Bundesamt
für Strahlenschutz

Anlagen

zum Ressortforschungsbericht zum Strahlenschutz

Workshop über die Wirkung elektrischer, magnetischer und elektromagnetischer Felder auf oxidativen Stress

Vorhaben 3621EMF104

Valentum Kommunikation GmbH

Bundesamt
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International workshop

Impact of electric, magnetic and electromagnetic fields on oxidative stress – 16th to 18th February 2022 in Cottbus and online

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PROGRAMME

(All times refer to CET.)

Wednesday, February 16th

From 11.00 am	*Arrival, registration, welcome coffee & snacks*
12.00 pm	Official welcome – Gunde Ziegelberger, Federal Office for Radiation Protection
	Session 1: Basics of Oxidative Stress – Knowns and Unknowns Chair: Bernd Henschenmacher
12.15 pm	Pietro Ghezzi | Oxidative damage in toxicology and disease and the problem of biomarkers
12.45 pm	Margaret Ahmad | The role of cryptochromes in fauna and flora: light sensing, magnetic field sensing, and involvement in oxidative stress
1.15 pm	Henrik Mouritsen | The radical-pair mechanism of magnetoreception in migratory birds: magnetic and radio frequency effects
1.45 pm	*Coffee Break*
	Session 2: Measurements of Oxidative Stress as Major Criteria of Study Quality Chair: Felix Meyer
2.15 pm	Michael Jonathan Davies | Biomarkers of oxidative stress and their measurement
2.45 pm	Myrtill Simko | Quality of oxidative stress studies
3.15 pm	Robert Usselman | Biophysical methods - Quantum Effects in the Biological Production of Reactive Oxygen Species
3.45 pm	Discussion
	Keynote lecture
4.30 pm	Helmut Sies | Oxidative Stress: Oxidant Sources from Cell Metabolism and from the Exposome
from 7.00 pm	*Informal Get-together*

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Thursday, February 17th

Morning Lecture

9.30 am Henry Jay Forman | What is oxidative stress?

10.00 am Coffee Break

Session 3: Biophysical Mechanisms and Effects of Oxidative Stress |Chair: Alex Leymann

10.30 am Pietro Ghezzi | Inflammation and oxidative stress

11.00 am Alex Jones | Magnetic field effects on neuronal activity

11.30 am Daniel Kattnig | The Radical Pair Mechanism and Reactive Oxygen Species?

12.00 pm Lunch Break

Session 4: Systematic Reviews | Chair: Blanka Pophof

1.00 pm Jos Verbeek | WHO project on adverse health effects of radiofrequency electromagnetic fields

1.30 pm Katya Tsaioun | Assessment of evidence in scientific studies

2.00 pm Rob Wright | Literature Searching in Systematic Reviews: A Case Study

2.30 pm Coffee Break

Session 5: RF-EMFs and Oxidative Stress | Chair: Jens Kuhne

3.00 pm Gernot Schmid | Radio Frequency (RF) Exposure Generation and Assessment in Experimental Studies

3.30 pm Bernd Henschenmacher | The WHO systematic review on radio-frequency fields and biomarkers of oxidative stress

4.00 pm Discussion

from 7.00 pm Conference Dinner

Friday, February 18th

Session 6: ELF-EMFs and Oxidative Stress | Chair: Julia Ketteler

9.00 am Ilkka Laakso | Exposure assessment for ELF-EMF in humans

9.30 am Dmitrij Sachno | A systematic review of the effects of exposure to static- and low frequency electromagnetic fields on biomarkers of oxidative stress

10.00 am Jonathan Woodward | Radical pair based magnetic field effects on the autofluorescence of living cells

10.30 am Coffee Break

11.00 am Discussion and Outlook

from 12.00 pm Lunch and farewell

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SESSION 1: BASICS OF OXIDATIVE STRESS – KNOWNS AND UNKNOWNS

Oxidative damage in toxicology and disease and the problem of biomarkers

Pietro Ghezzi | University of Urbino, Italy and Brighton & Sussex Medical School, United Kingdom

The main biochemical pathway for the metabolism of molecular oxygen in aerobic organism is represented by its reduction to water, and four electrons are needed to reduce one molecule of molecular oxygen to two molecules of water. There are also several enzymes, such as NADPH oxidases, that evolved to generate intermediate forms of reduction of oxygen (for instance superoxide radicals and hydrogen peroxide) that are called reactive oxygen species (ROS) as they can easily oxidize electron donors to be reduced to water, which is the most stable form. Reactivity in a biological system is often predictive of toxicity, and ROS can oxidize, and potentially damage, different biological molecules; proteins, lipids and nucleic acids can all be oxidized by ROS.

This is very important in toxicology as physical and chemical toxicants can increase ROS generation in biological systems. High oxygen concentrations (hyperoxia) and ionizing radiations can produce ROS (the latter via water radiolysis), which contributes significantly to their toxicity. Some chemicals, such as the herbicide paraquat or certain drugs such as doxorubicin, can generate ROS by redox cycling and this is important for their toxicity.

The idea that an unbalance between the production of ROS and the capacity of the antioxidant systems to detoxify them (defined as “oxidative stress”), and that this unbalance is at the basis of several pathologies or degenerative conditions, was first hypothesized by Harman in 1956 with the free radical theory of ageing. That is probably the earlier mention of what I would define the “oxidative stress theory of disease”. By now, “oxidative stress” has been implicated in the pathogenesis of probably every known disease, based on the following types of evidence: 1) measurement of biomarkers of oxidative stress in patients or animal models; 2) the ability of ROS to induce, in vitro or in vivo, changes like those observed in the disease; or 3) the protective effect of antioxidants and reducing agents in models of disease.

Many of the studies on the role of ROS in disease predict that administration of antioxidants can be a therapeutic avenue to treat or prevent those diseases. However, despite a huge number of studies published on the pathogenic role of ROS, there are no antioxidants approved by regulatory agencies for any specific indication, and their use is so far mainly in the field of complementary and alternative medicine, with various nutraceuticals and supplements, that can be marketed even in the absence of clinical trials showing their efficacy.

Several issues affect the clinical translation of the oxidative stress theory of disease. These include practical reasons and epistemological weaknesses of the theory, and I will list some of them.

1) Most studies done using “antioxidant molecules” do not consider that these molecules are often nonspecific and have other properties.

2) ROS are important signaling molecules (redox signaling) and administration of antioxidants may block cellular signaling, not only the possible pathological overproduction of ROS by specific enzymes in specific tissues.

3) It is always difficult to extrapolate from very well-defined experimental model, that try to study specifically this mechanism (reductionism) to the clinical situation where many diseases are multifactorial.

4) Studies measuring OS in patients are reporting an association. This could be explaining not only hypothesizing that OS is a cause of the disease but could also that OS is caused by the disease (reverse causation).

5) It is also possible that an association between OS and disease is due to a confounder which induces both. For instance, many diseases have inflammation in their causal pathway. If inflammation caused both the disease and OS, then OS is not necessarily in the causal pathway for the disease.

6) Measuring ROS in vivo is technically difficult.

The latter point could be defined as “the problem of biomarkers”. Unlike other theories of disease where the causal agent can be directly measured (for instance, microbes in the germ theory or cytokines like TNF in the cytokine theory of disease), it is very difficult to measure ROS because, being reactive, they have very short half-lives (nano- to milliseconds). For this reason, we usually resort to measure the traces left by their formation, that is products of oxidation of cellular constituents (proteins, lipids or DNA).

This talk will discuss on some problems related to the use of indirect biomarkers as proxies. To do so, I will make some considerations based on the theories of signs focusing on some aspects: ambiguity, actionability and the need to measure more than on biomarker.

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SESSION 1: BASICS OF OXIDATIVE STRESS – KNOWNS AND UNKNOWNS

The role of cryptochromes in fauna and flora: light sensing, magnetic field sensing, and involvement in oxidative stress

Margaret Ahmad | Institute de Biologie Paris Seine, Sorbonne Université, France and Xavier University, Cincinnati, Ohio, USA

*Marootpong Pooam (1,2), Blanche Aguida (1), Soria Drahy (1), Nathalie Jourdan (1), Hakim Karoui (3), Olivier Ouari (3); *1): Sorbonne Université, Paris, France; 2) Naresuan University, Phitsanulok Thailand; (3) ICU, Aix-Marseille Université France; 4) Xavier University, Cincinnati, Ohio USA.*

Short Summary

Cryptochromes are evolutionarily conserved flavoprotein receptors found in plants and animals which mediate multiple and diverse signaling functions and play a role in perception of electromagnetic fields. Here we describe how plant cryptochromes are activated by light-driven photoreduction of protein-bound flavin (from FADox to FADH° and FADH-), followed by restoration of the resting state via a reoxidation reaction that consumes molecular oxygen and occurs independently of light (from FADH- to FADox). Importantly, the cryptochrome reoxidation reaction is accompanied by formation of both superoxide (02°-) and hydrogen peroxide (H202) which are ROS (reactive oxygen species) that themselves have important cellular signaling roles, particularly in response to stress and cellular damage. Similarly in human cell cultures, the primary effect of exposure to both static and oscillating magnetic fields (from 10Hz to GHz range), are transient change in the concentration of intracellular ROS (reactive oxygen species) and induction of ROS signaling pathways. Some of these effects in human cells have been linked to the function of cryptochrome and the Radical Pair mechanism, whereas others appear to occur by distinct mechanisms. We discuss the possible negative consequences of man-made electromagnetic noise on plants and animals in the context of ROS induction, as well as demonstrate novel medical applications of magnetic fields to treat diseases such as SARS-COVID 19, that respond to modulation of intracellular ROS by externally applied magnetic fields.

Introduction

Cryptochromes are an evolutionarily ancient and diverse family of flavoprotein blue light receptors found throughout the biological Kingdom. They are related to photolyases, which catalyse the light-dependent repair of UV-damaged DNA. However, unlike photolyases, cryptochromes have for the most part lost DNA repair function and instead have evolved new roles in cellular signaling including in plant growth and in the animal circadian clock. Intriguingly, cryptochromes have also been implicated in the perception of electromagnetic fields (static and oscillating) in plants, animals and man. These effects include modification of behavioral responses in flies, changes in the growth and development of plants, and magnetosensitivity of the avian magnetic compass. Illumination of plant and insect sensory cryptochromes induces photoreduction of protein-bound flavin (from FADox to FADH° and FADH-), which is followed by reoxidation (FADH- to FADox) to restore the resting (dark adapted) redox state. The process of flavin reduction induces conformational changes in the cryptochrome protein to achieve the biologically active 'lit' state, which can interact with downstream signaling partners to initiate many different cell signaling reactions. This cycle of cryptochrome flavin reduction/reoxidation, which is referred to as the cryptochrome photocycle, furthermore produces H202 as a byproduct, which can itself function as a potent cell signaling and stress trigger.

