (experimental) in 3 experimental media (during 10 min) by using a sensitive assay based on enhanced chemiluminescence in peroxidase–luminol–*p*-iodophenol system (Bruskov et al., 2002). The method allowed the investigators to detect the H_2O_2 content at a range of nanomolar concentration. The chemiluminescence of samples was quantified with 1450 Wallac 1450 liquid scintillation and luminescence counter (PerkinElmer, Wallac Oy, Turku Finland). For luminescence counting the 24-well sample plates (1450–402) set on 1450–102 cassettes were used. The ratio of sample and "counting solution" volumes in each well of plat was 1:1 (v/v). The "counting solution" contained 10 mM Tris–HCl buffer ($pH = 8.5$), 50 μmol *p*-iodophenol, 50 μmol luminol, and 1 nmol horseradish peroxidase (Sigma-Aldrich Chemie Gmbh, Steinheim, Germany). The "counting solution" was prepared immediately prior to the measurement. The H_2O_2 concentration in mM was quantified according to the calibration curve.

Determination of Oxygen Content in PS Samples

The content of O_2 in non treated (sham) and IS-treated by 8 Hz 30d B IS (experimental) PS samples (per 10 ml) was quantified by a Dissolved Oxygen Meter (World Precision Instruments Inc., Sarasota FL, USA). The data was expressed in g/l.

Statistics

The SPSS 13.0 PC program was used for data analysis. The mean value and standard error of the mean (SEM) were calculated. The statistical probability was determined by Paired-Samples T Test and expressed in the figures with the help of asterisks (*).

RESULTS

To investigate the modulating effects of illumination- and BGR on IS induced changes of physicochemical properties of PS in the first step of experiments the frequency-dependent (1–10 Hz) effect of 10 min 30 dB IS on HFP at room temperature (18°C), daylight illumination ($E_v = 500 - 550$ lux) and NBGR ($R = 17$–18 μRoentgen/hour) condition were studied. Findings presented in Fig. 2 show that a more pronounced effect of IS was observed at 3 Hz (which shortened the HFP by 24.7%), 5 Hz and 8 Hz (which prolonged the HFP by 18.5% and 38.4% correspondingly). It is worth noting that the frequency dependent effect of IS on

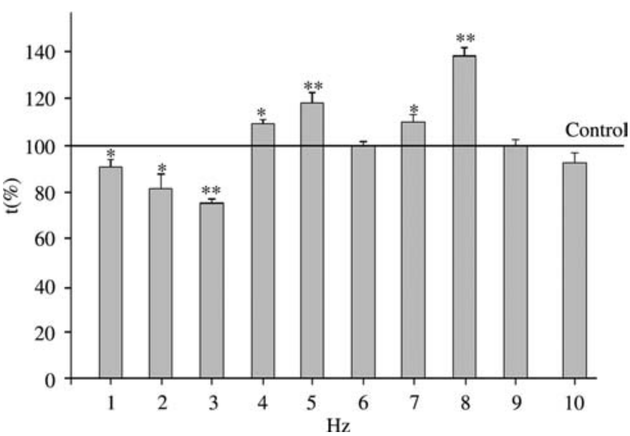

FIGURE 2 Frequency-dependent effect of IS on HFP of 10-min IS-treated PS.

