Ученые записки Таврического национального университета им. В. И. Вернадского
Серия «Биология, химия». Том 24 (63). 2011. № 2. С. 261-267.

УДК 577.3

EFFECT OF ELECTROMAGNETIC FIELD WITH 8 Hz FREQUENCY ON THYMOCYTES NUCLEUSES INJURY CAUSED BY NANOSTRUCTURED SILICON AND HYDROGEN PEROXIDE

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Electromagnetic fields of low frequency are known to modulate cells functioning both in physiological and pathological conditions, on what their effective application in medicine is based. Using the reliable and sensitive method of comet assay the nucleuses DNA damages were studied in the suspension of isolated rat thymocytes after three hour incubation with nanostructured silicon, hydrogen peroxide and exposure to electromagnetic fields, considering both individual and combined effects. After the comet assay analysis we confirmed the results of previous research that indicated the DNA damages in cells after combined influence of nanostructured silicon, 0.1 mM hydrogen peroxide and electromagnetic fields. It was found that the exposure of the suspension of thymocytes to electromagnetic fields with frequency of 8 Hz combined with the action of hydrogen peroxide and nanostructured silicon in the presence of light leaded to the increased number of DNA breaks in nucleuses that might be due to a protective response to peroxide damage.
Keywords: apoptosis, electromagnetic fields of low frequencies, comet assay, single- and double-strand breaks of DNA, hydrogen peroxide, nanostructured silicon.

INTRODUCTION

During the past few decades, research of the electromagnetic waves influence has developed into specialized sub-disciplines, from basic physics to tumor biology and experimental therapy. One of actual problems of modern biophysics is to evaluate the possibility of electromagnetic fields of low frequency to induce and modulate (modify) cell death [1]. Recently it was shown that the combined influence of 8 Hz frequency EMF with the action of oxidative factors such as hydrogen peroxide and nanostructured silicon in the presence of light reduces viability of thymocytes by increasing the number of apoptic cells compared to control [2]. It was supposed that energy of photoexcited silicon nanocrystals is transferred to the interacting cell's molecular system and results in tissue damage mainly through generating of reactive oxygen species (R.O.S.), which induce oxidative damage and genetic instability. The combined influence of 8 Hz frequency EMF with the action of hydrogen peroxide and photoexcited nanostructured silicon is considered to induce physical, chemical and molecular damage to tissues leading to genomic instability and can cause cell death, particularly apoptosis [3, 4]. Nevertheless received results are not absolutely reconcilable and further research are indispensable.

As a model for studying the influence of electromagnetic waves of low frequency range (8 Hz) on the processes caused by the action of silicon and hydrogen peroxide (0.1

mM) as an oxidative factors suspension of isolated thymocytes were selected because they were not fully differentiated cells, characterized by greater instability of genome than other cells, and lower activity of DNA reparation systems of single strand breaks, which facilitates the activation of apoptosis while they were exposed to various factors. The use of isolated thymocytes for analysis of apoptosis in vitro allowed morphological control of thymocytes and to identify cells with fragmented chromatin and apoptotic bodies, whereas in vivo they were quickly absorbed by phagocytes [5].

Since a reliable and sensitive method was necessary for our research the comet assay was chosen. The comet or single-cell gel electrophoresis assay is now widely used as a quick, sensitive and cheap method for measuring DNA stand breaks in eukaryotic cells for investigation of genetic damage associated with exposures to potentially genotoxic agents. The comet assay was first described by Ostling and Johansson (1984) and in its independent modification by Singh et al. (1988). One great advantage of this method is that it is possible to measure the level of single- and double-strand breaks of DNA in individual cells. The comet assay detects very reliably the level of single- and double-strand breaks of DNA at low levels of damages [6, 7, 8].

The aim of our present study was to investigate DNA damages induced by monoinfluences of chosen factors and the combined influence of 8 Hz frequency EMF with the action of oxidative factors such as hydrogen peroxide and nanostructured silicon in the presence of light compared to control.

MATERIALS AND METHODS

Thymocytes were obtained from the thymus of Wistar rats with weigh about 120-150 g which were kept in standard vivarium diet. Dedicated thymus was grinded through the filter of synthetic fibers (Ø = 0,1 mm) in buffer solution with following composition (g\1): NaCl – 6,796; KCl – 0,274; $CaCl_2$ – 0,288; $NaHCO_3$ – 2,091; KH_2PO_4 – 0,299; $MgSO_4$ – 0,144; glucose – 1,8; (pH 7.4). The number of cells was counted by light microscope in the chamber of Horyayev using dye (0,4% solution of trypan blue). If the membrane integrity has been compromised (dead cells), the cells absorb the dye and appear blue. When the cell viability was less than 80%, the cells were discarded and a new batch was started.