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In this talk I will discuss the possible mechanisms by which weak magnetic fields ranging from near-earth strength static fields to oscillating fields in the mHz and GHz range can impact on cryptochrome in plants and animals and describe the physiological consequences. I will discuss both light-dependent and –independent mechanisms for magnetosensing via the Radical Pair mechanism, as well as environmental consequences and novel biomedical applications of EMF in the context of their effect on cellular ROS and oxidative stress mechanisms.

Materials and Methods

Arabidopsis Cry1 cryptochrome, avian Cry1a, and HsCry1 cryptochrome proteins and their response to electromagnetic fields are analysed in insect cell cultures, in plants, and in human HEK and primary alveolar epithelial cell culture systems. Details of our methodology and assay systems are summarized in our recent papers and references therein (1 – 4).

Results and Discussion

The magnetically sensitive reaction of the cryptochrome photocycle occurs in the course of flavin reoxidation in the dark. In the case of plant cryptochromes, we verified one of the predictions of the Radical Pair mechanism by demonstrating that RF fields in the 1 – 10 MHz range could entirely abolish the response of plant cryptochrome to the earth's magnetic field. The radical pair mechanism predicts that external magnetic fields can alter the rate constants of biochemical reactions that generate radical pair intermediates, in this way altering the biological activity of the receptor. There are two possible steps in the cryptochrome photocycle where radical pair intermediates can be formed; one occurs during flavin photoreduction to generate a short-lived radical pair (Trp°/FADH°). This radical pair is only generated during illumination. A second radical pair (O2°-/ FADH°) can be made during flavin reoxidation, which takes place in darkness over an extended time frame.

These two possibilities were distinguished by subjecting plants to alternating pulses of light and dark, and providing magnetic field exposure only during the dark interval in the absence of illumination. The result was that the plants show magnetic sensitivity even in the absence of simultaneous illumination. This eliminates the Trp/FADH° radical pair as a candidate for mediating magnetic sensitivity, and indicates the magnetosensitive radical pair is formed in the course of flavin reoxidation. The results were further extended to migratory birds, in which Cry1a cryptochrome has been suggested as a possible magnetosensor. Birds were subjected to light pulses such that magnetic field exposure occured only during the dark intervals between light pulses. The birds were still able to orient to the magnetic field under these conditions, in which the Trp/FADH° radical pair is not formed (5). Birds were furthermore oriented when magnetic field exposure was provided in the presence of monochromatic green light (565nm), a condition in which the Trp/FADH° radical pair is not formed at all (5 and references therein). Therefore, the many reports attempting to link the Trp/FADH° radical pair to avian biological magnetosensing are categorically and demonstrably false.

Increase in intracellular ROS is a consequence of magnetic field exposure. One important consequence of magnetic sensitivity occurring in the course of the flavin reoxidation reaction is the formation of reactive oxygen (ROS) and H202 as byproducts, which have many biological signaling roles. Assuming a possible common underlying response mechanism, we accordingly evaluated the production of ROS in human cell cultures exposed to static (Low Level Field (5 milliGauss)), 10Hz pulsed field (1.2 mT), and 1.8 GHz oscillating fields. In each and every case, a marked increase in intracellular ROS was an immediate consequence of

even very brief (10 – 15 min) exposure. Intriguingly, k/o mice lacking human cryptochrome showed no response to the 10Hz Pulsed magnetic fields, indicating a role for cryptochrome in the formation of ROS. Such an effect could occur independently of light as long as other forces (e.g. cellular metabolites or reductants) were able to trigger the cryptochrome redox cycle. Whether or not cryptochrome is the primary magnetosensor, or else may play secondary roles in redox signaling pathways triggered by other magnetosensing mechanisms, still remains to be established.

Magnetic Field effects on ROS signaling pathways – medical implications. Our results indicate that man-made electromagnetic noise is not physiologically inert and can induce a transient change in intracellular ROS in many different human cell types. This raises the question of re-examining the possible health consequences of exposure to weak EMFs that are currently considered harmless. For example, there may be effects of chronic exposure to magnetic fields over a long-term basis. In particular, complicating factors like host hypersensitivity to oxidative stress, or else simultaneous exposure with other types of stressors that may act synergistically with magnetic fields, need to be taken into account. Safety data on exposure levels may therefore have to be re-evaluated.

Nonetheless, the most exciting implication of our findings is with respect to the enormous potential for novel biotechnological and biomedical applications. Living things are exquisitely sensitive to temporal fluctuations in cellular ROS, which trigger cellular defense and repair pathways at moderate concentrations as well as causing oxidative stress and damage (e.g. to cancer cells) at high exposure. We were particularly intrigued by the known effects of ROS on the immune response and by historical reports that pulsed electromagnetic field exposure (PEMF) at defined intervals had been found effective against various types of inflammation. We therefore decided to test the idea of whether pulsed magnetic field exposure could be used to treat hyperinflammatory response triggered by COVID 19.

As proof-of-principle, we induced an inflammatory response in modified HEK cells, primary alveolar lung cells, and macrophage cell lines that mimic the hyperinflammatory pathology caused by COVID-19-infection. We used magnetic field exposure conditions (10 Hz, 2mT) that we had previously found to induce cellular ROS and ROS-regulated gene expression in human cell cultures. Indeed, after exposing diseased cell cultures to pulsed electromagnetic field for just 10 minutes per day, twice a day, over a 2 – 4 day period, inflammatory cell markers including for TNF-alpha, chemokines and cytokines were considerably reduced as compared to non-treated control cells. Although these findings need to be confirmed by proper clinical trials, they provide a promising proof-of-principle for what may become possible in the near future by controlled applications of magnetic fields to achieve new therapies in medicine.

Conclusion

An increasing body of evidence has implicated cryptochromes in magnetic field responses, which can occur both in the presence or absence of light. The radical pair mechanism can explain effects of static and oscillating (MHz) magnetic fields on plant growth and avian navigation, resulting from changes in reaction rate constants of the cryptochrome photocycle. The magnetically sensitive radical pair in plant cryptochromes is formed during the process of flavin reoxidation, during which both flavin (FADH°) and superoxide (O2°-) radicals are formed. Our results also help to explain a recurring theme in the EMF literature, that a large variety of electromagnetic field exposure conditions appear to modulate intracellular ROS (reactive oxygen species). Cryptochromes could account for such modulation of ROS in response to static and pulsed magnetic fields, but not in the GHz range where the radical pair mechanism does not appear to hold. Therefore,

other cellular mechanisms must also be involved response to magnetic fields. Although weak EMF exposure is considered safe, our results raise the possibility that long-term chronic exposure to man-made electromagnetic noise may be harmful to susceptible individuals, particularly in combination with additional stressors that induce ROS. However, the potential beneficial effects of modulating cellular ROS by such non-invasive methods are also considerable, especially in devising new therapies for diseases that may be responsive to manipulating oxidative stress and/or cellular ROS (such as chronic and acute inflammation).

References:

1. Pooam M et al. Exposure to 1.8 GHz Radiofrequency field modulates ROS in human HEK293 cells as a function of signal amplitude. DOI: 10.1080/19420889.2022.2027698. KCIB-Communicative & Integrative Biology, 2022, in press.

2. Pooam et al. Therapeutic application of light and electromagnetic fields to reduce hyper-inflammation triggered by COVID-19. KCIB-Communicative & Integrative Biology, 2021.https://doi.org/10.1080/19420889.2021.1911413

3. Albaqami M et al. Arabidopsis cryptochrome is responsive to Radiofrequency (RF) electromagnetic fields. Sci Rep. 2020. https://doi.org/10.1038/s41598-020-67165-5

4. Pooam M et al. HEK293 cell response to static magnetic fields via the radical pair mechanism may explain therapeutic effects of pulsed electromagnetic fields. PLoS One. 2020 Dec 3;15(12):e0243038. https://doi.org/10.1371/journal.pone.0243038

5. Wiltschko et al. Light-dependent magnetoreception in birds: the crucial step occurs in the dark. J R Soc Interface. 13(118). pii: 20151010. doi: 10.1098/rsif.2015.1010.

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SESSION 1: BASICS OF OXIDATIVE STRESS – KNOWNS AND UNKNOWNS

The radical-pair mechanism of magnetoreception in migratory birds: magnetic and radio frequency effects

Henrik Mouritsen | Institute of Biology and Environmental Sciences, Universität Oldenburg, Germany

In my talk, I will shortly introduce the radical-pair mechanism of magnetoreception which seems to be used by night-migratory songbirds. Night-migratory songbirds are the most numerous migratory birds in Europe, and they migrate over thousands of kilometers alone, at night, and without any contact to their parents. One of the key cues they use to find their way is the Earth's magnetic field. Their magnetic compass is detecting the inclination of the magnetic field lines. Magnetic compass information detection requires the presence of light and seems to be based on a radical pair mechanism. The sensory molecule behind this sense seems to be a protein called cryptochrome (Cry) and by far the best candidate we have among the six crypto chromes known to exist in birds is Cryptochrome 4. Recently, my laboratory managed to express and purify Cry4 from a night-migratory songbird, the European robin (*Er*Cry4), for the first time. Using advanced spectroscopic techniques, our physical chemistry colleagues in Oxford, could measure that radicals are indeed formed when *Er*Cry4 absorbs blue light. Furthermore, strategic mutations of amino acids involved in the electron transport chain within *Er*Cry4 made it possible to understand exactly how the electrons jump with *Er*Cry4 and where the magnetic sensitivity is generated in the protein *in vitro*.

After presenting the magnetic field effects measured on isolated *Er*Cry4, I will turn my attention to the effects that very weak RF-fields have on the orientation behaviour of night-migratory songbirds and discuss which frequency range affect the birds. I will also present some theoretical considerations made in collaboration with our physics colleagues that might explain these findings. I will also touch upon why previous claims that there should be very specific effects of "Larmor-frequency" RF fields only are very unlikely to be true.

