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Extremely low frequency electromagnetic field affects the expression of heme oxygenase 1 in the retina of European roe deer (*Capreolus capreolus L*.)

Abstract: *This paper presents the results of studies on the effect of extremely low frequency electromagnetic field on the expression of heme oxygenase 1 in the retinal tissues of European roe deer (Capreolus capreolus L.). The aim of this paper was to investigate the effect of 50 Hz electromagnetic field on the retina of the roe deer. Heme oxygenase (HO-1) plays a key role in the response to oxidative stress. Additionally, the expression of DNA repair protein, XRCC1, was analyzed. Retinal tissues were exposed to 50 Hz extremely low electromagnetic field for 15 or 30 minutes, and the control group consisted of tissues not treated with the field. Western Blot ana Extremely low frequency electromagnetic field affects the expression of heme oxygenase 1 in the retina of European roe deer (Capreolus capreolus L.)lysis showed a significant increase in HO-1 expression in both EMF-treated groups compared to the control, while no significant differences were observed in the expression of XRCC1 protein.*

Streszczenie: *W niniejszej pracy zostały przedstawione wyniki badań oddziaływania pola elektromagnetycznego ekstremalnie niskiej częstotliwości na ekspresję oksygenazy hemowej 1 w tkankach siatkówki sarny europejskiej (Capreolus capreolus). Celem niniejszej pracy było zbadanie wpływu pola elektromagnetycznego o częstotliwości 50 Hz na siatkówkę oka sarny.. Szczególną uwagę poświęcono oksygenazie hemowej (HO-1), która odgrywa kluczową rolę w odpowiedzi na stres oksydacyjny. Dodatkowo przeanalizowano ekspresję białka naprawczego DNA, XRCC1. Tkanki siatkówki poddano 15- lub 30-minutowej ekspozycji na pola elektromagnetyczne o ekstremalnie niskiej częstotliwości 50 Hz, a grupę kontrolną stanowiły tkanki nietraktowane polem. Analiza Western Blot wykazała istotny wzrost ekspresji HO-1 w obu grupach traktowanych EMF w stosunku do kontroli, natomiast w przypadku ekspresji białka XRCC1 nie zaobserwowano istotnych różnic. (**Pole elektromagnetyczne o ekstremalnie niskiej częstotliwości wpływa na ekspresję oksygenazy hemowej 1 w siatkówce sarny europejskiej (Capreolus capreolus L.))***

Keywords: electromagnetic field of extremely low frequencies; heme oxygenase 1; European roe-deer.
Słowa kluczowe: pole elektromagnetyczne ekstremalnie niskiej częstotliwościach; oksygenaza hemowa 1; sarna europejska.

Introduction

The process of vision is a set of interactions between the environment, the eyes and the brain, enabling the perception of stimuli caused by electromagnetic radiation in a specific range of wavelengths. Vision includes recognizing shapes, distinguishing colors, light and dark, and assessing the direction of the light signal and the distance of the observed object from the eye. (Sánchez López de Nava et al., 2024). The eyes of roe deer – the animal model used in the research presented in this paper – are set laterally, which is why these animals do not have the ability of stereoscopic vision. In roe deer, compensation for the inability to see stationary objects and blurred vision is a very wide field of vision and the ability to notice moving objects from great distances. This is particularly important in situations of danger, e.g. a predator attack, and allows for a quick attempt to escape (Pielowski, 1988). Ultraviolet (UV) radiation can cause damage to the visual apparatus, as evidenced by numerous scientific studies. The degree of this damage depends on, among other things, the length of the electromagnetic wave, the time of exposure, the intensity of the beam, and the height of the sun (long or short daylight). (Sliney, 2001). Due to its function, the retina is characterized by high oxygen consumption, which is why it is exposed to reactive oxygen species (ROS) of endogenous origin. (Saccà et al., 2013). ROS populations can also be generated by external factors.

Heme oxygenase (HO) is a microsomal enzyme that breaks down heme into biliverdin with the release of carbon monoxide (CO) and ferrous ions. (Fe2+) (Fig. 1) (Cukiernik et al., 2003). Heme oxygenase 1 belongs to the group of heat shock proteins and is an isoform of HO induced under stress conditions. Heme oxygenases are expressed in gonads, brain, liver, spleen, kidneys and heart, among others, with the spleen being the only organ where HO-1 is the dominant form under physiological conditions (Wagener., 2003). HO-1 activity in the retina is significantly higher at noon than at midnight, which is why it is believed that heme oxygenase plays a protective role in the retina (Zhao i in., 2012). HO-1 is strongly induced in response to oxidative stress factors, which include heat shock, UV radiation, heavy metals, low glutathione levels, bacterial lipopolysaccharides (LPS), nitric oxide (NO), carbon monoxide, and high concentrations of heme. (Bełtowski et al., 2004; Zhao et al., 2012).

