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BEMER Electromagnetic Field Therapy Reduces Cancer Cell Radioresistance by Enhanced ROS Formation and Induced DNA Damage.

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**Long term effects of Bio-Electromagnetic-Energy Regulation therapy on fatigue in patients with multiple sclerosis.**

Haase R¹, Piatkowski J, Ziemssen T.

**Author information**

**Abstract**

**BACKGROUND:** Electromagnetic-field therapy has beneficial short-term effects in multiple sclerosis (MS) patients with major fatigue, but long-term data are lacking. PRIMARY STUDY OBJECTIVES: To evaluate the long-term effects of a specific electromagnetic therapy device (Bio-Electromagnetic-Energy-Regulation [BEMER]) on MS-related fatigue, we designed a crossover control of a previously performed randomized controlled trial and a long-term open-label follow-up trial.

**DESIGN AND SETTING:** Crossover and open-label follow-up trials at a single neurological outpatient center.

**PARTICIPANTS:** Patients with relapsing-remitting MS who had major fatigue (N = 37 patients).

**INTERVENTION:** After a previous randomized controlled trial (exposure to low-frequency pulsed magnetic fields for 8 min twice daily or to placebo treatment for 12 wk), a crossover from control to treatment for another 12 weeks, followed by an open label follow-up trial to 3 years, were done.

**PRIMARY OUTCOME MEASURES:** The outcome criteria were the Modified Fatigue Impact Scale (MFIS), Fatigue Severity Scale (FSS), German long version of the Center for Epidemiologic Studies Depression Scale (CES-D), Multiple Sclerosis Functional Scale (MSFC), and Expanded Disability Status Scale (EDSS).

**RESULTS:** Patients previously on placebo during the randomized controlled trial experienced significant reductions in fatigue after crossing over to treatment. The MFIS and FSS scores were significantly lower in the open-label group than in the control subjects after follow-up. Participation in the open-label treatment was the strongest predictor of low fatigue outcome after followup. Electromagnetic-field therapy was well tolerated.

**CONCLUSIONS:** In this long-term study, a beneficial effect of long-term BEMER therapy on MS fatigue was demonstrated. Electromagnetic-field therapy may be a useful therapeutic modality in MS patients with severe fatigue.

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Synergistic effect of EMF-BEMER-type pulsed weak electromagnetic field and HPMA-bound doxorubicin on mouse EL4 T-cell lymphoma.

Bfhova B, Ety:ycb r, $rrova M, Eims11<j Ulbricb k ts:ovaf M.

Abstract
We have investigated the effects of low-frequency pulsed electromagnetic field (LF-EMF) produced by BEMER device on experimental mouse T-cell lymphoma EL4 growing on conventional and/or athymic (nude) mice. Exposure to EMF-BEMER slowed down the growth of tumor mass and prolonged the survival of experimental animals. The effect was more pronounced in immune-compromised nude mice compared to conventional ones. Acceleration of tumor growth was never observed. No measurable levels of Hsp 70 or increased levels of specific anti-EL4 antibodies were detected in the serum taken from experimental mice before and at different intervals during the experiment, i.e. before solid tumor appeared, at the time of its aggressive growth, and at the terminal stage of the disease. A significant synergizing antitumor effect was seen when EL4 tumor-bearing mice were simultaneously exposed to EMF-BEMER and treated with suboptimal dose of synthetic HPMA copolymer-based doxorubicin, DOX(HYD)-HPMA. Such a combination may be especially useful for heavily treated patients suffering from advanced tumor and requiring additional aggressive chemotherapy which, however, at that time could represent almost life-threatening way of medication.

Synergistic effect of EMF-BEMER type pulsed weak electromagnetic field and HPMA-bound doxorubicin on mouse EL4 T cell lymphoma

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Material and methods

Pol:mer conjugate DOXfYD_HPMA was prepared according to Etrych ct al. (2008). It is a doxorubicin bound to N-(2-hydroxypropyl)methylacrylamide (HPMA) copolymer carrier through a hydrazon bond with a MW-34000.

EMF source and exposure

The BEMER device is a certified medical instrument. The control unit works with an operating voltage from 12-15V. In the connected coil mat the special multidimensional pulsating current generates a weak, pulsating electromagnetic field. The basic BEMER impulse starts at a frequency of 0 Hz, is constantly increasing and within 30ms reaches its maximum of 2 kHz. From there it falls back to 0 Hz and the impulse starts again. Parallel the magnetic flux intensity begins at 0 µT and pulses upwards until it reaches its maximum, according to the chosen level. From there, like the frequency, it falls back to 0 µT and the impulse starts again (Fig. 1). For the experiment were chosen maximum levels of 3,5 µT; 1 0,5 rLT; 21 µT and 35 µT. The BEMER device neither offers the choice of only one constant frequency nor only one constant intensity.