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SESSION 2: MEASUREMENTS OF OXIDATIVE STRESS

Biomarkers of oxidative stress and their measurement

Michael Jonathan Davies | Department of Biomedical Sciences, Panum Institute, University of Copenhagen, Denmark

Biological systems are exposed to a wide variety of oxidants – both free radicals and two-electron species. These species are often termed 'reactive oxygen species' (ROS), though this is a misleading term, as it does not refer to a single species, but rather a complex and very diverse group of species whose reactivity, selectivity and effects vary enormously. These oxidants are generated both deliberately (e.g. as signaling molecules, to kill invading pathogens, or as intermediates in enzymatic reactions) or unintentionally (e.g. via metabolism of drugs, exposure to chemicals, pollutants, radiation).

Oxidants can generate both positive effects ('oxidative eustress', 'good stress') when these are generated deliberately and in a controlled fashion, with a classical example being the inter- and intra-cellular signaling events mediated by nitric oxide ($NO^{\cdot}$) and hydrogen peroxide (H_2O_2). A number of mechanisms have been elucidated by which such signaling occurs, including binding to heme groups (for example, in the case of) $NO^{\cdot}$ or by the selective oxidation of particular thiols (R-SH, the reactive side chain of the amino acid cysteine, Cys) on proteins by H_2O_2. The latter class of reactions can generate sulfenic acids (R-SOH) and disulfides (R-S-S-R) that alter the structure and function of the oxidized protein. Oxidation can be transmitted from one initial protein to another, with repair of the initial lesion and the modification transmitted to the second protein: these transfer reactions are often termed 'redox relays', and can result in transmission of signals between biological compartments (between organelles within cells, or between cells, and between tissues). Ultimately, the oxidation is removed, or repaired, by the abundant 'antioxidant' defence systems within cells and tissues, with this mainly mediated by enzymatic systems. Low molecular mass antioxidants, such as ascorbic acid (vitamin C), tocopherols (vitamin E) and carotenoids can also play a role in some circumstances.

In other cases, oxidation can be negative and damaging ('oxidative distress') when oxidants are generated at elevated levels, or at the wrong time, or in the wrong place. This can result in molecular, cellular, tissue and organismal dysfunction. Damage can either be highly specific and localized, or non-specific and widespread. Thus, highly reactive oxidants, such as the hydroxyl radical, react indiscriminately will all biological molecules within cells, including DNA, RNA; proteins, lipids, carbohydrates etc. In contrast, other oxidants can give rise to highly specific modifications at particular sites. An example of such selectivity, is the superoxide radical anion ($O_2^{\cdot -}$), a species formed by one electron reduction of molecular oxygen (O_2). This species is generated by multiple pathways in cells, with major routes being via single electron transfer to O_2 by Complexes I and III of the mitochondrial electron transport chain, or via the action of multiple isoforms of NAPDH oxidases (NOXs). $O_2^{\cdot -}$ is poorly reactive with nearly all biological targets, with the exception of other radicals (including $NO^{\cdot}$ to give a secondary, and powerful oxidant, peroxynitrous acid/peroxynitrite, $ONOOH/ONOO^-$), and the iron-sulfur clusters ($Fe\text{-}S_x$) of specific proteins, of which aconitase is a well-established example.

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As proteins are highly abundant and react rapidly with many oxidants, they are major targets for damage. This can result in changes to protein structure, function and turnover, and a loss or (occasional) gain of activity. Accumulation of modified proteins, due to either increased generation and/or decreased removal, has been associated with both aging and multiple diseases. It is clear, that different oxidants produce a wide variety of changes, and at vastly different rates on proteins, and that a broad spectrum of post-translation modifications (PTMs) can be generated. These can therefore be useful biomarkers of damage, but it is important to understand that the level of a particular product (biomarker) is dependent on a number of critical and important factors. In particular, the levels of a specific product are determined by the difference between the rate of formation and removal (or repair) of the damage. If the latter is fast and efficient, then the steady state level (concentration) at a particular time point may be low, even though the rate of generation (and flux down a pathway) is high. It is also important to understand that different oxidants have different targets, and that the same oxidant will give very different types and levels of products with different targets. Therefore, there are few species that are 'universal' biomarkers of damage that can be reliably used to give evidence for oxidative stress in all situations. Understanding the nature and chemistry of the wide family of oxidants is therefore very important in the choice of biomarkers of modification. Indeed, it is often very sensible to examine a wide group of different species to avoid false information.

There is therefore a pressing need for reliable and robust methods for the detection, identification and absolute quantification of products formed by oxidants, and especially in complex systems. This presentation will summarize some of the methods that are available to detect modifications – particularly on proteins, and some of the advantages and disadvantages of different species and approaches to the detection of these.

Key references:

C. L. Hawkins and M. J. Davies, Detection, identification, and quantification of oxidative protein modifications**,** J Biol Chem, (2019) 294, 19683-19708. DOI: 10.1074/jbc.REV119.006217. PMID: 31672919

C. L. Hawkins, P.E. Morgan and M. J. Davies, Quantification of protein modification by oxidants, Free Radic Biol Med, (2009) 46, 965-88. DOI: 10.1016/j.freeradbiomed.2009.01.007. PMID: 19439229

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SESSION 2: MEASUREMENTS OF OXIDATIVE STRESS

Quality of oxidative stress studies

Myrtill Simkó | SciProof International AB, Östersund, Sweden

EMF research has been ongoing for many decades and has produced numerous publications showing countless results with or without findings of effects, using different (from static to radiofrequency) electromagnetic fields (EMF) as exposure sources, using different living systems, exposure durations, and so on. However, the issue of EMFs in environmentally relevant exposures and possible health effects may still cause controversy. The question remains, why can we not answer the question of whether there is a health-related or a biologically relevant effect caused by any type of EMF exposure at levels below the exposure guidelines?

First of all, a "non-effect" cannot be proven, because the question will always remain whether the test was appropriate. So only real or statistically relevant effects can be proven.

Secondly, there are research groups that find so-called bioeffects, which on the one hand worries some citizens and on the other hand gives arguments to the activists.

Moreover, some results could never be reproduced by other independent research groups, and it is a fact, that even different commissions/expert groups or institutions sometimes interpret the available publications differently.

The problem lies in the quality of the studies performed. It is well known that many studies are of poor quality in terms of exposure conditions, but also in terms of experimental procedures, which is the main reason for the lack of reproducibility and contradictory results. Many published studies have not used appropriate controls for the biological as well as for the physical conditions. In addition, scientists often do not use hypothesis-based studies, but "hunt" for effects and often misinterpret them. And this, in turn, is due to the use of statistical analysis, which is often inappropriate. A statistical analysis is a data interpretation tool. A statistically significant difference does not necessarily mean a real effect, just a mathematically observable feature!

Study quality issues are independent of the endpoint being studied. However, oxidative stress seems to be a "sexy" endpoint to study, with similar study quality issues as other endpoints. Even when an effect is detected, the interpretation of the changes, which are usually very small (compared to controls, if any), is not clear. So how does one deal with small effects that are not understood, and with poor study quality?

The goal of EMF research in this setting is to evaluate all available data and allow performing risk assessment. However, this task is almost impossible due to poor data quality.

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SESSION 2: MEASUREMENTS OF OXIDATIVE STRESS

Biophysical methods – Quantum Effects in the Biological Production of Reactive Oxygen Species

Robert Usselman | Biomedical and Chemical Engineering and Sciences, Florida Institute of Technology, USA

One of the great challenges of modern science is to bridge the gap between atomic and cellular level phenomena that determine the condition and outcomes of our cells. A potentially transformational facet of this challenge is quantum biology (QB). QB may be thought of as the signatures of molecular-level quantum phenomena observed in biological systems at functional, cellular, or organism levels. For example, quantum effects in biological systems have been implicated in key mechanisms for bird navigation, olfactory sensing, and photosynthesis. Here, we investigate a novel domain of quantum biology: the control of the biological production of reactive oxygen species (ROS) by influencing coherent spin dynamics in a radical pair (RP) reaction between reduced flavin (enzymes) and the activation of molecular oxygen. The RP mechanism in ROS production can be potentially harnessed as an entry point into oxidative signaling channels in living systems by radiofrequency magnetic resonances. We will discuss the observation and identification of quantum signatures written into cellular oxidative signaling channels and emergent collective behavior of RP outcomes in this domain.

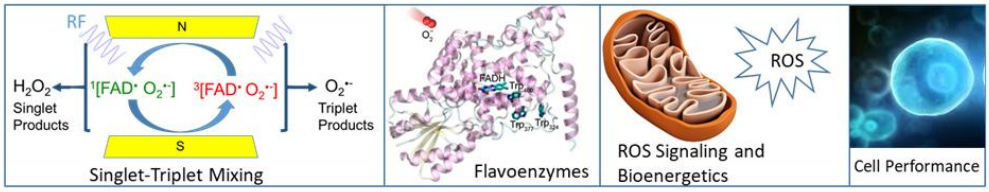

Figure 1. Multi-scale representation of non-classical effects in biological systems. A spin-correlated radical pair is created, where the ROS spin dynamics and ROS products can be altered by magnetic field stimulation. Certain flavoenzymes are the local terminal points of radical pair creation. ROS partitioning affects mitochondrial bioenergetics between glycolysis and respiration, thus affecting cellular performance.

References

Usselman RJ, Chavarriaga C, Castello PR, Procopio M, Ritz T, Dratz EA, et al. The Quantum Biology of Reactive Oxygen Species Partitioning Impacts Cellular Bioenergetics. Sci Rep. 2016;6:38543. doi: 10.1038/srep38543. PubMed PMID: 27995996; PubMed Central PMCID: PMCPMC5172244.

Usselman RJ, Hill I, Singel DJ, Martino CF. Spin biochemistry modulates reactive oxygen species (ROS) production by radio frequency magnetic fields. PloS one. 2014;9(3):e93065. doi: 10.1371/journal.pone.0093065. PubMed PMID: 24681944; PubMed Central PMCID: PMC3969378.

