REJUVENATION RESEARCH Volume 17, Number 2, 2014 © Mary Ann Liebert, Inc. DOI: 10.1089/rej.2013.1487

Efficiency of Cellular Growth When Creating Small Pockets of Electric Current Along the Walls of Cells

Gunther Kletetschka,^{1,2} Vojtech Zila,¹ and Lucie Klimova^{1,3}

Abstract

Pulses up to 11 Tesla magnetic fields may generate pockets of currents along the walls of cellular material and may interfere with the overall ability of cell division. We used prokaryotic cells (*Escherichia coli*) and eukaryotic cells (murine fibroblast, embryonic stem cells) and exposed them to magnetic pulses of intensities ranging from 1 millitesla (mT) to 11,000 mT. We found prokaryotic cells to be more sensitive to magnetic field pulses than eukaryotic cells.

Introduction

DEVELOPMENT OF METHODS OF delivering living organisms with ferromagnetic iron-bearing nanoparticles (NPs) uses strong magnetic gradients for NPs to transport within the organisms.¹ Magnetic resonance imaging (MRI) uses magnetic field in excess of several Tesla to monitor degenerative diseases.^{2,3} There is a likely relation between magnetic power and cancer growth. A magnetic field of 50 Hz and 100 microtesla enhanced tumor development and growth in the breast cancer in female Sprague-Dawley rats.⁴ It has been hypothesized that the increased breast cancer rates in industrial societies is related to greater exposure to power-frequency electric and magnetic fields (EMFs). EMFs are thought to reduce the circulating level of melatonin, increase estrogen levels, and stimulate the turnover of epithelial stem cells that may possibly lead to malignant transformation,⁵ possibly due to telomere abrasion that limits the life span not only mammalian somatic cells^{6,7} but also pine trees.⁸ Furthermore, the frequency rhythmic electrical modulation system (FREMS) showed a positive response in treatment of venous leg ulcers.⁹

There is no consensus about the effect of magnetic fields. Therefore, we used three different cell cultures, bacteria *(Escherichia coli)*, nurine 3T6 fibroblasts, and murine embryonic fibroblasts (MEFs) for exposure to various magnetic field peak intensities from 1 millitestla (mT) to 11,000 mT. The peak of magnetic flux was responsible for setting up the inducing currents within the cells. Generated currents quickly decayed with time due to ohmic losses of the saline cellular solution.

Material and Methods

Pulses of various magnetic field peak intensities were generated by a magnetic pulse–generating system (Magnetizer Model IM-10-30, ASC Scientific). The coil used for this experiment was custom made to achieve 11,000 mT maximum pulse density ever the space of 5 mm in diameter and 5 cm of length within 3–4 sec.

For experiments with *E. coli*, we used Petri dishes with agar, micropipettes, tips, incubator, Bunsen burner, glassware for holding solutions, chemical hood, optical microscope, camera, and plating wands. Tubes containing *E. coli* cultures were placed into the magnetizing coil interior so that the full content of the *E. coli* culture was receiving a magnetic pulse. Cells were exposed one time or 10 times with a pulse of 11,000 mT. Cells not exposed were used as a control. Five hours after exposure, cells were plated on agar in Petri dishes at 37°C. Colonies were counted using an optical microscope. Experiments took place at the Department of Biology of Johns Hopkins University (Baltimore, MD).

Swiss albino mouse fibroblast 3T6 cells (American Type Culture Collection [ATCC] CCL-96) or MEFs were grown on 10-cm Petri dishes at 37°C and 5% CO₂ in complete Dulbecco modified Eagle medium (DMEM) (Sigma-Aldrich, St Louis, MO) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich) and GlutaMAX-I (Gibco, Life Technologies). Cells were harvested by incubation with 0.25% trypsin/0.53 fmM EDTA for 3–5 min to detach the cells from the Petri dish and centrifuged at $250 \times g$ for 5 min. Pelleted cells were re-suspended in DMEM, and their concentration was determined using a Bürker chamber. Cell

¹Faculty of Science, Charles University in Prague, Czech Republic.

²Institute of Geology, ASCR, v.v.i., Prague, Czech Republic.

³Institute of Molecular Genetics, ASCR, v.v.i., Prague, Czech Republic.

2

KLETETSCHKA ET AL.



FIG. 1. Bacteria or murine eukaryotic cell conditions when experiencing magnetic pulses, each lasting 1–4 sec. (A) *Escherichia coli* cells were exposed 10 times (C-3) or 1-time (C-4) to a magnetic intensity field of 11 T_i and plated on Petri dishes for 5 hr. As a control, non-exposed cells were plated (C-1, C-2). Photographs of *E. coli* colonies at 5 hr after exposure are shown. (B) Concentrations of *E. coli* colonies. Data represent mean values \pm standard deviation (SD) from three independent experiments. (C–E) 3T6 or MEF cells were exposed five times to various magnetic intensity fields, seeded on to six-well dishes, and cultivated for 72 hr. (C) Bright-field microscopy images of control, non-exposed cells, or cells after exposure to 10,000 mT, the highest magnetic field intensity used in experiment. (D and E) Counts of 3T6 (D) or MEF (E) cells 72 hr after exposure to various magnetic intensity peaks. Data in graphs represent mean values \pm SD from three independent experiments.

suspensions were transferred to 0.25-mL tubes and exposed five times with peak magnetic field intensities of 1, 10, 100, 1000 and 10,000 mT using an ASC Magnetizer. Between each exposure, cells were kept at 37°C. After exposures, equal numbers of 3T6 cells (2×10^3 cells/well) or MEFs (5×10^3 cells/well) were seeded on six-well dishes and grown for 72 hr. After that time, cells were harvested by trypsinization and re-suspended in culture medium. Their concentrations were determined by microscopy using a Bürker chamber. Experiments took place at Faculty of Science, Charles University in Prague.