Cancer cell line

Mouse T-cell lymphoma EL4 cells were obtained from American Type Culture Collection (ATTC).

Culture conditions

EL4 cells were grown at 37° C with 5% CO2 in RPMI 1640 medium (Gibco BRL) supplemented with heat-inactivated 10% v/v fetal calf crum (FCS) selected for low mitogenicity, 4 mM L-glutamine (Gibco BRL), 1 mM Na-pyruvate, 50 mM 2-mercaptoethanol, 4.5 g/l glucose, 100 U/mL penicillin and 100 µg/mL streptomycin (Sibilla).

Animals

All experiments were done either on conventional eight-week-old female mice of inbred strain C57BL/6 (H-i) purchased from the Animal Center of the Institute of Physiology, A.cademy of Sciences of the Czech Republic, v.v.i. or on eight-week-old female immunodeficient athytnic nu/nu CD-1 mice obtained from AnLab Ltd., Prague. The mice were randomly assigned to either experimental or control groups and housed in accordance with approved guidelines. Food and water were given ad libitum. The animal room was maintained at 22° C. The experimental designs were in accordance with the Czech Republic Act for Experimental Work with Animals (Decrees No.311/97, 117/87, and Act No. 246/96), which is fully compatible with the corresponding European Community Acts.

In vivo tumor growth

a) Exposure to EMF only

On day zero, 1 x 10^5 EL 4 T-cell lymphoma cells in 0.1 mL RPMI 1640 medium were injected s.c. (subcutaneously) on right back of C57BU6 or nu/nu CD-1 mice. The experimental animals were exposed to low-energy EMF - BEMER. Controls were
three independent experiments were conducted and differences between exposed and control animals with an error probability of $P < 0.05$ were considered to be statistically significant.

**Results and discussion**

Conventional C57BL/6 mice were first exposed to pulsed EMF - BEMER (30 min every four hours with the intensity of $0.5 \mu T$, $21 \mu T$ or $35 \mu T$ intensity or permanently with the intensity $3.5 \mu T$) on day four before s.c. transplantation of cancer cells and then every day until the end of the experiment. Figure 2A illustrates mean tumor volume change for each of the four treatment groups and Figure 2B documents survival of experimental animals. Both these show significant retardation of tumor growth and prolongation of lifespan in mice exposed to EMF - BEMER with an intensity of $21 \mu T$. The experiment was repeated three times with similar results.

The slight anti-tumor activity demonstrated in the experimental group exposed to EMF-BEMER 3000 of the intensity of $2 \mu T$ could be, among others, related to the activation of the immune system. To elucidate a tentative involvement of innate (natural or native) and/or adaptive (specific) immunity in the mechanism of action of EMF - BEMER on tumor growth, we used for further experiments immunocompromised athymic nude (nu/nu) mice. Athymic mice suffered from an extremely limited number of $T$ cells, which is the reason why they have only marginal specific immunity and are routinely used to define the role of $T/B$ lymphocytes in immunity and disease.

Similarly as in conventional mice, we have repeatedly observed in nude mice that EMF - BEMER to which the animals were exposed slowed down the growth of experimental EL4 T-cell lymphoma (Fig. 3A) and significantly extended their average lifespan (Fig. 3B). Interestingly enough, the exposure to EMF BEMER gave a better result in terms of the tumor growth retardation and prolongation of survival time in immunocompromised nude mice, where the effect was more pronounced than in conventional animals. This suggests that either innate immunity, that is strong in athymic mice, or absence of $T$ suppressive activity may contribute to the protective effect of EMF - BEMER.

Taken together, the results point to slight but clear-cut anti-tumor effects of low-power EMF - BEMER on EIA mouse T cell lymphoma or at least could be taken as a proof that exposure to EMF - BEMER is not a risk factor intensifying the development of experimental mouse T-cell lymphoma EL4.

There are numerous data confirming not only the safety but a certain antiproliferative effects of EMF treatment (Beneducci et al., 2005; Jimenez-Garcia et al., 2010). Williams et al. (2001) were the first to report the reduction of tumor angiogenesis after exposure of mice with experimental cancer to pulsating electromagnetic fields. As a result, tumor growth was significantly reduced in female C57H/HcJ mice bearing mammary adenocarcinoma. Tofani et al. (2002) documented that the treatment of tumor-bearing nude mice with daily exposure to extremely low-frequency magnetic fields for 4 weeks caused significant tumor growth inhibition. Mice suffering from cancer xenografts in nude mice exposed to EMF either alone or in combination with gamma radiation. Similarly, a slight inhibition of the formation of chemically induced neoplastic foci in rat livers was observed when the animals were exposed to the EMF (Rannug et al., 1993)
The anti-cancer effects of EMF could result from inhibition of cell proliferation, targeted apoptosis induction, regulation of cellular homeostasis, affecting pathways associated with heat stress and/or activation of the immune system.

