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Action of extremely low frequency electromagnetic fields on the expression of heme oxygenase 2 in the retina of European roe deer (*Capreolus capreolus L.*)

Abstract: *In this paper, we present the results of studies on the effect of an extremely low frequency electromagnetic field on the expression of heme oxygenase 2 in the retina of the European roe deer (Capreolus capreolus). Retinal tissue slides were exposed to an electromagnetic field of 50 Hz for 15 or 30 minutes. The analysis showed that the extremely low frequency electromagnetic field did not affect the viability of retinal cells. A significant decrease in the activity of heme oxygenase 2 in the tissues after the field exposure was also demonstrated. No statistically significant differences in the expression of PARP protein were observed, which indicates the lack of apoptotic changes in the cells.*

Streszczenie*: W niniejszej pracy zostały przedstawione wyniki badań oddziaływania pola elektromagnetycznego ekstremalnie niskiej częstotliwości na ekspresję oksygenazy hemowej 2 w siatkówce sarny europejskiej (Capreolus capreolus). Skrawki tkanki siatkówki zostały poddane oddziaływaniu pola elektromagnetycznego o częstotliwości 50 Hz przez 15 lub 30 minut. Przeprowadzona analiza wykazała iż pole elektromagnetyczne o ekstremalnie niskiej częstotliwości nie wpływa na żywotność komórek siatkówki. Wykazano również, znaczny spadek aktywności oksygenazy hemowej 2 w tkance siatkówki sarny po oddziaływaniu pola. Nie zaobserwowano statystycznie istotnych różnic w ekspresji białka PARP, co wskazuje na brak zmian apoptotycznych w komórkach. (**Pola elektromagnetyczne o ekstremalnie niskiej częstotliwości wpływa na ekspresję oksygenazy hemowej 2 w siatkówce sarny europejskiej (Capreolus capreolus L.)*)

Keywords: electromagnetic field of extremely low frequencies; heme oxygenase 2; European roe-deer.
Słowa kluczowe: pole elektromagnetyczne ekstremalnie niskiej częstotliwościach; oksygenaza hemowa 2; sarna europejska.

Introduction

The electromagnetic field is an environmental factor that influences organisms. In the electromagnetic field spectrum, frequencies ranging from 0 Hz to 300 GHz can be distinguished (Kasprzyk i Butlewski, 2013). Higher-range UV, X-ray and gamma radiation are capable of ionizing particles, which can result in DNA damage. Electromagnetic waves in the extremely low range, and, among others visible light and infrared, do not produce enough energy to cause changes in DNA and are an example of non-ionizing radiation (Hamedani i in., 2022). The most common electromagnetic field in the environment is extremely low frequency, which is emitted by mains-powered devices, as well as by transmission devices and the power infrastructure. It is assumed that the energy generated by extremely low electromagnetic fields is not sufficient to have a genotoxic effect, because it is not able to directly damage DNA. However, its action can generate oxygen radicals and destabilize the action of antioxidant mechanisms. However, non-ionizing electromagnetic fields cause biological effects even at very low intensities. (Henrykowska i in., 2014). Therefore, it can be used and utilized in the treatment of many diseases. One example is the use of extremely low electromagnetic fields in the treatment of hepatocellular carcinoma, where therapy using extremely low frequency electromagnetic fields resulted in disease stabilization and in some patients triggered a partial positive response (Baharara i in., 2016).

The response to light is common to most life forms, but only animals with eyeballs are capable of spatial vision. (Land, 2005). This happens by stimulating the visual receptors, which causes depolarization of the optic nerve by breaking down rhodopsin and shaping the image in the central nervous system.. (Youssef i in., 2011). The structure of the retina is similar in all vertebrates. It is a thin, semi-transparent layer of nervous tissue (Ansari i Nadeem, 2016) lining the eyeball. (Purnyn, 2013). The visual and iris parts of the retina are distinguished. In vertebrates, the photosensitive receptors are directed to the pigment epithelium (inverted retina), and light reaches them after penetrating the dioptric centers and the entire retina (Purnyn, 2013). Photoreceptors (cones, responsible for daytime vision, and rods, which are not involved in color vision) are responsible for phototransducing the light signal into an electrical impulse (Willoughby i in., 2010).