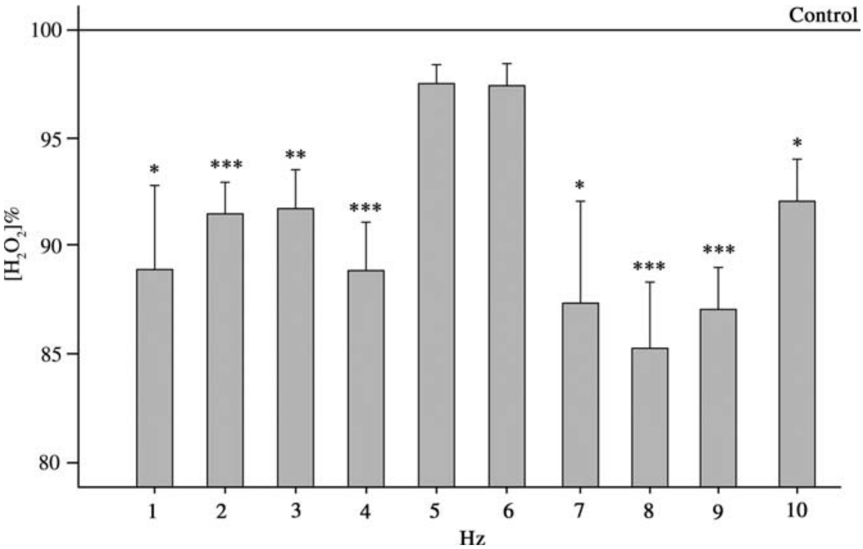

FIGURE 3 The frequency-dependent effect of IS on H_2O_2 content in PS exposed to 1–10 Hz 30 dB IS during 10 min.

HFP of PS had a nonlinear character and the maximum effect of infrasound was displayed after 8 Hz treatment.

The study of the frequency-dependent effect of IS on H_2O_2 content in PS had shown that this dependency also has a nonlinear character as in the case of HFP. As can be seen in Fig. 3, the 10-min treatment of PS samples by IS with 30 dB intensity in a range of 1–10 Hz frequencies had a depressing effect on H_2O_2 formation in PS in all cases. While the maximum effect was observed after treatment by 8 Hz frequency (by 85.28%), in the case of 5 and 6 Hz this depression was less pronounced (Fig. 3).

The above presented data had shown that the 8 Hz frequency has the maximum effect on HFP of PS and H_2O_2 formation in it. Thus, the findings allowed us to conclude that 8 Hz frequency served as an effective exposition parameter for 10-min IS treatment. Therefore, for the study of the effect of illumination- and background radiation on IS-induced changes of physicochemical properties of PS in the next series of experiments the 8 Hz was chosen as the treatment-frequency.

In the following experiments the dependency of 8 Hz-induced changes of PS's HFP, H_2O_2 and O_2 contents at different light intensity and BGR was studied.

Experiments on the modulating influence of illumination and BGR on 8 Hz IS-induced effect on HFP of IS-treated (Fig. 4B) and non treated (Fig. 4A) PS samples were performed at room temperature. As can be seen in Fig. 4 in normal room condition (when G = 17 μR, and illumination = 500–550 lux) in case of non treated samples (Fig. 4A) NBGR in Dark and LBGR in Dark prolonged HFP of PS frozen crystals by 14% and 23% correspondingly, compared to NBGR in Light (Fig. 4A). However, as a result of 8 Hz IS-treatment this effect was fully reversed (Fig. 4B), i.e., NBGR in Dark and LBGR in Dark caused the HFP of PS to be reduced.

The comparative study of illumination- and background radiation-induced effects on H_2O_2 content in PS demonstrated that these factors have different types of influence. The incubation of non treated PS samples in ***NBGR in Dark*** causes decrease of H_2O_2 content by 43% ($p < 0.005$, n = 10), while ***LBGR in Dark*** condition has an elevating effect on peroxide content by 14% ($p < 0.05$, n = 10) (Fig. 5A). It is remarkable to note that 8 Hz IS at ***NBGR in Dark*** and ***LBGR in Dark*** conditions results in a similarity of these factors-induced effects compared to ***NBGR in Light***