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KEYNOTE LECTURE

Oxidative Stress: Oxidant Sources from Cell Metabolism and from the Exposome

Helmut Sies | Institute for Biochemistry and Molecular Biology I, Heinrich-Heine-Universität Düsseldorf and Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany

The global concept of „Oxidative Stress" is defined as „an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage".[1,2] Substantial research on the molecular basis of redox regulation identified an essential role of oxidants as second messenger, which revealed that oxidative stress is two-sided: maintenance of a physiological (low) level of oxidant challenge is essential for governing life processes through redox signaling, termed "oxidative eustress", whereas excessive oxidant challenge causes damage to biomolecules, termed "oxidative distress".[1,3] Hydrogen peroxide (H2O2) and superoxide (O2.-) are the major reactive oxygen species (ROS) in redox signalling[4], and their sources are endogenous, i.e. generated from cellular metabolic reactions, or exogenous, i.e. from nutrition, lifestyle, toxins and physical factors such as radiation, collectively termed "exposome".[5]

Ionizing radiation is one of the exposomal modes. We examined the role of H2O2 which, as a stable product of water radiolysis, occurs at nanomolar concentration upon low-dose ionizing radiation (LDIR) (<100 mGy), which is within the normal cellular H2O2 concentration range. This led us to propose a role for H2O2 in radiation hormesis[6]. LDIR is capable of utilizing known molecular redox master switches such as Nrf2/Keap1 or NF-κB/IκB to effect adaptive resilience. This leads to the hypothesis that, as a normal component of the exposome, LDIR mediates hormetic effects by H2O2 signaling. Radiation hormesis is the biological consequence of low-dose exposure. This is contrasted by the linear-no-threshold (LNT) concept, which is solely based on physical principles, ignoring the beneficial biological response at low-dose. It will be of interest to further investigate the physiological impact of other modes of exposomal radiation, e.g. electromagnetic fields[7] or noise[8]. A pivotal issue to address will be to identify the transition from beneficial effects, eustress, to detrimental consequences, distress, i.e. to characterize the "tipping point" between health and disease processes.

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MORNING LECTURE

What is oxidative stress?

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When first used by Helmut Sies, oxidative stress was defined as an imbalance between production of oxidants and antioxidant defenses that may result in damage to biological systems. Since the 1980s, the term has evolved beyond lipid peroxidation and protein and nucleic acid oxidation to also include oxidant-induced alterations in physiology and disease. Nonetheless, only a few diseases involve oxidative stress as a major cause while in most diseases oxidative stress exacerbates pathology or is secondary. The secondary importance explains the failure of antioxidant therapies to have a major impact on many diseases even when there is some evidence of oxidative stress having occurred. Regardless, both endogenously produced and exogenous agents can cause oxidative stress. Reactive oxygen species (ROS) is an abbreviation that is too often misused by treating it as a chemical entity. ROS includes superoxide $O_2^{\cdot-}$, hydrogen peroxide (H_2O_2), and hydroxyl radical (•OH) among others. Peroxynitrite ($ONOO^-$), another contributor to oxidative stress is produced from $O_2^{\cdot-}$ and nitric oxide ($^{\cdot}NO$). Major sources of $O_2^{\cdot-}$ include the mitochondrial electron transport chain and NADPH oxidases. The major source of H_2O_2 is dismutation of $O_2^{\cdot-}$ by superoxide dismutases although some enzymes can produce H_2O_2 directly. A large number of enzymes are involved in H_2O_2 removal. In contrast, the only effective defense against $^{\cdot}OH$ is to prevent its production. H_2O_2 is also a major participant in physiological cell signaling and prolong oxidant production contributes to chronic inflammation. But, while overproduction of H_2O_2 can therefore result in abnormal cell function, it may also induce antioxidant defenses. Thus, preventing damage or malfunction depends upon maintaining redox homeostasis.

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SESSION 3: BIOPHYSICAL MECHANISMS AND EFFECTS OF OXIDATIVE STRESS

Inflammation and oxidative stress

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In the 1980s it became clear that most of the effects of inflammation are mediated by inflammatory cytokines like interleukin-1 or TNF, a finding that soon led to the development of anti-cytokine antibodies as therapeutic agents for chronic inflammatory disease which are not the top selling biological. The hallmarks of inflammation, as defined by Celsus and Galenus are: rubor, tumor, dolor, calor and functio lesa (redness, swelling, pain, heath, and loss of function), and most of them can now be explained by the action of cytokines on the vasculature through increased permeability and leukocyte extravasation and migration, and by leukocyte activation.

A large body of literature identified reactive oxygen species (ROS) as mediators of inflammation, both as inducers of cytokine synthesis and as effector, cytotoxic molecules produced by activated leukocytes. This has led to the widely accepted idea that ROS are pathogenic mediators and antioxidants could therefore ameliorate inflammation, and to view “oxidative stress” as an underlying mechanism of the complications of systemic inflammation such as in acute respiratory distress syndrome and sepsis.

However, the inflammatory response is the main mechanism of innate immunity, the first line of defense against pathogens. Inflammation is also an essential response to injury and an important step in wound healing. In this context, ROS are one of the effectors by which leukocytes kill pathogens, as well as a signaling mechanism in wound healing. Chronic granulomatous disease, an X-linked deficiency in the enzyme that produces ROS, causes serious susceptibility to infection.

This lecture will discuss the role of ROS in immunity and tissue damage, highlighting the inevitable trade-off between pathogen control and damage control.

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SESSION 3: BIOPHYSICAL MECHANISMS AND EFFECTS OF OXIDATIVE STRESS

Magnetic field effects on neuronal activity

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Introduction

Cryptochrome (CRY) is a promiscuous signalling molecule found in plants and animals.[1] CRY from *Drosophila melanogaster* (dCRY) is a case in point;[2] there is evidence to support light-dependent roles for it in the circadian clock, the visual system, ageing, and in the regulation of neuronal activity and metabolism. There is mounting evidence that CRY also serves as a light-dependent magnetoreceptor protein that facilitates the ability of some animals to sense the Earth's magnetic field (MF) to aid with behaviours such as navigation and migration.[3] The strongest direct evidence for this from *in vivo* experiments is from *Drosophila*, where genetic alteration has shown dCRY to be necessary for various measures of magnetic-sensitivity.[4-10]

It is proposed that changes to the ambient MF conditions could alter the concentration of the active state of CRY through interaction with spin-correlated radical pair intermediates.[11] The dCRY structure[12, 13] has a domain that binds the blue light chromophore, flavin adenine dinucleotide (FAD), the radical form of which correlates with the active state of the protein.[14] This active state makes the partially disordered C-terminal tail (CTT) available for binding to interaction partners.[15] It is therefore plausible that magnetic effects on the population of this radical state could influence signals transduced by active CRY. For a magnetic effect on a protein to influence behaviour, however, this signal must presumably trigger a nervous system response.

Results and Discussion

In my lecture, I will present dCRY-dependent effects of MF on neuronal activity in *Drosophila*. I will discuss data from a behavioural measure that reflects effects of MF on the whole larval nervous system[8] and data from individual nerve cells that begin to reveal mechanistic detail.[9] I will go on to discuss a radical pair model that could be consistent with these observed effects that involves the oxidation of the reduced FAD bound to dCRY and the generation of ROS intermediates. In summary:

Cryptochrome-dependent magnetic field effect on seizure response in Drosophila larvae[8]

If the developing nervous system in *Drosophila* experiences inappropriate neural activity during a critical period in embryogenesis, the resultant third instar larvae are prone to seizure.[16] When exposed to blue light (peak emission ~ 470 nm) during this period, seizure duration is roughly doubled compared to when embryos are kept in the dark. This effect is substantially and significantly increased further by the presence of a 100 mT MF during blue light exposure. Knocking out the genes that express dCRY removes the effects of both blue light alone and blue light plus MF.

MF Modulate Blue-Light-Dependent Regulation of Neuronal Firing by dCRY[9]

The data from seizure response are consistent with dCRY-dependent stimulation of the developing nervous by blue light and MF during embryogenesis. It is known that blue light increases the firing rate of large lateral ventral arousal neurons in *Drosophila*, a process regulated by dCRY.[17] Similarly, we discovered an increase in both membrane potential and action potential firing of aCC motoneurons exposed to blue light. This effect on motoneurons is significantly enhanced in the presence of a 100 mT MF. Again, the effect of blue light and its potentiation by MF are only manifest in motoneurons ectopically expressing dCRY. Interestingly, our data are consistent with the closing of ion channels, potentially resulting from an interaction between dCRY and a channel redox subunit.[18]

A possible model involving ROS?

What our data don't appear to be consistent with, however, is the canonical radical pair mechanism of CRY-dependent magnetoreception. This mechanism describes a magnetically-sensitive radical pair in CRY that is formed in the singlet spin-state during the photoreduction of oxidised FAD to its radical form. In the absence of evidence to the contrary, it is reasonable to assume that the increase in blue-light and CRY-dependent neuronal firing that results from exposure to a 100 mT MF is the result of a magnetically-induced increase in active CRY. In other words, a magnetically-induced increase in the concentration of FAD radical. As I will explain, the effect of a 100 mT MF on a singlet born radical pair during photoreduction would be to decrease the FAD radical concentration, and hence the amount of active CRY. An increase would require a triplet-born radical pair, which is not consistent with the kinetics of CRY photoreduction.[19] In principle, however, a triplet-born radical pair could be generated in CRY during reoxidation of FAD following photoreduction.[20] If so, this is likely to generate ROS intermediates, the concentration of which would themselves become magnetically-sensitive.

Conclusions

The data summarised here reveal MF effects on neuronal activity in *Drosophila*. These observations are consistent with a radical pair mechanism in CRY that could be realised through a process involving ROS intermediates. It is therefore possible that these CRY-dependent effects of MF on neuronal activity are relevant to oxidative stress resulting from MF exposure. I will discuss the implications, challenges, and likelihood of this mechanism.

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SESSION 3: BIOPHYSICAL MECHANISMS AND EFFECTS OF OXIDATIVE STRESS

The Radical Pair Mechanism and Reactive Oxygen Species?

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Introduction

The radical pair mechanism (RPM) is widely considered *the* canonical non-thermal mechanism to explain effects of weak magnetic fields on chemical reactions and biological processes. According to this mechanism, magnetic field effects (MFEs) can arise during the singlet-triplet spin evolution of two radicals, which are in a joint non-equilibrium state, i.e. spin-correlated. Applied magnetic fields can affect the temporal evolution of the singlet-triplet interconversion, and thereby modulate relative reaction rates. This process is quantum coherent in nature, i.e. it builds on the system's ability to exist in several distinct states, singlet and triplet states, simultaneously. The RPM is distinguished from alternative models insofar as MFEs due to radical pair spin dynamics are well-understood and well-explored for many elementary chemical reactions. The mechanism furthermore provides the theoretical underpinning for one of the central hypothesis of quantum biology, the compass sense of certain animals, most notably migratory songbirds (*1*). This elusive sense is thought to be attributed to spin dynamics in a photo-generated radical pair in the flavo-protein cryptochrome.