The retina is responsible for the generation and transmission of visual signals, which requires a fast metabolism. Mitochondria responsible for cellular respiration therefore produce large amounts of reactive oxygen species (ROS) (Hsueh i in., 2022). Furthermore, this tissue is constantly exposed to the light radiation, which also leads to increased ROS production. In conditions that do not allow the full use of the potential to counteract oxidative stress (OS), many pathological physiological reactions occur in the body, such as tissue inflammation. (Jadeja i Martin, 2021).

European roe deers are small mammals, weighing between 28 and 32 kg. (Koziorowska i in., 2024). The area inhabited by European roe deer is often in close proximity to human habitation – small towns and housing estates. They also rarely move longer distances, as this can be risky and cost a lot of energy (Lovari i in., 2017). The European roe deer is a popular and widespread species throughout most of Europe and adapts very well to its environment. The electromagnetic field is one of the factors that are constantly present in the lives of many organisms, including roe deer. Roe deer habitats are often located near high-voltage lines or power infrastructure, which can emit electromagnetic fields at extremely low frequencies. Our previous in vitro studies have shown that exposure of living organisms to such a field can affect their physiological processes, including the reproductive system and the regulation of the biological clock.

Heme oxygenases are cytoprotective enzymes. They are found in the endoplasmic reticulum and their main role is to break down heme into iron, carbon monoxide and bilirubin. (Intagliata i in., 2019). There are three isoforms of these

proteins, inducible heme oxygenase 1 (HO-1), heme oxygenase-2 (HO-2) – the constitutive form (Kikuchi et al., 2005), and heme oxygenase-3 (HO-3). The latter is considered a processed HO-2 transcript, and therefore a pseudogene. Heme oxygenase (HO) is an enzyme which important role in the body is to break down heme, especially in the eye area, where the sunlight, which is the catalyst for this process is most readily available. The breakdown of heme into carbon monoxide (CO), iron, and biliverdin is of great importance in maintaining the internal stability of the body, because heme in moderate amounts is essential for many biological functions. The resulting heme degradation products can initiate oxidative processes in erythrocytes, however, at low concentrations they exhibit cytoprotective properties. Heme degradation products have anti-inflammatory, antioxidant and antiapoptotic properties and are important biologically active compounds. Heme oxygenase 2 is found in high levels in the brain and testes. It plays a neuroprotective role and influences male reproduction. The presence of this enzyme in high levels in the brain suggests a role in oxidative stress defense and CO-mediated vasodilation in the brain (Intagliata i in., 2019).

Research objective

The aim of the research presented in this paper was to investigate the effect of an extremely low frequency electromagnetic field of 50 Hz exposition on the activity of heme oxygenase 2 in the retina of the European roe deer (*Capreolus capreolus L*.).

Materials and methods

Retinal tissues were isolated from the eyes of roe deer collected during selective hunts in the vicinity of Kolbuszowa (Poland), during the long light day (July), and then exposed to an electromagnetic field with an extremely low frequency of 50 Hz for 15 and 30 minutes. The viability of cells after exposure to an electromagnetic field was examined and compared to the control group cells (maintained in the same conditions, without exposure to an electromagnetic field). The next step was to isolate total protein from the tested tissues and from control tissues, which were denatured to take a linear form. Proteins were examined using the Western Blot technique. After visualization of the results, based on the images of the membrane, it was checked whether there were any differences between the activity of heme oxygenase 2 in the studied groups and the control group. In addition, the presence of PARP protein and changes in the expression of this protein after exposure to an electromagnetic field were also examined. PARP protein is involved in the repair of double-stranded basic break. The last stage of the study was the LDH test on homogenates isolated from retinal tissues.