Incubation of thymocytes (2-4 × 106 cells / ml) was performed in a water thermostat at 37°C in a stationary medium RPMI-1640 with the addition of 2.05 mM glutamine. Incubation was been carried out for 3 hours with light.

Samples were subjected to the magnetic field treatment, which was created by the Helmholtz rings. Impulses were rectangular with different polarity. Frequency of the magnetic field was 8 Hz whereas induction of the magnetic field was 25 μT. Frequency of the magnetic field was selected on the basis of its ecological and geophysical significance [9]. Vector of induction generated by the magnetic field was parallel to vector of the geomagnetic field. Investigated samples were set down in Helmholtz rings. Control samples were in the conditions of the electromagnetic fields background commonly appropriate to the laboratory (20-65 nT). To assess the reliability of the impact of the

electromagnetic fields of low frequencies we used Student's t-test for independent samples linked in pairs.

In this experiment we used the following conditions: control cell suspension, cell suspension, which has been subjected to the effects of nanocrystalline silicon with the size of pores and crystallites of 2-5 nm activated by light, the electromagnetic wave with frequency of 8 Hz during incubation, with the addition of hydrogen peroxide to a final concentration of 0.1 mM in the incubation medium, a combination of electromagnetic interference with hydrogen peroxide one.

To estimate DNA damage comet assays was chosen because of its quickness and sensitivity. The neutral version of comet assay was carried out based on the work of Syvolob et al as follows: on the day of electrophoresis, an aliquot of 50 µl freshly prepared suspension of cells was mixed with 100 ml 1 % low- melting-agarose. 25 µl of the mixture was layered on top of an ordinary microscope slide precoated with 1 % normal-melting -agorose, which was allowed to dry at room temperature protected from dust and other particles. After low-melting-agarose was solidified in a refrigerator for 3-5 minutes, the coverslip was carefully removed and the slide was gently immersed in a freshly prepared lysing solution (2.5MNaCl, 100mM EDTA, 10mM Tris-HCl (pH=8,0), with 1 % Triton-100 added just before use). Lysis was carried out at least for 2 h [10].

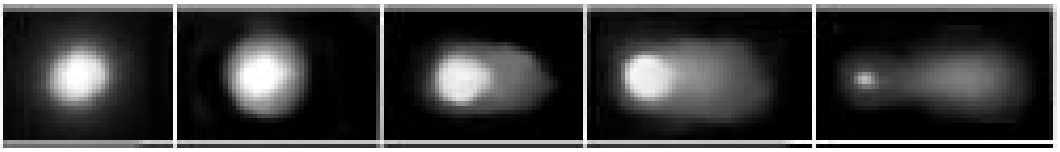
This assay is based on the separation from supercoiled DNA of DNA loops containing strand breaks (specifically DSBs) that become free to migrate out of the nucleus towards the anode during a neutral electrophoresis in a TBE buffer (0,089 M Tris, 0,089 H_3BO_3, 2 mM EDTA, pH=7,5). After lysis slides were washed away with electrophoresis' buffer and were incubated for 10 minetes. Single cell gel electrophoresis was performed for 20 min under following conditions: 4^oC, 1V/sm, 300 nA) [10].

DNA images were captured after staining with DAPI with a phluorescent microscope. For quantification, the comets were classified into different categories. We subdivided cell DNA damage into five stages (0–4) according to the length and the intensity of the comet tail as illustrated in Fig. 1. Stage 0 (no tail) and stage 1 (halo around the nucleus) corresponded to cells without a significant number of DNA strand breaks. Stages 2–4 corresponded to a gradual increase in DNA damage. We measured the comet score in 100 randomly selected cells per slide. Results were, first, expressed as the percentage of each stage of comets per slide. In a second step, a comet score was calculated with the help of the programe CometScore, following a modification of Collins' method, as the sum of the percentage of each comet stage multiplied by n (from 0 to 4) (Collins 2004). The scores were expressed in arbitrary units on a scale from 0 (all the comets are in stage 0) to 400 (all the comets are in stage 4) [11].