Heme degradation products have cytoprotective effects and are also involved in many physiological and pathological processes. (Bełtowski et al., 2004; Gozzelino et al., 2010).

DNA repair protein XRCC1 (X-ray repair cross-complementing protein 1) is a so-called scaffold protein and interacts with many enzymes involved in the repair of single-strand DNA breaks (SSBR). The lack of XRCC1 protein results in the loss of key elements of the DNA repair mechanism, which leads to the accumulation of permanent damage. The weakening of repair mechanisms may be the cause of, among others, the induction of the aging process, carcinogenesis or activation of programmed cell death pathways, e.g. apoptosis. (Clementi et al., 2020; London, 2020).

Electromagnetic fields are used in the treatment of many diseases, for example electroceutical therapy in the treatment of urinary incontinence. Using fields with a dominant electric or magnetic component, measurable effects can be achieved. (Mróz et al. 2020).The electromagnetic field (solar radiation) activates heme oxygenase 1, which results in increased production of carbon monoxide, which is a strong vasodilator. This increases blood flow to the brain structures, significantly increasing the

possibilities of synthesis of hormones involved in reproductive processes - GnRH, gonadotropins (FSH, LH, PRL), and testicular steroids. The effect of this is increased libido, increased spermatogenesis activity and thus fertilization of females. At the same time, females go into heat, which allows fertilization.

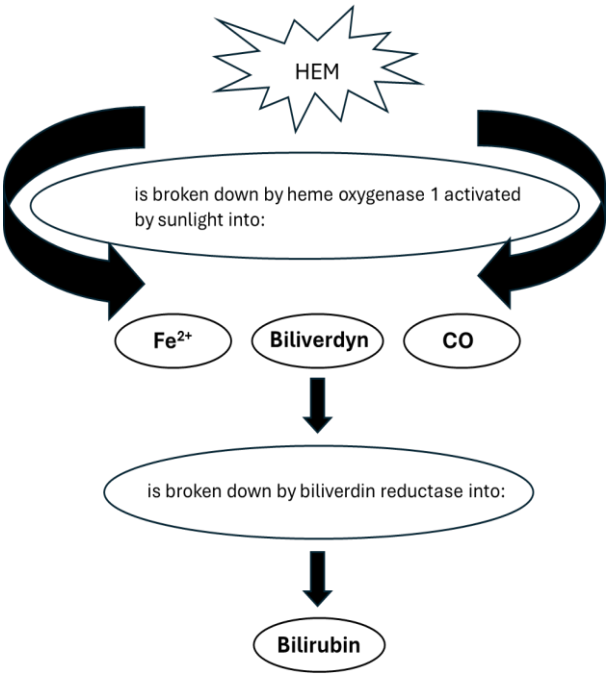

Fig. 1. Scheme of heme degradation by heme oxygenase 1.

Purpose of research

The aim of the research presented in this paper is to determine the effect of a 50 Hz electromagnetic field on the expression of heme oxygenase 1 (HO-1) in the retinal tissues of the European roe deer. This research is intended to enable a better understanding of the process of regulation of heme oxygenase activity in the context of exposure to an extremely low-frequency electromagnetic field.

Materials and methods

Fragments of the retinal tissue of the roe deer eye were exposed to an electromagnetic field with a frequency of 50 Hz and a magnetic induction value of 1.2 mT for a period of 15 or 30 minutes. Cell viability analysis was performed from the tested homogenates by staining with trypan blue and performing the LDH test. Additionally, in order to determine the occurrence of possible relationships between exposure to electromagnetic fields and HO-1 expression and the induction of DNA damage, an analysis of the expression of the DNA repair protein XRCC1 was performed. The Western Blot technique was used to evaluate the expression of HO-1 and XRCC1 proteins. Appropriate statistical tests were performed to demonstrate the presence of statistically significant differences in cell viability and expression of the studied proteins between the study groups and the control group.

µPulse 10 generator and field interaction

The research used the µPulse 10 electromagnetic field generator, which is placed inside the incubator. The circular generator applicator is equipped with a cooling and temperature control system, and at its base there is an aluminum block that allows for stable positioning of the culture plate and even heat transfer across the plate. A heating and cooling circulator is used to maintain the optimal temperature of the applicator coil.