To be sensitive to a magnetic field as weak as the Earth's, both the lifetime and the electron spin relaxation time of the radical pair must approach or exceed ≈ 1 µs. Such slow spin relaxation indeed appears feasible for flavin radicals bound to cryptochrome (*2*). On the other hand, this requirement challenges the possibility of spin dynamics-derived MFEs for radical pairs involving reactive oxygen species ROS, at least in moderate magnetic fields (< 1 T). The most relevant members of the ROS family are the superoxid, nitric oxide, and hydroxyl radical and molecular oxygen. These species have in common that they are subject to exceedingly fast spin relaxation, which manifests as a consequence of their electronic structure (*3*). Specifically, the $^2\Pi$ state of free ROS radicals implies a non-quenched angular momentum, large spin-orbit coupling and fast relaxation by the spin rational coupling when tumbling in solution. Molecular oxygen is a triplet state ($^3\Sigma$), for which the zero-field splitting results in equally fast spin relaxation. For the RPM this implies quickly lost spin-correlation and no magnetosensitivity. As a consequence, manifestations of spin effects on the reactions of $OH^\bullet$, $NO^\bullet$ and $O_2^{\bullet-}$ are extremely rare and have only been clearly demonstrated for chemical systems in strong magnetic fields (*4*).

Many studies on complex biological systems have provided circumstantial evidence for the involvement of ROS in magnetic field-affected phenotypes in weak magnetic fields. Specifically, the putative magnetosensitivity of flavin/superoxide dyads is a recurring feature. It has been hypothesized to explain magnetosensitive and magnetic isotope-responsive traits in cryptochrome magnetoreception, cellular ROS production, cellular bioenergetics, the circadian clock, lithium effects on hyperactivity, etc. The model is popular not only because its direct connection with ROS, which appears to be an overarching experimental finding, but also because it predicts large magnetosensitivity in weak magnetic fields (as long as spin relaxation are excluded from the analysis). This is a direct consequence of the hyperfine coupling topology in the radical pair, which collects all hyperfine interactions in one radical and none in the other (*5*). $FH^\bullet/O_2^{\bullet-}$ appears to be the only biologically viable example of such a "reference-probe" radical pair system. However, the spin relaxation properties of superoxide question the model. In the context of magnetoreception, the resulting dichotomy of wish and

reality has led to the postulation of the $Z^{\bullet}$-radical, which retains superoxide favourable traits, but not its spin relaxation. Unfortunately, $Z^{\bullet}$ appears to be a hypothetical construct only.

Results

The ostensible issues and challenges of the RPM in the context of ROS-related magnetic field effects can be overcome by:

1. three-radical systems (*6-8*) for which the (primary) radical pair undergoes a spin selective scavenging reaction with a third, initially uncorrelated radical (a phenomenon dubbed the chemical Zeno effect).
2. three-radical systems coupled via the electron-electron dipolar interaction as their predominant interaction mode (*9, 10*), and thus undergoing spin dynamics not as a consequence of hyperfine interactions (such as for the RPM) but their mutual dipolar coupling.

I will demonstrate how these effects can enable significant MFEs in weak magnetic fields in the presence of swiftly relaxing radicals, such as ROS. For illustration, I will focus on the phenomena of

1. adult hippocampal neurogenesis and hippocampus-dependent cognition in mice, which is impaired in hypomagnetic fields (*11*),
2. the reoxidation pathway of cryptochrome (*12*), and
3. lipid peroxidation (*13*)

For the example of hippocampal neurogenesis we have demonstrated that the experimental findings (*11*) are in qualitative agreement with a model extending the previously assumed $FH^{\bullet}/O_2^{\bullet-}$ radical pair by a third radical derived from a radical scavenger. We argue that the ascrobyl radical ($A^{\bullet-}$) would be well suited to act in this role, as it is abundant in neurons (ascorbic acid concentration: up to 10 mM) and its chemical properties favour its enhancing engagement in the three-radical process. This model is resilient to fast spin relaxation in the $O_2^{\bullet-}$. In fact, it retains, magnetosensitivity even in the limit of infinitely fast spin relaxation in $O_2^{\bullet-}$, whereupon its spin dynamics formally reduce to that of a radical pair with effective rate constants determined by the three-radical processes. Furthermore, unlike the previously suggested $FH^{\bullet}/O_2^{\bullet-}$ radical pair model, the sign of the effect is correctly predicted without unnatural assumption on the initial spin state of the primary radical pair (a singlet initial state is required by the RPM model).

For the example of cryptochrome magnetoreception, we have suggest a three-radical model for the re-oxidation pathway (*12*). For models agnostic to the identity of the third radical, we find that the three-radical processes can strongly enhances the directional sensitivity to the geomagnetic field, even when properly accounting for the (unavoidable) electron-electron dipolar interactions, which have usually been neglected from comparable treatments, but strongly attenuate the effect in radical pairs (*10*). We probe into the possibility that the third, scavenging radical could be produced in the photo-reduction, stored as persistent tyrosine radical, and made available to engage in the three-radical spin dynamics upon reoxidation of the fully reduced flavin cofactor by molecular oxygen. This model too is found suitable in the limit of swift spin relaxation in the formed ROS intermediates. However, we also notice that a model assuming a freely diffusing radical scavenger, such as $A^{\bullet-}$, might be preferable.

The immobilization of superoxide (or comparable ROS species) cannot overcome the sensitivity issue. Even if strong environmental interactions quenched the orbital angular momentum and slowed the rotational tumbling (and thus the spin relaxation), immobilization would suppress MFEs in radical pairs due to exceedingly strong inter-radical interactions at distances suitable for radical recombination (*10*). Again, we find that

this limit can be overcome by a third radical, which could be an inert bystander (*10*), or reactive (*8*). Eventually, we demonstrate that the electron-electron dipolar coupling is not always detrimental. In systems of more than two radicals, it can promote magnetic field effects even in the absence of hyperfine interactions. This model could substantiate MFEs in lipid peroxidation, which involve radicals devoid of strong hyperfine coupling interactions (*13*).

Discussion & Conclusion

In relation to ROS, magnetic field effects due to the established radical pair mechanism appear unlikely in realistic reaction scenarios. Yet, ROS related effects have been widely postulated. We suggest that these effects can be accommodated in extended models for which the primary radical pair undergoes a competitive reaction with a third radical, such as a persistent radical derived from a radical scavenger. These models predict large magnetosensitivity of dark-state radical reactions involving ROS, despite their unfavourable properties precluding magnetosensitivity via the classical RPM. Ultimately, the credible possibility of such MFEs, suggest that the redox homeostasis could be linked to the geomagnetic field via the magnetosensitivity of the ROS-generated processes in competition with radical scavenging. This possibility raises the exciting prospect to manipulate biological processes by applied static and oscillatory fields via direct modulation of ROS levels. This could be relevant for space travel, but also in the context of many neurodegenerative diseases, which are frequently accompanied by redox imbalances leading to increased ROS levels. Eventually, redox homeostasis could be regarded as a, possibly indirect/coincidental, quantum effect in biology.

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SESSION 4: SYSTEMATIC REVIEWS

WHO project on adverse health effects of radiofrequency electromagnetic fields

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The World Health Organization (WHO) has a long-standing history in reviewing the findings of research on the health effects of exposure to electromagnetic fields. For radiofrequency electromagnetic fields, the latest overview dates to 1993. An update is being conducted that will result in a scoping review of all reported adverse health outcomes. Based on the adverse health outcomes published around the world, a priority setting exercise was conducted that led to six priority topics to be systematically reviewed, namely on cancer, fertility, cognitive functioning, symptoms, heating, and oxidative stress. The first four can be considered health outcomes, whereas the latter two explain mechanisms of action.

Ten systematic review teams were commissioned with conducting reviews of various evidence streams on the priority topics. Each team was provided with a draft protocol and asked to write a protocol to be published in Environment International and then conduct the systematic review. The publication of a protocol with a a-priori specification of the methods will greatly reduce bias.

In contrast to the scoping review, the systematic reviews will not provide an overview of what has been published on a topic but try to answer specific questions. An example of such a so-called PECO question, stated in terms of the Population, Exposure, Comparator and Outcome, is: “What are the effects of localised and whole-body RF-EMF exposure (E) on male infertility; sperm morphology; motility; concentration or count, and time to pregnancy (O) compared to no/low level of exposure (C) in adult males (P) within human observational studies?”. Finally, the answers to these questions will enable conclusions on overarching health outcomes such as ‘Does RF EMF lead to problems with fertility’. These conclusions will be based on the systematic reviews of human observational and experimental studies, animal studies, and in-vitro studies that explain mechanisms of action.

Nine protocols have now been published in a special issue of the journal *Environment International* (https://www.sciencedirect.com/journal/environment-international/special-issue/109J1SL7CXT). The results of the systematic reviews are expected in the first half of 2022 and the final conclusions of the project in the second half of 2022.

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SESSION 4: SYSTEMATIC REVIEWS

Assessment of evidence in scientific studies

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Evidence-based methodologies, developed in the field of clinical research, are now the standard for assessment of the entirety of available evidence published in clinical studies and, moreover, are the basis for evidence-based medical practice. The adaptation of evidence-based methods, including their principal instrument, systematic reviews, to public and environmental health have started in the last decade with the Navigation Guide[1], followed by the US National Toxicology Program Handbook for Conducting Systematic Reviews for Health Effects Evaluations[2]. The Primer on application of systematic review to the field of toxicology was developed by a multi-stakeholder EBTC working group, where the key steps of systematic review have been outlined, as well as the gaps in methods and challenges in application of available tools discussed[3].

History of evidence-based methodologies and examples of their adaptation to the field of toxicology and environmental health will be presented, key recent advancements and challenges discussed, as well as lessons learned from training basic scientists, regulators and academic groups in presenting and publishing their work such that the evidence could be extracted and used to develop public health guidelines.