Conducting tissue culture and the influence of the electromagnetic field on tissues

Making our study we used a new, innovative µPulse10 generator placed inside the incubator as a source of electromagnetic field. Thanks to this, the tissues during the culture were provided with constant and controlled environmental conditions (temperature, humidity, atmospheric composition) and controlled electromagnetic field parameters - magnetic induction and current amplitude value.

The generator is controlled by an external control panel or a computer with dedicated software, which allows to modify the parameters of the generated field. These include the waveform (rectangular, triangular), operating mode (continuous or pulsed), amplitude of the generated wave, wave frequency (from 2 to 20,000 Hz). The cells or tissues tested on plates were placed inside the generator on a metal plate. During the tests, the temperature, atmospheric composition, and magnetic induction value were controlled. This ensured constant parameters and certainty of the data provided.

Fig. 1. Photo of the innovative µPulse10 generator

Applied research methods

Viability assay was performed using Trypan Blue solution. The samples were transferred to glass slides and cell viability was counted in triplicate for each sample and the results were statistically analyzed. Western Blot method was used to assess protein expression. Protein content was measured using Pierce™ BCA Protein Assay Kit, compared to a standard curve. Based on the results, the amount of sample was determined in Western Blot method.

The Western blot method is graphically presented in Figure 2.

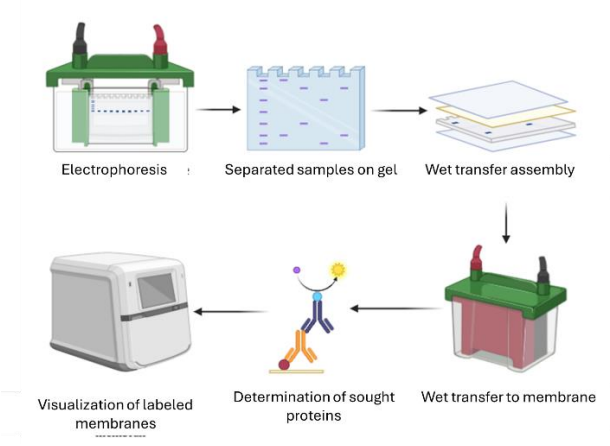

Fig. 2. Graphical representation of the Western Blot method. Created with www.biorender.com.

Statistical analysis of results

GraphPad Prism 9.0.0 (GraphPad Software Inc., San Diego, California, USA; www.graphpad.com) was used for statistical analysis of both viability and LDH assays. After checking the normality of the results, one-way ANOVA was

used for viability and LDH assays. Tukey's test was used to compare differences between groups.

Densitometric analysis was performed using ImageJ (version 1.54i March 3, 2024; U.S. National Institutes of Health, Bethesda, Maryland, USA). Then, the results of HO-2 protein and PAPR protein were equated to β-actin. In this case, two-way ANOVA and Tukey's test were used as a parametric statistical technique.

The results of the research

The results of the obtained tests were presented in the form of graphs comparing the test groups and the control and the test groups with each other. The graph showing the viability of cells stained with Trypan Blue solution (Figure 3.), tested using an automatic cell counter, indicates statistically insignificant differences in both groups compared to the control. The studied groups also do not show any significant differences when compared with each other.

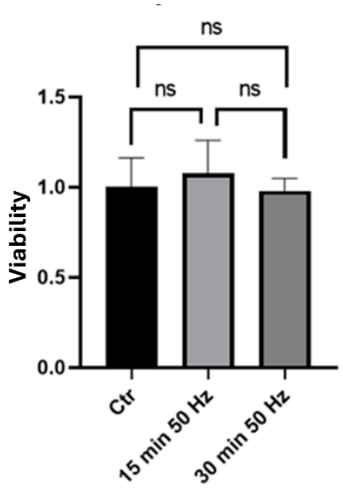

Fig. 3. Changes in the viability of retinal cells exposed to an electromagnetic field of 50 Hz for 15 and 30 minutes compared to the control group.

After performing the LDH test (Figure 4.), no statistically significant differences were found between the study groups or in comparison with the control group. A slight decrease was observed after 15 minutes of exposure to the stress factor, which was the electromagnetic field, as well as another decrease after 30 minutes of exposure, but they were not statistically significant.