The prepared retinal tissue slices in the culture plate were placed inside the electromagnetic field generator on the aluminum block in the central part of the applicator. From the control panel, the following parameters were selected: continuous emission; frequency 50 Hz; sinusoidal shape; magnetic induction value 1.2 mT; amplitude 20%. The study group included tissues incubated in an electromagnetic field for 15 or 30 minutes in an atmosphere of 5% CO_2, 595% O_2 and a temperature of 37±0.1°C. For each study set, corresponding control sets were prepared, which were incubated for the same period of time, under identical conditions of temperature and gas content, but without the exposure to the electromagnetic field.

Western Blot Method

The Western Blot (WB) method was used for specific detection of proteins. The main steps of this technique include: sample preparation, electrophoretic separation of proteins, electrotransfer of proteins to the membrane (Fig. 1), blocking of nonspecific antibody binding sites, antibody attachment and protein detection. Electrophoresis was carried out for about 10-20 minutes at a voltage of 80 V until the samples entered the separating gel, then at a voltage of 120 V until the sample front reached a height of about 1 cm above the lower boundary of the gel (about 40-60 minutes). Electrotransfer was carried out under cooling conditions at a voltage of 100 V for 90 minutes. After the electrophoresis was completed, wet transfer of the separated proteins to a PVDF membrane was carried out.

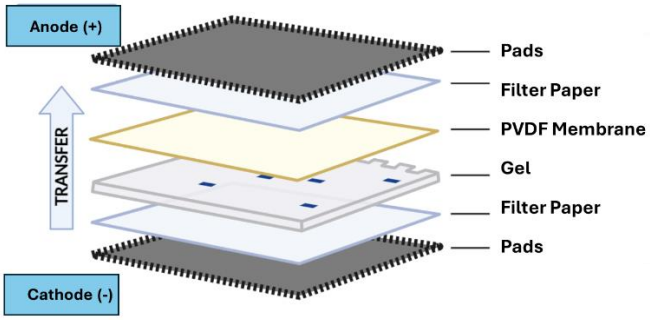

Fig. 2. Distribution of the sandwich elements for protein transfer onto the PVDF membrane. The transfer direction is marked with an arrow. Created in BioRender.com

Densitometric analysis of the samples was performed using ImageJ software (U.S. National Institutes of Health, Bethesda, Maryland, USA). Based on the images of the membranes, the intensity of the bands obtained as a result of the reaction with antibodies was measured. The obtained chemiluminescent signals for HO-1 and XRCC1 proteins were normalized to β-actin.

Statistical analysis of results

Statistical analysis was performed using GraphPad Prism 9.0.0 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com). All data were tested for probability distribution and homogeneity of variance, and appropriate statistical tests were selected for each set of results.

The results of the research

The first studies determined the effect of a 50 Hz electromagnetic field on retinal cell viability. The results of the retinal cell viability assay with trypan blue indicate no

statistically significant differences (P>0.05) between the experimental groups. Exposure to the field for 15 minutes caused a slight increase in the population of viable cells, but after exposure for 30 minutes there was a further decrease in viability.

Further studies have determined the effect of electromagnetic field on the expression of HO-1 and XRCC1 proteins in the roe deer retina (Fig. 4 and Fig. 5). In the case of heme oxygenase 1, there are statistically significant (P<0.05) differences between the control group and both groups treated with an electromagnetic field at a frequency of 50 Hz. HO-1 protein expression increased significantly in both the 15-minute and 30-minute treatment groups compared to the control group. No statistically significant differences were observed between the 15- and 30-minute treatment groups, while HO-1 expression was slightly higher in the shorter-term electromagnetic field group.

Analysis of XRCC1 protein expression (Figure 3) showed no statistically significant differences (P>0.05) between the control and study groups. Only a slight increase in XRCC1 activity was observed in both groups treated with 50 Hz EMF compared to the control. This increase was higher in the group treated with 15 min EMF. No statistically significant differences were observed in XRCC1 expression depending on the time of tissue treatment with electromagnetic field.

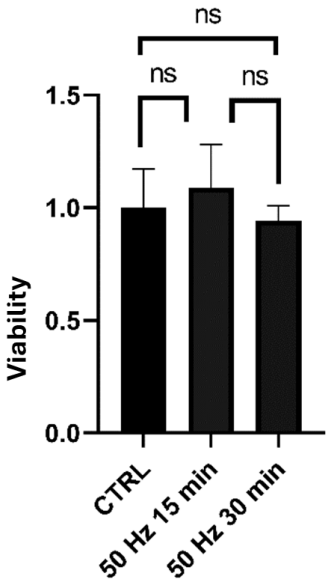

Fig. 3. Changes in retinal cell viability (normalized to control - control value was assumed as 1) for each experimental group. Bars on the graph represent mean values ± SD. "ns" - statistically insignificant relationship.