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SESSION 4: SYSTEMATIC REVIEWS

A Case Study of its Application for a Systematic Review of the Effects of Radiofrequency Electromagnetic Fields on Biomarkers of Oxidative Stress

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Short Summary

Documents discovered through comprehensive searches of the literature constitute a large and important part of the body of evidence examined by systematic reviews. Guidelines for the conduct of systematic reviews, including from Cochrane, the National Academy of Sciences, and the World Health Organization (WHO), stress the importance of including an information specialist on the review team and outline in broad terms the roles that information specialists play in developing and running searches in bibliographic databases. These guidelines also do a good job of summarizing some of the complexities that may be encountered when developing a search strategy. They also allude in general terms to the fact that information specialists work with subject matter experts on the review team to craft effective searches. This talk will go beyond these generalities to present a detailed description of the search development process for an on-going systematic review of the effects of radiofrequency electromagnetic fields (RF-EMF) on biomarkers of oxidative stress. Challenges encountered when building searches for specific databases will be highlighted, as well as the ways that the review team examined multiple iterations of the search and provided critical input on search terms. Finally, evidence that validates the considerable time and effort invested in this thorough and systematic approach to searching will be presented.

Introduction

The search development process described here was part of an on-going systematic review funded by the WHO as one of ten related systematic reviews designed to assess the potential health effects of exposure to RF-EMF. Search strategies were developed to address the objective of the systematic review – to assess the effects of exposure to RF-EMF on the biomarkers of oxidative stress in experimental *in vivo* and *in vitro* studies. Major elements of the search strategies were derived from a PECO (population, exposure, comparator, outcome) statement that provides the framework for the review.

Five databases were searched to help ensure a comprehensive coverage of the literature – PubMed, Embase (via Embase.com), Scopus, the Web of Science Core Collection, and the EMF-Portal. PubMed and Embase provided a thorough coverage of the biomedical literature. Scopus and Web of Science have broad, multidisciplinary emphases and so contributed to the biomedical coverage while adding unique content in related, potentially relevant fields, such as bioelectrochemistry, biomedical engineering, biomedical materials, biotechnology, high voltage engineering, microwave technology, and radio science. The EMF-Portal, in contrast to the other four databases searched, has a much narrower range, systematically curating a collection of documents focused on the health effects of electromagnetic fields, including RF-EMF. This added a unique, specialized slice of the literature to our sources of evidence.

The search strategies were developed by two information specialists working within a systematic review team comprised of experts in systematic review methods, statistical analysis, RF-EMF exposure, and biomarkers of oxidative stress.

Materials & Methods

A two-concept approach was adopted for the search strategies as a way of effectively capturing relevant studies across a wide range of populations (human and animal), study designs (*in vivo* and *in vitro*) and comparators (sham or no exposure). The two concepts, or main elements, of the search strategies relate to the exposure and outcome components of the PECO statement developed by the review team. Review team experts provided the information specialists a specific frequency range for RF-EMF and a detailed list of oxidative stress biomarkers. These reference points were used in building out the search terms for each concept. A search strategy was first developed for PubMed and then translated into the other four databases.

Search concepts were built as strings of synonyms connected with the Boolean OR operator. Controlled vocabulary terms (i.e. terms that are part of database-specific, hierarchically-arranged thesauri) were identified for the PubMed search using the MeSH Database and for the Embase search using Emtree. Both parent and child terms in MeSH and Emtree hierarchies were examined for inclusion. Controlled vocabulary terms were supplemented with extensive collections of keyword terms derived from MeSH and Emtree synonym lists, words encountered in on-target articles, and expert knowledge.

The two search concepts were run individually using the advanced search features of the databases as a way to check for errors. These two concepts were then combined in the advanced search interface using the AND Boolean operator, generating a final result set whose documents all had an element related to RF-EMF and an element related to biomarkers of oxidative stress. One exception to this approach involved the EMF-Portal, which required splitting the oxidative stress biomarker concept into 36 separate searches (see below).

Multiple iterations of the PubMed search strategy were developed and run in order to achieve an effective balance of precision and recall in the results. The specific search strings of these iterations were shared with the review team, as were sample sets of results. Feedback from the review team led to the addition of missing terms that generated new relevant results and the removal of existing terms that generated off-target results.

After a draft final PubMed search strategy was created, all of its elements were translated as faithfully as possible into search strategies for each of the other databases, starting with Embase. Additional relevant terms and terms generating off-target results were discovered during this translation process. This necessitated changes to the PubMed search strategy. As a penultimate step before running the final searches in all databases, all search strategies were checked to ensure an equivalent set of terms was being used.

Search quality was judged based on the precision of the results and whether or not the searches retrieved a pre-specified set of 39 on-target, gold standard articles.

The final searches were run on June 28, 2021. No date or language restrictions were applied.

Results & Discussion

Searches of the five databases yielded 22,097 results according to the following breakdown: PubMed (2,972); Embase (4,418); Scopus (7,629); Web of Science (4,636); and EMF-Portal (2,442). After the removal of duplicates, these results were reduced to 10,177 and formed the set of studies for title-abstract screening.

Nine iterations of the PubMed search strategy were developed over the course of five months. As part of this process, more than twenty RF-EMF exposure-related terms or term families suggested by review team members were tested. A number of these terms were found to add potentially relevant results. A similar set of twenty oxidative stress biomarker terms or term families recommended for removal were tested. Most of these terms had no impact on the results. For those terms that lead to fewer results when removed, none of the missed results were relevant. PubMed's interface changed during this time, as did the way it processed search terms. Additional testing was performed to ensure that search results were stable across PubMed versions, and some search syntax had to be changed.

A number of important discoveries were made during the process of translating the PubMed search strategy for use in the other databases. Synonyms found in Embase's controlled vocabulary sometimes gave off-target results and needed to be considered carefully. The use of a field code for searching for chemical entities in Scopus was found to generate large numbers of off-target results, was identified as redundant based on other field codes used in the search and was removed. Microwave-related exposure terms were found to generate many off-target results in Web of Science, owing in part to hits on studies where microwaves were used as a chemical synthesis method. These microwave-related terms were adapted to reduce this impact on the search. Finally, the EMF-Portal's searching rules required splitting a search strategy of more than 250 terms into 36 eight- to nine-term mini-strategies. Additional searching rules required the removal of both Boolean operators and truncation symbols (for finding word variants). The EMF-Portal automates both the insertion of the OR Boolean operator and the generation of word variants.

The value of the search development process designed by the information specialists and supported by the review team has been validated in four ways. First, our RF-EMF exposure terms matched those developed independently by other WHO RF-EMF review teams. Second, all 39 pre-identified, gold-standard articles were found by our searches. Third, our final set of search results have unique contributions from all five searched databases. Fourth, a set of 34 included studies related to oxidative stress outcomes that were found independently by a different WHO RF-EMF review team were also found by our searches.

Conclusions

Four main conclusions can be drawn from the search development process described above. One is that the involvement of subject matter experts is highly valuable for assessing the appropriateness of terms and the relevance of the results of test searches. Second, iteration in search building is important to optimize search strategies and to achieve a good balance of precision and recall. Third, the translation of search strategies across databases is a complex task that requires mapping across multiple controlled vocabularies and adjusting search strategies to account for differences in database coverage. Fourth, the search development process for systematic reviews is time- and labor-intensive but ultimately pays off by helping to ensure that relevant evidence is not missed.

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SESSION 5: RF-EMFS AND OXIDATIVE STRESS

Radio Frequency (RF) Exposure Generation and Assessment in Experimental Studies

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1. Introduction

The propagation and absorption of radiofrequency electromagnetic fields (RF-EMF) follows relatively complex physical principles. Therefore, with regard to experimental studies investigating possible health effects of RF-EMF, an interdisciplinary collaboration of biologists/medical scientists and RF engineers is essential to realize a study design on an adequate quality level. Unfortunately, in many cases this is not considered in practice and the complexity of RF dosimetry is often underestimated, with the consequence that the actual exposure applied during the experiments cannot be quantified or can only be quantified with great uncertainty. Nonetheless, such studies still find their way into respected scientific journals, showing that even in the review process prior to publication, insufficient attention is paid to the issue of adequate RF dosimetry. While it is probably impossible to get a manuscript published dealing with possible effects of a chemical substance or pharmaceutical agent without correctly quantifying the dose administered, this is apparently still possible for studies dealing with possible effects of RF-EMF, although the technical possibilities for an adequate recording of exposure are available and have undergone an enormous increase in quality over the past two decades.

2. Basic properties of RF-EMF relevant for the design of experimental studies

One of the basic phenomena in this context is the fact that in the frequency range of several hundred MHz and above the wavelength λ is already in the same order of magnitude or smaller than the typical system dimensions of the experimental setup, and therefore the propagation of the RF EMF increasingly follows optical laws, i.e. reflections, diffraction, refraction and partial absorption of the radiated energy occur at all objects. An immediate consequence of this is that multipath propagation occurs and, as a result of destructive and constructive interference, the field energy is distributed in space in a complex and heterogeneous pattern. Therefore, one can no longer assume that the field strength decreases monotonically with distance from the source. Rather, a complex spatial field distribution arises in which field strength maxima and minima alternate within distances of approximately $\lambda/4$, whereby the ratio of maximum to minimum field strength in highly reflective environments can be significantly more than a factor of 10 (corresponding to significantly more than a factor of 100 for the power or energy, respectively). Considering, for example, a typical cage for rats or mice (dimensions of the footprint about 20 cm x 30 cm) and a frequency around 1800 MHz ($\lambda/4 \approx 4$ cm), it is clear that several maxima and minima of field strength can occur within the cage and the exposure of the animals, depending on their location within the cage, may vary greatly.

Another important aspect is the fact that the extent of absorption (i.e., how much RF energy is absorbed inside the body) depends not only on the field strength at the respective location, but additionally on a whole range of other parameters, such as frequency, size and shape of the body, orientation of the body in relation to the field vector, dielectric properties of the body (body tissues), direction of wave incidence, etc. Finally, in the case of freely moving animals, shadowing effects due to closely neighboring animals must also be considered as a possible influencing factor.

In the case of exposure in the reactive near field of the RF source, i.e. when the source is in close proximity (typically within a few centimeters) to the body, the situation becomes even more complicated, since in this case the body can also significantly influence the properties of the source.