RESULTS AND DISCUSSION

The experiment designed to measure DNA damage was performed with rat thymocytes suspension and showed nuclear DNA damage in samples exposured to different factors and their combinations. By using scoring method of Collins (2004), the DNA damage due to different influences shows different responses with certain regularity (Table 1, Fig. 2). Sufficient discrimination about the degree of damage in DNA according to comet appearance [Type 0 (no tail) to type 4 (almost all DNA in tail)] was given by

Collins (2004) (Fig.1.). A total of 100 comets were scored from each sample and each comet assigned a value of 0 to 4 according to its type. The total score for the sample gel is given between 0 to 400 "arbitrary units". Most of the comets of unexposed samples belonged to type 0 and type 1(Table 1, Fig. 2). The frequency of type 2, 3 and 4 increased with respect to the increase of damaging impact strength to thymocytes, which further correspondingly effects the arbitrary unit, showing increase from 31.0 in control sample to 178.0 in sample exposed to the combined effect of nanoporous silicon with H_2O_2 and 8 Hz frequency EMF.



Stage 0 Stage 1 Stage 2 Stage 3 Stage 4

Fig. 1. Different stages of DNA damage measured by comet assays.

Some baseline damage is always there which may be because of unwanted exposure to so many environmental mutagens in day to day life and partially may be due to processing error. The data presented in Figure 2 and Table 1 indicate that after tree hour exposure the monoinfluence of electromagnetic field (EMF) with frequency of 8 Hz, silicon and hydrogen peroxide increase the level of DNA damages in different ways. The largest augmentation of DNA damages was observed under action of hydrogen peroxide, namely 132, driven by oxidative injuries to cells. Under the monoaction of electromagnetic field (EMF) with 8 Hz frequency insignificant increase of 37 "arbitrary units" was observed. When nanostructured silicon was activated by light, the degree of DNA damages amounts 40, which might be due to the formation of reactive oxygen species.

Table 1

Score of DNA damage measured by comet assay in thymocytes immediately after three-hour exposure to different factors and their combination. DNA damage was in neutral conditions (DSBs). Data are expressed as arbitrary units on a scale from 100 to 400; TC – total number of cells scored. The experiment was carried six times, and the average values presented in the table. Statistical analyses were made by comparison of control values and values obtained after treatments

Sample №	Factor of exposure	TC	Cells with comet	Comet type					Arbitrary unit
				0	1	2	3	4	
1	control	100	21	79	11	10	0	0	31±1,75
2	n.s.silicon	100	28	72	18	8	2	0	40±1,83
3	8 Hz	100	30	70	24	5	1	0	37±2,08
4	H_2O_2	100	76	24	42	23	7	4	132±2,58
5	n.s.silicon+8 Hz	100	37	63	26	9	1	1	51±2,25
6	n.s.silicon+H_2O_2	100	93	7	48	27	12	6	162±2,42
7	H_2O_2+8 Hz	100	88	12	50	23	10	5	146±1,58

8	n.s.silicon+H_2O_2+8 Hz	100	98	2	43	38	9	8	178±2,5
Total		800	471						

In some cases a significant enhancement of DNA damages level was observed when we combined different factors of influence. After the combined action of 0.1 mM H_2O_2 and silicon the amount of DNA damages is 162; after the combined action of EMF with 8 Hz frequency and silicon the number of DNA damages makes 51 "arbitrary units"; the combined influence of H_2O_2 and EMF augment the level of DNA damage, which is 146; the combined influence of silicon, H_2O_2 and EMF with 8 Hz frequency has the strongest impact on DNA damages, whish amounts 178 "arbitrary units". It should be mentioned that in all samples nanostructured silicon was activated by light, thus, it could efficiently transfer their energy to the molecules of O_2 adsorbed on the surface of nanocrystals. As a result, the transition of the molecule from the triplet to the singlet state was observed and nanoporous silicon could demonstrate oxidative effect [12]. Therefore, comparing the results we can conclude that the amount of DNA damages was considerably increased by influence of H_2O_2 and photoactivated silicon due to its oxidative properties and the effect was enhanced by the EMF with frequency of 8 Hz, which is known to enhance oxidative damages [13].