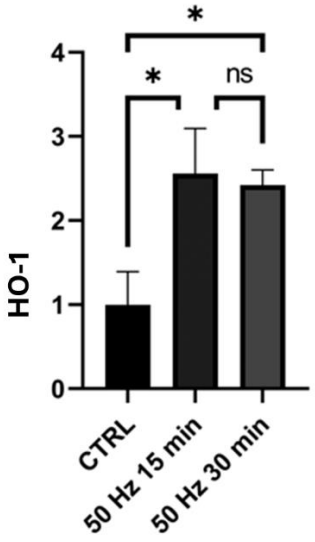

Fig. 4. Results of Western Blot analysis of heme oxygenase 1 proteins after exposure to electromagnetic field in roe deer retinal tissues. Bars in graphs represent mean values ± SEM, normalized to the reference protein β-actin (control value was taken as 1). "*" – P < 0.05; "ns" – statistically insignificant relationship.

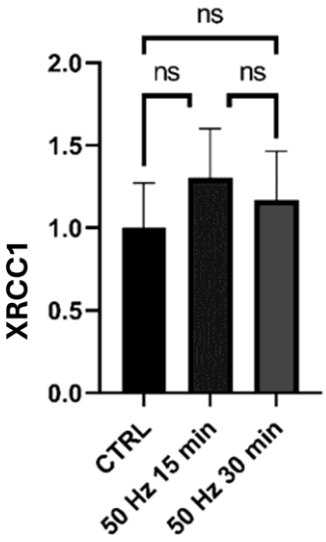

Fig. 5. Results of Western Blot analysis of XRCC1 proteins after exposure to electromagnetic field on roe deer retinal tissues. Bars in graphs represent mean values ± SEM, normalized to the reference protein β-actin (control value was taken as 1). "*" – P < 0.05; "ns" – statistically insignificant relationship.

Conclusions

Changes in the expression of heme oxygenase 1 may be the result of the approaching reproductive season. The effect of oxygenase action is the production of carbon monoxide, which causes vasodilation, increased blood flow in the brain area, and thus increased activity of the hypothalamic-pituitary-testicular axis, the effect of which will be an increase in the level of GnRH hormones, gonadotropins and steroids.

Solar radiation is the electromagnetic radiation. If the activity of solar radiation increases, the amount of carbon monoxide produced increases by increasing the activity of oxygenase 1. If the activity of oxygenase 1 increases after the impact of the electromagnetic field, we can assume by analogy that this is a similar situation as before the reproductive season. There are no studies in the available literature that would explain this phenomenon and it requires further research.

Due to the rapid technological development, many scientific studies have focused on the potential health effects of extremely low frequency electromagnetic field exposure in recent years. However, the mechanisms of response of living organisms at the cellular and molecular level to this impact remain poorly understood. Maintaining redox homeostasis is crucial for preventing damage caused by reactive oxygen species, and the enzyme heme oxygenase 1 plays an important role in this process. HO-1 is induced under conditions of oxidative stress and catalyzes the degradation of heme to biliverdin, carbon monoxide, and free iron, which play an anti-inflammatory and antioxidant role. The retina is a structure particularly susceptible to oxidative stress, due to its very high metabolic rate and constant exposure to UV radiation, hence studies of the impact of the electromagnetic field on this tissue can provide valuable information in the context of potential mechanisms of damage and protection

of nerve cells. The study used the European roe deer model, which we have used previously (Koziorowska et al., 2024), because roe deer lead a sedentary lifestyle and the impact of the electromagnetic field occurring in its habitat occurs for a long time and may affect the health and physiology of these animals.

The study may have important implications for better understanding the mechanisms of protection against oxidative stress in the retina. This stress is a significant factor in the pathogenesis of eye diseases such as diabetic retinopathy and age-related macular degeneration (AMD). In this study, we have shown that a 50 Hz electromagnetic field can stimulate the expression of HO-1, an antioxidant enzyme. Modulation of HO-1 expression can potentially be used as a new therapeutic approach, and further research in this direction may lead to the development of feasible strategies to prevent oxidative damage to the retina.

The obtained results also constitute a basis for conducting further studies on long-term exposure of the visual organ to extremely low-frequency electromagnetic fields. Despite the demonstrated lack of influence of short-term exposure of the 50 Hz field on the activation of DNA repair mechanisms, long-term exposure of the field could presumably cause a genotoxic effect. The obtained results also constitute a basis for conducting further studies on long-term exposure of the visual organ to extremely low-frequency electromagnetic fields. Despite the demonstrated lack of influence of short-term exposure of the 50 Hz field on the activation of DNA repair mechanisms, long-term exposure of the field could presumably cause a genotoxic effect.

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