3. Quantification of RF exposure

From the fact that the RF energy which is finally absorbed in the body or cell culture depends not only on the field strength measurable at the subsequent location of the body or cell culture, but also on many other factors, it is immediately apparent that the specification of these (body-external) field strength values alone is generally not a reliable method for quantifying exposure. Rather, it is necessary to determine the RF energy or RF power actually absorbed inside the body (e.g., in the form of the specific absorption rate SAR in W/kg). SAR is not only a measure of the RF energy or RF power absorbed inside body tissues (finally converted to heat) but is also directly related to the field strength occurring inside the tissue. The methods for a determination of body-internal exposure metrics (e.g. SAR) are way more complex than the measurement of external field strengths, and require relatively large amounts of expertise and computer hardware, software, and measurement resources. An accurate determination of the SAR inside the body or in different organs or in cell cultures is only possible on the basis of computer simulations using appropriately representative detailed numerical models of the exposed body and the radiation source (antenna) used. However, for validation of the computational model, especially with respect to the numerical model of the antenna and the influence of reflective objects in the field space, an experimental validation of the computer model is necessary (e.g. SAR measurements in simplified homogeneous body phantoms).

4. RF-induced temperature elevation

Another essential aspect of experimental studies investigating possible effects of RF-EMF on various biological endpoints is that of RF-induced temperature increase in the exposed tissues or cell cultures. Temperature elevation is, on the one hand, a direct consequence of RF energy absorption, but on the other hand, it is known to be a mediator of virtually all chemical and biological processes. The consideration of how much RF-induced temperature elevation inside the tissue or cell dish is acceptable in an experimental design, to still exclusively assess the effects of RF absorption should therefore be central from the very beginning of the study design. As a further complicating fact, it has to be noted that it is almost impossible to achieve the same relation between SAR and tissue temperature elevation in an experimental animal model as it occurs in real-world human exposure. In any case, it is essential to quantify as precisely as possible the temperature increases to be expected during the experiment.

5. Concepts of exposure systems

Over the years, many different concepts for exposing animals, cell cultures or human subjects to RF-EMF in experimental studies have been published. Unfortunately, the majority of these concepts were far from what could be called well thought out, i.e., the basic properties of RF-EMF described above have not been sufficiently taken into account. In such cases the uncertainty of exposure in terms of the actually absorbed RF-energy inside the exposed sample is often not better than a factor of 10-100, and therefore often causing doubts about sufficient exposure contrast between different experimental groups or conditions, respectively.

However, there have also been reported highly sophisticated exposure systems along with a detailed dosimetric characterization. For example, for large scale animal studies in the frequency range above several hundred MHz, concepts based on the principle of radial waveguides, with the animal cages located along a

circle around a central antenna have been reported for frequencies up to approx. 2 GHz (e.g. [1], [2]). A more recent approach is based on the exposure inside reverberation chambers (e.g. [3], [4]).

Similarly, there are also well characterized exposure apparatuses available for in vitro investigations. Such system concepts include (resonant) waveguides (up to approx. 3-4 GHz) (e.g. [5], [6]) or exposure in front of the aperture of directional antennas, applicable up to millimeter wave frequencies (e.g. [7],[8]).

However, independently of the chosen concept for the exposure facility, it is important to thoroughly check the appropriateness for the actually considered study design. Even changes in the study design that appear to be of low relevance from the biological point of view may cause the necessity for a re-evaluation with respect to RF-exposure of the considered animals, cell culture or human subjects, respectively.

6. Conclusions

From what has been said above, it is evident that a qualitatively adequate exposure setup can only be developed in close cooperation between biologists/medical scientists and experienced RF engineers in order to find an appropriate compromise between biological/medical requirements for the study design and a well quantifiable exposure of the experimental animals or cell cultures or subjects. Otherwise, there is a great risk that the study results cannot be interpreted or can only be interpreted with difficulty due to confounding factors or large uncertainties concerning the actual applied exposure level, resulting from an insufficiently well thought out RF exposure system.

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SESSION 5: RF-EMFS AND OXIDATIVE STRESS

The effects of radiofrequency exposure on biomarkers of oxidative stress, a systematic review of experimental studies

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Background: Oxidative stress is conjectured to be related to many diseases, and there is the hypothesis that radiofrequency fields may induce oxidative stress in various cell types and thereby compromise human and animal health. This systematic review (SR) aims to summarize and evaluate the literature in this field.

Objectives: The main objective of this SR is to evaluate the associations between the exposure to radiofrequency electromagnetic fields and oxidative stress in experimental models (in vivo and in vitro).

Methods: The SR framework has been developed following the guidelines established in the WHO Handbook for Guideline Development and the Handbook for Conducting a Literature-Based Health Assessment). We will include controlled in vivo and in vitro laboratory studies that assess the effects of an exposure to RF-EMF on valid markers for oxidative stress compared to no or sham exposure. The protocol is registered in Prospero.

We will search the following databases: PubMed, Embase, Web of Science, Scopus, and the EMF-Portal. The reference lists of included studies and retrieved review articles will also be manually searched.

Study appraisal and synthesis method: Data will be extracted according to a pre-defined set of forms developed in the DistillerSR online software and synthesized in a meta-analysis when studies are judged sufficiently similar to be combined. If a meta-analysis is not possible, we will describe the effects of the exposure in a narrative way.

Risk of bias: The risk of bias will be assessed with the NTP/OHAT risk of bias rating tool for human and animal studies

We will assess the certainty of the conclusions (high, moderate, low, or inadequate) regarding the association between radiofrequency electromagnetic fields and oxidative stress using GRADE.

References:

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SESSION 6: ELF-EMFS AND OXIDATIVE STRESS

Exposure assessment for ELF-EMF in humans

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Short summary
This talk will give an overview of computational dosimetry methods for exposure assessment of extremely low frequency electromagnetic fields and will focus on dosimetry modelling using realistic anatomical models. The latest results on individualized dosimetry for exposure assessment will also be presented.

Introduction
The international guidelines and standards for limiting human exposure to extremely low-frequency electromagnetic fields (ELF-EMF) are based on preventing adverse effects in both the central and peripheral nervous systems (ICNIRP 2010, IEEE 2019). These effects include retinal phosphenes (which are not treated as adverse on their own, but effects in the brain could occur via similar mechanisms) and activation of axons of peripheral nerves and the central nervous system. As these effects are related to the electric field induced in the tissue, the limits on human exposure are given in terms of the electric field strength.

In typical exposure scenarios, the international bodies provide reference levels for the external electric and magnetic field strengths, which can be easily used to verify that the induced electric field in the body is weaker than the exposure limit. However, if the exposure is strong and/or a more accurate assessment of the induced electric field strength is needed, detailed dosimetry modelling is needed, as the induced field strongly depends on both the exposure scenario and anatomy. This talk will give an overview of the dosimetry modelling techniques used for exposure assessment, starting with the physical and mathematical background and computational methods. The talk will also introduce the latest results from our lab on inter-individual variability and experimental validation of dosimetric models.

Materials & methods
In the ELF range, the body is transparent to the external magnetic field and opaque to the external electric field. Exposure to a magnetic field induces an electric field in the body via Faraday's law of induction, and the exposure to an external electric field generates an internal electric via electroquasistatic induction. Dosimetry of the internal electric fields induced by these coupling mechanisms can be performed using computer simulations, e.g., using the finite element method. For precise estimation of the induced electric field strength, realistic models of the human body are needed. Whole body models of adults and children various ages have been developed by several research groups and have been widely used for dosimetry modelling. Recently, we and others have also developed computational pipelines that can be used for the automatic generation of individualized anatomical models from magnetic resonance images of the head. We have recently used these models for both studying the inter-individual differences in the induced electric fields, and, in combination with magnetic or electrical stimulation techniques, to validate the accuracy of dosimetry models.

Results
To characterize the inter-individual in the electric field induced in the brain, we recently performed a large-scale computational study featuring 118 individual head models generated from magnetic resonance images of adults (Soldati et al 2020). The head models were exposed to uniform magnetic fields at 50 Hz. As

expected from Faraday's induction law, the results showed increased induced field strength in individuals with larger head sizes. We also found a statistically significant effect of age, older people producing increased electric field strength in the brain. Despite the systematic effects of size and age, the overall variability was still relatively small; standard deviation among the whole study group was approximately 10% of the mean.

A project is currently ongoing to validate the predictions produced by dosimetric models. For this purpose, we are using transcranial magnetic stimulation, which uses strong pulsed magnetic fields that induce electric fields strong enough to directly activate neurons in the cerebral cortex. When the motor cortex is stimulated, the stimulation produces motor responses that can be objectively measured. When individualized head models are used, the electric field strength calculated in the motor cortex can be used to predict the motor threshold in each participant, which can be directly compared to the measurements. Initial data in ten participants suggest that the measured motor threshold data fits within the prediction intervals produced by dosimetry modelling.

Discussion & conclusions

Relatively few anatomical models have conventionally been used for exposure assessment due to limited availability of detailed anatomical models. New developments in image processing techniques have made it possible to perform dosimetry at the individual level. Individual dosimetry allows the exposure assessment at the population level considering anatomical variability, and opens new possibilities for the validation of dosimetry modelling. The data on inter-individual differences can be highly useful for deriving safety factors for ELF-EMF exposure.

References

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SESSION 6: ELF-EMFS AND OXIDATIVE STRESS

A systematic review of the effects of exposure to static- and low frequency electromagnetic fields on biomarkers of oxidative stress

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Introduction

The human population nowadays is extensively exposed to electric, magnetic and electromagnetic fields (EMF) of all frequency ranges in different residential and occupational settings. Many scientific studies postulate oxidative stress as a possible mode of action in the development of putative adverse effects. However, the extent of the observed oxidative effects claimed to be caused by EMF are often low and their health relevance unclear. In addition, according to SCENIHR (2015), many of these studies show qualitative deficiencies, such as insufficient information on dosimetry or a complete lack of blinding. To assess the effect of EMF exposure on oxidative stress from the perspective of radiation protection, a systematic literature assessment on the influence of electric, magnetic and electromagnetic fields on oxidative processes in humans as well as in animal and laboratory studies is being performed in this project. As the effects of radiofrequency exposure on biomarkers of oxidative stress in humans as well as in animal and laboratory studies are being assessed in a separate review (Henschenmacher et al., 2022; PROSPERO ID: 235573), this review focuses on the exposure to EMF with frequencies below 100 kHz, namely static and low frequency electric, magnetic and electromagnetic fields.