Densitometric analysis showed significant statistical differences between the control group and both study groups for heme oxygenase-2 (Figure 5.). The results showed a significant decrease in protein expression in the groups exposed to the electromagnetic field. There were no statistically significant differences between the study groups. A slight decrease in expression can be seen between the group treated for 30 minutes compared to the group treated for 15 minutes.

The PARP protein was tested to determine whether a stress factor (in this case an extremely low-frequency electromagnetic field) had an effect on the increased occurrence of programmed cell death – apoptosis – in retinal cells. The analysis was also carried out to check whether changes in HO-2 protein expression would affect the concentration of the PARP form in the homogenates studied (Figure 5.). However, this protein does not show significant changes in expression between the control group and the groups after exposure to an electromagnetic field with a frequency of 50 Hz. In the group treated for a longer time (30 min.), a slight increase can be observed compared to the group exposed for 15 minutes. However, this change is not statistically significant.

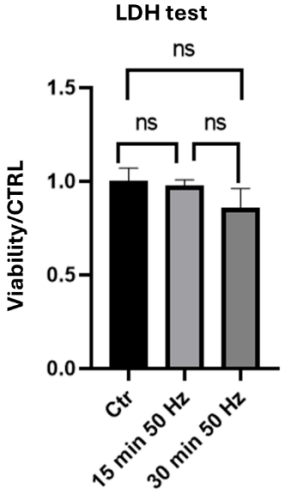

Fig 4. Results of the LDH test performed on protein homogenates of retinal cells after exposure to an electromagnetic field for 15 and 30 minutes.

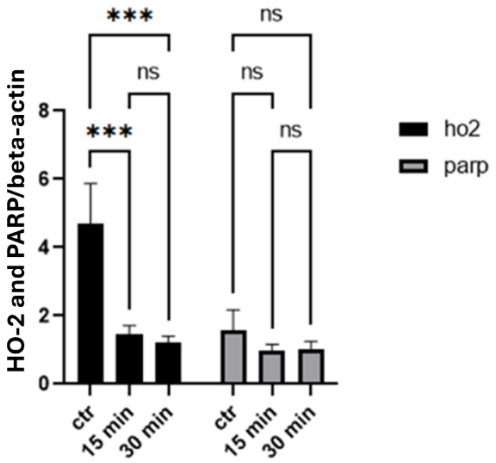

Fig. 5. Results of Western Blot analysis of heme oxygenase 2 proteins and PARP protein following exposure to electromagnetic field on roe deer retinal tissues.

Conclusions

The influence of extremely low-frequency electromagnetic fields on organisms has been studied by many authors for years (Wdowiak and Mazurek, 2016). The impact on humans, animals and the natural environment is studied (Koziorowska et al., 2020). Roe deer lead a relatively sedentary lifestyle and can be an ideal research model for analyzing the effects of extremely low-frequency electromagnetic radiation on living organisms. Extremely low frequency electromagnetic field is not a factor influencing the viability of retinal cells. Moreover, no statistically significant differences in PARP protein expression were observed, which indicates the lack of apoptotic changes in cells. However, the stress factor caused a decrease in HO-2 protein activity after both 15 and 30 minutes of exposure.

The research presented in this paper indicating a reduction in the expression of heme oxygenase-2 after exposure of tissue to an extremely low-frequency electromagnetic field may be used in the future in therapies aimed at counteracting diseases of the visual sense or the central nervous system caused by oxidative stress. HO-2 is

considered a regulator of heme oxygenase-1 – one of the main antioxidant proteins. Reduction of HO-2 activity leads to overexpression of HO-1 and, consequently, to increased cellular response to oxidative stress Since the basis of many vision-related diseases, such as glaucoma or diabetic retinopathy, is the disruption of homeostasis between ROS and antioxidants, it is possible in the future to use EL-EMF and overexpression of HO-1 in combination with other mechanisms of cell defense against ROS for the treatment of these diseases.

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