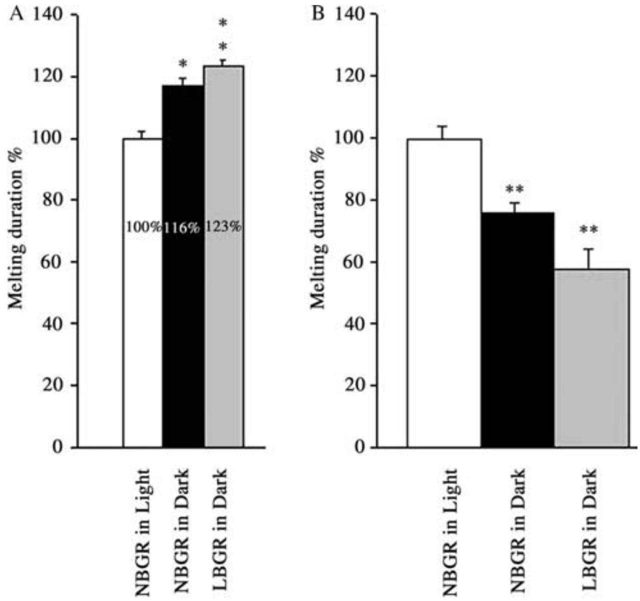

FIGURE 4 The effect of 10 min incubation in **NBGR in Light** (white), **NBGR in Dark** (black), and **LBGR in Dark** (grey) conditions on HFP of non treated and IS treated PS samples. Bars indicate the mean of 10 independent measurements, vertical bars represent the standard error of mean (SEM), *p $<$ 0.05, **p $<$ 0.01 as compared with the control (**NBGR in Light**).

one. These facts indicate that 8 Hz does not modulate the ***NBGR in Dark***-induced effect, while it can strongly reverse the ***LBGR in Dark***-induced effect, i.e., it can remove the background radiation-induced effect on H_2O_2 content in PS.

It is known that the rate of H_2O_2 generation depends on the degree of water ionization and O_2 content in the medium (Voeikov, 2006). So, to find out whether the observed variation of H_2O_2 level is due to O_2 content change in solution, in the next

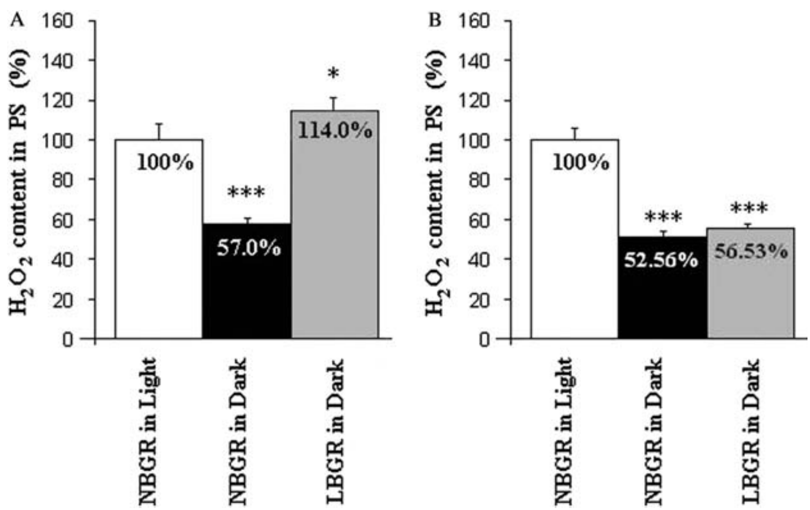

FIGURE 5 The effect of 10-min incubation in **NBGR in Light** (white), **NBGR in Dark** (black) and **LBGR in Dark** (grey) conditions on H_2O_2 content in non treated and IS-treated PS samples. Bars indicate the mean of 10 independent measurements, vertical bars represent the standard error of mean (SEM), *p $<$ 0.05, **p $<$ 0.01 as compared with control (**NBGR in Light**).