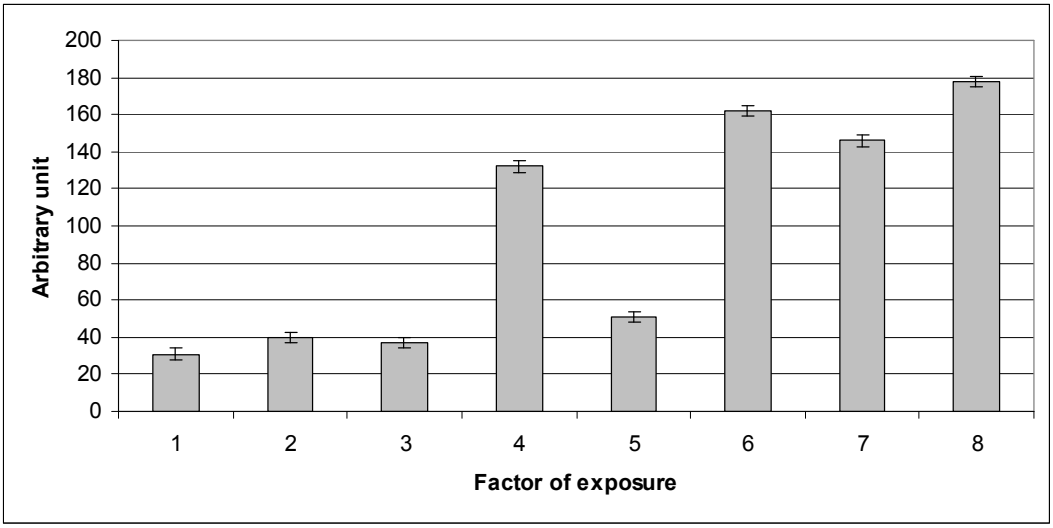


Fig. 2. DNA damage in thymocytes after three-hour exposure to different factors and their combination expressed as arbitrary units on a scale from 100 to 400. 1- control; 2 - n.s.silicon; 3 - 8 Hz; 4 - H_2O_2; 5 - n.s.silicon+8 Hz; 6 - n.s.silicon+H_2O_2; 7 - H_2O_2+8 Hz; 8 - n.s.silicon+H_2O_2+8 Hz.

Overall it should be noted that the mechanisms of biological effect of 8 Hz frequency EMF have been insufficiently researched, but despite this fact, magnetic fields have been used quite effectively in modern medical practice. In particular they have been used for

anti-inflammatory therapy, accelerating tissue regeneration and improvement of microcirculation. The generation of singlet oxygen caused by illumination of ensembles of silicon nanocrystals was proposed to be used for the suppression of reproduction of cancer cells. Presumably the suppression efficiency would be higher at the combined impact of nanostructured silicon and EMF of 8 Hz frequency. It was believed that the physical basis of this action was to coordinate the motion of charged particles [14]. The result of this interaction was primarily the change in membrane potential and activity of lipid peroxidation. In addition, the magnetic field affected the physical and chemical properties of water, free-radical chemical reactions, macromolecule of large anisotropic diamagnetic compounds [14].

CONCLUSION

The amount of DNA damages was considerably increased by influence of H_2O_2 and photoactivated silicon due to its oxidative properties and the effect was enhanced by the EMF with frequency of 8 Hz, which is known to enhance oxidative damages.

ACKNOWLEDGEMENTS

The authors are sincerely grateful to the Department of Genetics of Taras Shevchenko National University of Kyiv for providing laboratory facilities. The help rendered by Afanasieva K.S. and Zazhytska M. are thankfully acknowledged.

References

1. Barnes F.S. Biological and Medical Aspects of Electromagnetic Fields / Barnes F.S., Greenebaum B. // CRC Press. – 2007. – P. 1-256.
2. Sobko V. Effect of electromagnetic field with 8 Hz frequency on the cell injury and programmed cell death caused by nanostructured silicon and hydrogen peroxide / Sobko V., Martynyuk V., Shevchenko V., Ratushna O. // Physics of the alife. – 2010. – V. 18, No. 2. – P. 45-49.
3. Korenberg A. Radiation-induced genomic instability / Korenberg A. // Int J Radiat Biol. – 1994. – No. 65 – P. 57-64.
4. Chen T.C. Factors that influence the cutaneous synthesis and dietary sources of vitamin D / Chen T.C. Chimeh F. Holick MF. // Arch Biochem Biophis – 2007. V. 460, – P. 213-217.
5. Гринюк І.І. Зміни структурного стану хроматину в тимоцитах на ранньому етапі апоптозу за індукції пероксидом водню і радіацією / Гринюк І.І., Корнійчук Г.М., Капралов О.О. Матишевська О.П. // Укр. біохім. журн. – 2004. – Т. 76, вип. 5. – С. 90-95.
6. Stone JR. Hydrogen peroxide: a signaling messenger / Stone JR., Yang S. // Antioxidants & Redox Signaling – 2006. V. 8, – P. 243–270.
7. Coclet J. Cell population kinetics in dog and human adult thyroid / Coclet J, Foureau F, Ketelbant P, Galand P., Dumont JE. // Clinical Endocrinology – 1989. V. 31, – P. 655–665.
8. Driessens N. Hydrogen peroxide induces DNA single- and double-strand breaks in thyroid cells and is therefore a potential mutagen for this organ / Driessens N., Versteyhe S., Ghaddhab C., Burniat A. // Endocrine-Related Cancer. – 2009. – No 16. – P. 845-856.
9. Мартынюк В.С. У природы нет плохой погоды: космическая погода в нашей жизни / Мартынюк В.С., Темурьянц Н.А., Владимирский Б.М. - Киев: Издатель В.С. Мартынюк, 2008. - 179 с.