■EO1

Results and Discussion

A magnetic pulse with 11 Tesla did not compromise the bacteria's dividing potential. Exposure of cells to repeated

GROWTH OF CELLS AFTER ELECTRIC CURRENT EXPOSURE

magnetic pulses did not result in cell death. Bacterial cell growth was far more efficient when compared with controls. Magnetic field (11 Tesla) exposure resulted in increased cellular count by 260% when cells were exposed 10 times to the magnetic pulsing, and by 370% when exposed just one time. A single exposure resulted in maximum division yield. Exposure for 10 times was not as beneficial and probably may have caused some disruption, but overall the culture still outperformed the controls.

For both tested murine cell lines, the increase in EMF intensity did not result in cell death. Exposure of cells to all of the tested magnetic field intensities did not significantly affect their ability to grow, and the amounts of cells at the time of counting was comparable with the controls.

These experiments indicate a remarkable resistance of *E. coli* cells as well as <u>mammalian MEFs</u> to exposure to high magnetic field intensities. Extending these prokaryotic cell properties to mitochondria, we project that magnetic field activation may activate mitochondria genesis and thus influence aging.¹⁰ Observation of a dramatic enhancement of multiplication of *E. coli* cells was in strong contrast to an insignificant effect of magnetic pulses on growth of murine 3T6 or MEF cells, suggesting that effect of large magnetic field intensities may differ between prokaryotic and eukaryotic cells.

Despite the complexity of the cellular division process, physics allows predictions of the speed of proliferation and how much heat the bacterial cells may generate during the division process.¹¹ Theoretical predictions¹¹ suggest that the organism faces restrictive impediments to growth that arise from the speeds of rate-limiting reactions. Magnetic exposures indicate that for prokaryotic cell (*e.g., E. coli*) growth such reactions proceed at a significantly faster rate whereas changes in reactions during the growth of eukaryotic cells (murine 3T6 and MEF fibroblasts) are not measurable.

Acknowledgments

We thank Jocelyne DiRuggiero and her student Courtney Robinson for using their facilities at John Hopkins University. G.K. was supported by a Ministry of Education Youth and Sports grant no. LK21303.

Author Disclosure Statement

No competing financial interests exist.

References

 Kharlamov AN, Gabinsky JL. Plasmonic photothermic and stem cell therapy of atherosclerotic plaque as a novel nanotool for angioplasty and artery remodeling. Rejuvenation Res 2012;15:222–230.

- Serra L, Cercignani M, Petrosini L, Basile B, Perri R, Fadda L, Spanò B, Marra C, Giubilei F, Carlesimo GA, Caltagirone C, Bozzali M. Neuroanatomical correlates of cognitive reserve in Alzheimer disease. Rejuvenation Res 2011;14:143–151.
- 3. Prokopov AF. A case of recovery from dementia following rejuvenative treatment. Rejuvenation Res 2010;13:217–219.
- 4. Thun-Battersby S, Mevissen M, Loscher W. Exposure of Sprague-Dawley rats to a 50-Hertz, 100-mu Tesla magnetic field for 27 weeks facilitates mammary tumorigenesis in the 7,12-dimethylbenz a anthracene model of breast cancer. Cancer Res 1999;59:3627–3633.
- Graham C, Cook MR, Gerkovich MM, Sastre A. Examination of the melatonin hypothesis in women exposed at night to EMF or bright light. Environ Health Perspect 2001;109:501–507.
- Mendelsohn AR, Larrick JW. Ectopic expression of telomerase safely increases health span and life span. Rejuvenation Res 2012;15:435–438.
- Biotti D, Aho S, Bejot Y, Giroud M, Caillier M, Ragot S, Osseby GV, Moreau T, Teyssier JR. Leukocyte telomere length: A focus on cerebrovascular events. Rejuvenation Res 2012;15:274–80.
- 8. Flanary BE, Kletetschka G. Analysis of telomere length and telomerase activity in tree species of various lifespans, and with age in the bristlecone pine Pinus longaeva. *Rejuvenation Research* 2006; 9: 61–3.
- Santamato A, Panza F, Fortunato F, Portincasa A, Frisardi V, Cassatella G, Valente M, Seripa D, Ranieri M, Fiore P. Effectiveness of the frequency rhythmic electrical modulation system for the treatment of chronic and painful venous leg ulcers in older adults. Rejuvenation Res 2012; 15:281–287.
- Santiago C, Garatachea N, Yvert T, Rodríguez-Romo G, Santos-Lozano A, Fiuza-Luces C, Lucia A. Mitochondriogenesis genes and extreme longevity. Rejuvenation Res 2013;16:67–73.
- 11. England JL. Statistical physics of self-replication. J Chem Phys 2013;139:121923.

Address correspondence to: *Gunther Kletetschka Charles University in Prague Faculty of Science Albertov 6 Prague, 12843 Czech Republic*

E-mail: kletetschka@gmail.com

Received: September 3, 2013 Accepted: September 8, 2013

AUTHOR QUERY FOR REJ-2013-1487-VER9-KLETETSCHKA_1P

AU1: please confirm corresponding author name and address

EDITOR QUERY FOR REJ-2013-1487-VER9-KLETETSCHKA_1P

EQ1: Figure 1 is not cited in the text. Please check.