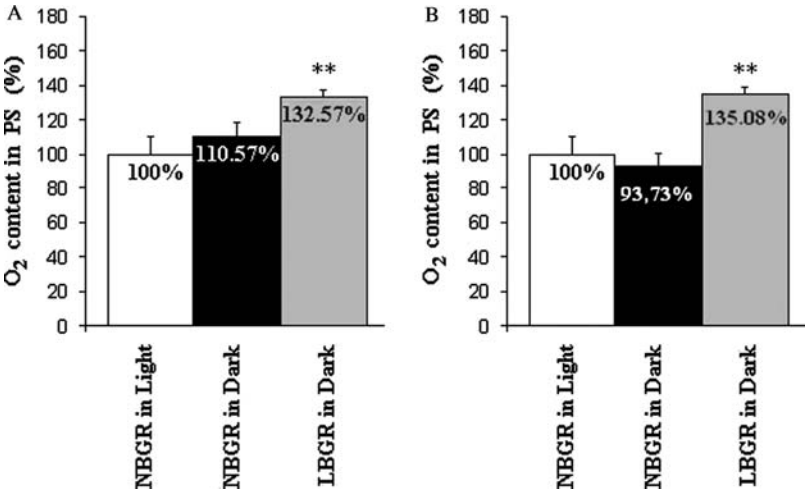

FIGURE 6 The effect of 10-min incubation in **NBGR in Light** (white), **NBGR in Dark** (black), and **LBGR in Dark** (grey) conditions on O_2 content in non treated and treated PS samples. Bars indicate the mean of 10 independent measurements, vertical bars represent the standard error of mean (SEM), *$p < 0.05$, **$p < 0.01$ as compared with control (**NBGR in Light**).

series of experiments the modulating influence of 8 Hz IS on ***NBGR in Light, NBGR in Dark***, and ***LBGR in Dark*** conditions-induced effects on oxygen content in non treated and IS-treated PS samples was studied.

The study of O_2 content of PS 10 min incubated in ***NBGR in Light***, ***NBGR in Dark***, and ***LBGR in Dark*** conditions showed that in ***NBGR in Dark*** conditions cause a slightly elevated effect in the case of non treated (by 10.57%; Fig. 6A) and a slightly depressed effect in the case of IS-treated PS samples (by 6.27%, Fig. 6B). While in the case of incubation in ***LBGR in Dark*** condition significant oxygen enrichment was observed in both IS-treated (Fig. 6B) and non treated (Fig. 6A) PS samples by 35.08% and 32.52% correspondingly compared to ***NBGR in Light*** one.

DISCUSSION

Although many properties of water are still mysterious, the fact that the extra sensitivity of hydrogen bonds makes water ionization one of the most variable water properties is considered to be well documented (Chaplin, 2011; Klassen, 1982). As the water ionization determines its physicochemical properties, it is suggested that it could serve as an extra-sensitive and universal sensor for environmental factors.

Because technical progress-induced environmental pollution brings the civilization towards ecological catastrophe and the level of BGIR has increased in a number of world locations, the problem of the study of biological effect of high background radiation becomes one of the most important global problems of modern life sciences.

Although the biological effect of NIR (such as IS, which is a progressive increasing component of environmental pollution) is well documented, our knowledge about the dependency of NIR on the BGIR-induced effects is rather limited. Modern research in biophysics has proved that water is a most essential target, through which the biological effects of ionizing and non ionizing radiations are realized.

In thermodynamics the changing of HFP of the solution is conditioned by the polarity of molecules and especially with the changing of the quantity and/or the length of the hydrogen bond (producing a reduction of the degree of water ionization in the case of shortening, and vice versa). On the other hand, it is well known that the increase of the degree of water ionization in the presence of O_2 could promote the formation of ROS (Chaplin, 2011). According to these statements a comparative study was performed on the effects of illumination and background radiation on physicochemical properties (heat fusion, which serves as a qualitative marker for polarity of aqua solution, H_2O_2 and O_2, which determine molecule ionization) of IS-treated and non treated PS samples.

The data on the frequency-dependent effect of 1–10 Hz IS on HFP (Fig. 2) and H_2O_2 content (Fig. 3) could be explained by the high sensitivity of hydrogen bonds (determining the polarity of solution) and ionization (determining the ROS formation) to infrasonic vibration. Considering the data in Figs 2 and 3, the above-mentioned sensitivity of PS to IS treatment is more pronounced at 8 Hz frequency. Therefore, in the next series of experiments, intended to estimate of the contribution of illumination and background radiation to the IS-induced effect on PS, the 8 Hz was chosen as the main frequency for infrasonic treatment.