10. Афанасьєва К. Швидкість виходу ДНК при нейтральному та лужному електрофорезі ізольованих клітин / Афанасьєва К., Шувалова Т., Зажицька М., Сиволоб А. // Вісник КНУ ім. Т. Шевченка. – 2007. – вип. 50. – С. 87-89.
11. Collins A.R. The comet assay for DNA damage and repair / Collins A.R. // Mol Biotech. – 2004. – No. 26. – P. 249-261.
12. Горячев Д.Н. Елекролитический способ приготовления пористого кремния с использованием внутреннего источника тока // Горячев Д.Н., Беляков Л.В., Сресели О.М. // Физика и техника полупроводников – 2003. – Т. 37(4). – С. 494-498.
13. Exley C. Silicon in life:A bioinorganic solution to bioorganic essentiality / Exley C. // Journal of Inorganic Biochemistry. – 1998. – V. 69. – P. 139.
14. Холодов Ю.А. Способы использования магнитных полей в медицине и пути воздействия этих полей на организм / Холодов Ю.А. // Магнитология - 1991. – вып. 1. - С. 6-11.

Собко В.М. Влияние электромагнитного поля частотой 8 Гц на повреждение ядер тимоцитов, вызванного действием наноструктурированного кремния и пероксида водорода / В.М. Собко, В.С. Мартынюк, В.Б. Шевченко, Н.В. Протопопов // Ученые записки Таврического национального университета им. В.И. Вернадского. Серия «Биология, химия». – 2011. – Т. 24 (63), № 2. – С.261-267

Электромагнитные поля сверхнизкой частоты способны изменять функционирование клеток как при физиологических, так и при патологических состояниях, на чем и основано их эффективное применение в медицине. С помощью метода кометного электрофореза было исследовано повреждение ДНК ядер в суспензии изолированных тимоцитов крыс после инкубации с наноструктурированных кремнием, H_2O_2 и при наличии электромагнитного поля, как при раздельном, так и комбинированном воздействии выбранных факторов. После анализа кометного электрофореза, были подтверждены результаты предыдущих исследований, которые указывали на повреждение ядра при действии кремния, H_2O_2 и электромагнитного поля. Показано, что воздействие поля частотой 8 Гц в сочетании с действием кремния и H_2O_2 на суспензию тимоцитов приводит к увеличению общего количества разрывов ДНК в ядре, что может быть обусловлено защитным ответом на пероксидное повреждение.
Ключевые слова: апоптоз, низкочастотное электромагнитное излучение, кометный электрофорез, разрывы ДНК, пероксид водорода, наноструктурированных кремний.

Собко В.М. Вплив електромагнітного поля частотою 8 Гц на ушкодження ядер тимоцитів, спричиненого дією наноструктурованого кремнію та пероксиду водню / В.М. Собко, В.С. Мартинюк, В.Б. Шевченко, М.В. Протопопов // Вчені записки Таврійського національного університету ім.В.І. Вернадського. Серія „Біологія, хімія”. – 2011. – Т. 24 (63), № 2. – С. 261-267.

Електромагнітні поля наднизької частоти здатні змінювати функціонування клітин як за фізіологічних, так і за паталогічних станів, на чому і засноване їхнє ефективне застосування в медицині. За допомогою методу кометного електрофорезу було досліджено пожкодження ДНК ядер у суспензії ізольованих тимоцитів щурів після інкубації з наноструктурованим кремнієм, пероксидом водню та при наявності електромагнітного поля, як при окремому, так і комбінованому впливі обраних факторів. Після аналізу кометного електрофорезу, було підтверджено результати попередніх досліджень, які вказували на пошкодження ядра при дії кремнію, пероксиду водню та при впливі електромагнітного поля. Показано, що вплив електромагнітного поля частотою 8 Гц у комбінації з дією кремнію та пероксиду водню на суспензію тимоцитів призводить до збільшення загальної кількості розривів ДНК у ядрі, що може бути зумовлено захисною відповіддю на перекисне пошкодження.
Ключові слова: апоптоз, низькочастотне електромагнітне випромінювання, кометний електрофорез, розриви ДНК, пероксид водню, наноструктурований кремній.

Поступила в редакцию 10.02.2011 г.