Material & Methods

Eligibility criteria

The eligibility criteria were defined using the Population, Exposure, Comparison, Outcome (PECO) strategy (NTP, 2015a). Articles are being included in this review when they report experimental human, in vivo or in vitro studies (P), with controlled exposure to static or low frequency electric, magnetic or electromagnetic fields in the frequency range of 0 Hz – 100 kHz (E). To be eligible for inclusion, studies further have to report a non-exposed or sham exposed control group (C). Furthermore, only studies are being included, that have examined at least one valid biomarker that represents a reliable and direct link to oxidative processes within the cell. Therefore, in vivo studies must report the measurement of at least one of the following biomarkers: *8-oxo-2'-deoxyguanosine, Chlorotyrosine, Glutathionylated protein / mixed protein disulfides, 4-hydroxy-2-nonenal /HNE / HNE-conjugated proteins, 15F2t-IsoProstane/ 8-iso-PGF2a, Methionine sulfoxide and cysteic acid, Nitrotyrosine (free or in proteins), Protein carbonyls/oxyblot technique, Dityrosine.* In vitro studies are being included, if one of the aforementioned biomarkers or one of the following biomarkers measured with a time course is reported: *Superoxide, Glutathione/ GSH/GSSG ratio, Lipid peroxides / lipid hydroperoxides, Peroxynitrite, Hydrogen peroxide* (O).

Excluded from this review are dosimetric studies, reviews, and commentaries as well as studies which investigated only co-exposures to different EF, MF and EMF or co-exposures with non-EMF stressors. Furthermore,

studies in which data have been obtained from less than three biological replicates in independent experiments, or the sample size and/or the number of replicates are not reported at all, are being excluded.

Information sources and search strategy

Only peer-reviewed articles are being considered, whereby no restrictions on the publication date and language are applied. The databases PubMed, Web of Science, Scopus, and EMF-Portal are being searched, using keyword terms and controlled vocabulary related to our PECO statement. To assure an optimal precision and recall in results, the search strategy was developed and validated on the base of an advanced set of on-target articles. The searches will be re-run just before the final analyses, to retrieve the most recent studies eligible for inclusion.

Study selection

Records and data are being managed using the DistillerSR web application for systematic reviews. Search results are being imported into DistillerSR for study selection from EndNote after de-duplication. Titles and abstracts of retrieved studies are being screened independently by two review authors. The full text of potentially eligible studies are being retrieved and independently assessed for eligibility by two review team members. Any disagreement over the eligibility of particular studies is being resolved through discussion with a third reviewer. The reasons for exclusions of full-text articles are being recorded in DistillerSR.

Data extraction

A standardized, pre-piloted form, implemented in DistillerSR, is being used to extract data from the included studies for assessment of study quality and evidence synthesis. One reviewer is extracting and recording the relevant features of each eligible study. A second reviewer proofs the extracted study information against the accompanying articles for completeness and accuracy. The detailed information in regard to the data that will be extracted in this study is available on the PROSPERO registration website under *CRD42021225170*.

Study appraisal

The validity and quality of included studies is being assessed using the “Risk of Bias (ROB) Rating Tool for Human and Animal Studies” developed by the NTP Office of Health Assessment and Translation (OHAT). Studies are being assessed across six domains, with detailed criteria elaborated for each domain in the form of risk-of-bias questions specific for each type of study design. The following domains are being covered by the risk of bias questions: selection, confounding, performance, attrition/exclusion, detection, and selective reporting. Each domain is rated as definitely low RoB, probably low RoB, probably high RoB, definitely high RoB, or no information available. RoB is being assessed by two reviewers. For studies with in vitro assays, where there is no validated risk of bias tool, the OHAT RoB framework will be used used and modified. Further, the quality of evidence is being assessed following the GRADE (Grading of Recommendations Assessment, Development and Evaluation) standards.

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Results and Discussion

The review protocol was registered in the International prospective register of systematic reviews (PROSPERO) and is available under the ID: CRD42021225170. After searching the databases PubMed, Web of Science, Scopus, and EMF-Portal and removing the duplicates 14.466 Articles have been identified as possibly relevant for this review. The title and abstract screening revealed 1006 Articles that are currently being assessed for eligibility in the full text level.

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SESSION 6: ELF-EMFS AND OXIDATIVE STRESS

Radical pair based magnetic field effects on the autofluorescence of living cells

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Summary

In this study, we demonstrate a robust, reproducible, real-time observation of the effect of millitesla order magnetic fields on the autofluorescence of living HeLa cells and provide evidence that the effect originates in radical pair mediated effects on the fluorescence of flavin molecules.

Introduction

Flavins are ubiquitous molecules in nature and in particular are important co-factors in blue-light photoreceptor proteins. One in particular, cryptochrome, is the putative magnetoreceptor at the heart of the radical pair hypothesis of animal magnetoreception [1]. Flavins readily undergo photoinduced electron transfer with electron donors such as tryptophan to generate spin-correlated radical pairs (RPs) which can undergo coherent evolution between singlet and triplet spin states and spin-selective back electron transfer. This renders their photocycle sensitive to externally applied magnetic fields. We recently demonstrated that flavin adenine dinucleotide (FAD) shows a magnetic field dependent photocycle even at physiological pH in the absence of an external electron donor [2].

It is well established that flavins are present in living cells and are responsible for cellular autofluorescence around 520nm. They are also known to be responsible for the production of reactive oxygen species in cells on exposure to visible light (particularly in the 400 nm – 500 nm range) [3]. To date there has been no direct evidence of radical pair based magnetic field sensitive photochemical processes at the cellular level. In this study, we used a magnetic field effect fluorescence-based microscope, to investigate the possibility of a magnetic field response of living cell autofluorescence based on the hypothesis that flavin photochemistry within cells might produce magnetically sensitive radical pairs and through the reaction cycle produce a magnetic field response in the autofluorescence signal. Such experiments are experimentally challenging due to the weak fluorescence signal, the small size of anticipated magnetic field responses and the rapid photobleaching of the autofluorescence signal.

Materials and Methods

Our self-constructed microscope utilizes a 100x oil objective lens with a numerical aperture of 1.49. Photoexcitation is provided via a 450 nm diode laser delivered through a single-mode fiber and beam-waist adjusted to provide an optimized, focused spot size for targeting HeLa cells. The magnetic field is supplied to the sample using a projected vector field electromagnet which is capable of generating a magnetic field in any arbitrary direction relative to the sample, and of amplitude and direction modulating said field. For autofluorescence magnetic field measurements, to avoid photobleaching of flavin autofluorescence, we first used bright-field illumination to identify a HeLa cell for observation and recorded an image of the cell prior to blue light photoexcitation. We then imaged the cell fluorescence under continuous irradiation with 1.0 mW of 450nm laser light, 100% amplitude modulated at 100 Hz with a 50 % duty cycle and an applied triangle-wave modulated magnetic field typically varying between +25 mT and -25 mT at much lower frequencies (from 0.05 to 0.25Hz).

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An extensive series of control experiments were performed and demonstrated clearly that our instrument and experimental protocol introduces no artefactual magnetic field responses.

Results and Discussion

We were able to repeatedly demonstrate consistent magnetic field responses in a large number of HeLa cells. Figure 1A shows a typical (not best) bright field image of a HeLa cell selected for measurement along with the overlaid fluorescence signal (the blue circle indicates the irradiation position). Figure 1B shows a portion of the time decay of the observed fluorescence (the inset shows the full time dependence recorded) in the presence of a 0.15Hz triangle wave magnetic field of amplitude 25mT. A corresponding oscillation is clearly observed in the fluorescence signal. By fitting a curve to the decay signal and subtracting it from the signal itself, the magnetic field response can be isolated. This is shown in figure 1C as a percentage of the total fluorescence. Other experiments measured i) the spectrum of the observed fluorescence, which was consistent with that produced by protein bound flavins and ii) the magnetic field strength dependence of the observed effect (the MARY curve) at different magnetic field modulation frequencies, which allowed us to abstract a $B_{1/2}$ (magnetic field at half saturation) value of 18.0 mT and a field effect saturation value of 3.7%.

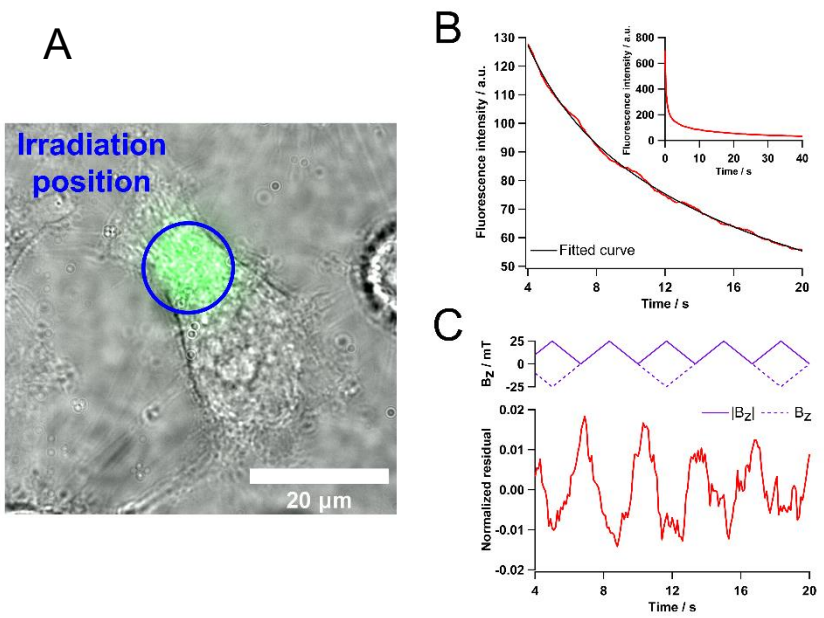

Figure 1. Magnetic field effect on the autofluorescence of HeLa cells. (A) Merged bright field and fluorescence images of a representative HeLa cell showing a magnetic field response. (B) Averaged autofluorescence change of the irradiated region of the HeLa cell with the application of a modulated external magnetic field (triangle wave, 0.15 Hz of frequency, 25 mT of amplitude). The insert figure shows the complete time period of the experiment. (C) Normalized residual intensity calculated by the average autofluorescence intensity divided by the value of the fitted curve representing the fractional MFE (adapted from [4]).

Conclusions

We demonstrated a direct response of the autofluorescence of native, living, cultured HeLa cells to the application of a modulated magnetic field of 25mT and less, and characterised its magnetic field dependence which was consistent with the radical pair mechanism observed through the fluorescence of protein bound flavin molecules.

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