These findings correspond with literature data according to which the 8 Hz infrasound has an obvious effect on cells (Wei et al., 2002), organisms (Ikuharu et al., 1996), enzyme activities (Dang et al., 2007), and sexual behavior (Zhuang et al., 2007).

Experiments on the effect of dark and null BGR on HFP of PS were shown that both ***NBGR in Dark*** and ***LBGR in Dark*** have an increasing effect compared to ***NBGR in Light*** one (Fig. 4A). Thus, the high sensitivity of heat fusion kinetics of PS to illumination and background radiation could be explained by the high sensitivity of saline ions hydration determining so called "clusters rigidity."

As could induce a structural change in liquid water (Shimokawa et al., 2009) and definite changes in the O—H stretching region (Yokono et al., 2009) as well as the ionizing radiation can cause of water ionization, so according to these facts it was predicted that the ionization degree of water will go down and correspondingly the HFP will be prolonged as a result of removing illumination and BGR (Fig. 4A). The fact that ***LBGR in Dark***-induced effect was stronger than ***NBGR in Dark***-induced effect can be explained by the absences of two factors simultaneously.

The treatment of PS samples by IS at ***NBGR in Light***, ***NBGR in Dark***, and ***LBGR in Dark*** conditions shows that null illumination and low background radiation have a shortening effect on HFP compared to the control one (Fig. 4B).

The reversion of ***NBGR in Dark***- and ***LBGR in Dark***-induced effects indicates that the illumination and background radiation (besides having an ionizing effect on water) can also influence the hydration degree of ions, which is more pronounced in case of IS treatment (because dipole vibration by IS).

Earlier it was shown that depending on the chemical composition of water, upon the effect of ionizing and non ionizing radiation, water ionization products could be transformed into reactive oxygen spaces (ROS, having strong modulating role for cell metabolic activity) like H_2O_2 (Beier et al., 1960). The formation of H_2O_2 and significant changes in the physical properties of water and water solutions were shown by exposure to static magnetic field (SMF) (Klassen, 1982), microwaves (Gudkova et al., 2005), and sound and ultrasounds (Domrachev et al., 1992). It was also shown that water freezing-melting, evaporation-condensation, sonication even with audible sound, and filtration through narrow capillaries lead to the generation of H_2O_2 even in ultra-pure and carefully degassed water (Domrachev et al., 1992, 1993; Hunanyan and Ayrapetyan, 2006).

Comparing the experimental data on H_2O_2 content of non treated and 8 Hz IS-treated PS samples has shown that in both cases ***NBGR in Dark*** decreased the amount of H_2O_2 in solution (Figs 5A and 5B, white and black columns) which was accompanied with no significant changes of O_2 content in it (Figs 6A and 6B, white and black columns). From the literature, it is known that the formation of H_2O_2 in water is accelerated under daylight during the first few hours which explains the low concentration of H_2O_2 in the case of ***NBGR in Dark*** treatment. According to Figs 5A, 5B, 6A, and 6B, the water processing by 8 Hz IS at ***NBGR in Dark*** condition does not change on H_2O_2 and O_2 contents in PS, while in the case of treatment of PS samples by IS at ***LBGR in Dark*** condition the H_2O_2 content was fully reversed without changing the content of O_2 in it.

CONCLUSION

Thus, based on the obtained data, it can be concluded that illumination and BGIR had a strong modulation effect on IS-induced impact on water physicochemical properties. Since the cell metabolic activity strongly depends on physicochemical properties (especially on peroxide content) of its bathing aqua medium, this effect could also have an adequate effect on living organisms.

From the other hand the variability of experimental data on the biological effects of non ionizing radiation, obtained by different laboratories can therefore be explained by various environmental conditions of experiments (especially light intensity and background radiation level), which are traditionally out of the consideration of researchers. However, our knowledge of the dependency of the modulating influence of IS on BGIR-induced biological effects on organisms is rather limited and for the final conclusion needs more detailed investigations in this field.

ACKNOWLEDGEMENTS

This work was supported by the Office of Naval Research Global, Research Grant Project No #1592P.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content of and writing of the article.

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