Tokalov & Gurzets (2003) demonstrated that EMF stimulated Hsp70 in human cells in response to extremely low-intensity electromagnetic fields alone and in combination with thermal stress. Since electromagnetic fields interact with moving charges, it is generally accepted that such treatment would stimulate the stress response by interacting directly with moving electrons in DNA (Blank & Goodman, 1999). The events mediating the electromagnetic field-stimulated stress response appear to be similar to those reported for other physiological stresses (e.g., hyperthermia, heavy metals, oxidative stress) and could well constitute the general mechanism of cell response to electromagnetic fields (Lim, Blank & Goodman, 1999). Detailed mechanisms of the processes of transduction of the electromagnetic signals into biological responses, especially changes in biosynthesis, are however still unknown.

We used an ELISA test to quantify the release of Hsp 70 into the surrounding mouse and to test whether heat shock protein expression is the positive anti-cancer reaction of mice exposed to EMF-BEMER 2000. The level of Hsp 70 in the serum taken 1 day after the experiments represented a control. Serum samples were then taken from individual mice on day zero, i.e. before transplantation of malignant EL4 cells, on day 9 after the transplantation, i.e. at the time when solid cancer is not yet palpable, on day 16, i.e. at the time of aggressive growth of the tumor and on day 30, i.e. in the terminal state of the disease. Using sensitive ELISA test we repeatedly failed to determine measurable levels of Hsp 70 in serum samples. The reason could be quantitative as the positive effect of EMF on the expression of the heat-shock protein genes HSP27, HSP60 and HSP70 was documented in tissue culture of human cells, malignant as well as normal, exposed to a wide range of environmental stimuli, including electromagnetic fields alone or in combination with thermal stress (Lim, Blank & Goodman, 1999; Orel et al. 2005).

Using a combination of low electric- and field cancer treatment and chemotherapy with 5-FU, Plotnikov et al. (2004) reported a significant tumor size reduction and prolongation of survival time in mice bearing murine colon carcinoma CT-26. Tumor growth inhibition was accompanied by an induction of anti-tumor immune reaction probably due to the antigenic material released from the deteriorating cancer cells.

Thus, we tested the formation of specific and EL4 antibodies in mice receiving EMF-BEMER 2000-7 cell lymphoma and exposed or not-exposed (controls) to EMF-BEMER. The antibodies were detected by indirect ELISA and their maximal level was seen between days 9 to 16 (Table 1, Table 2). Then the free antibodies from serum disappeared, probably due to their binding to metastasizing cells in peripheral blood and solid lymphatic tissues, including lymph nodes and spleen (Rabova et al. 2002). Antibodies detected in experimental animals before their first contact with EL4 cells (negative control) represent cross-reactive natural antibodies taken as a baseline. The sera of mice immunized five times with 7 x 10⁶ dead EL4 cells incorporated in a complete Freund’s adjuvant (CFA) were used as a positive control. In the end, no difference was recorded between experimental groups exposed to EMF-BEMER and that without EMF-BEMER exposure.

In several studies the exposure to EMF was combined with different anticancer drugs such as 5-FU (Plotnikov et al., 2004), anthracyclines (Liang et al., 1997; Orel et al. 2005) or methotrexate (Laque-Ruperez et al., 2003). Liang et al. (1997) report the enhancement of direct in vitro cytotoxicity of daunomycin by a pulsed magnetic field using multidrug resistant subline KB-ChR-8-5-11 while no such effects were seen by Laque-Ruperez et al. (2003) in MCF-7 breast cancer cells treated with methotrexate. The rare animal study explain a
positive effect of EMF given simultaneously with anti-cancer drugs by enhancing the drug delivery across biological barriers (Murthy, 1999).

The original reason for the conduction of this study was to document the effect, if any, of EMF - BEMER on the growth of cancer cell line ELA in vitro and on experimental ELA cancer model in vivo. The data presented in Figs. 2 - 3 which document slight but undoubted anti-cancer effect of EMF, substantiated the study of a hypothetical combinatorial effect of EMF - BEMER and a cytostatic drug. We decided to use its polymeric form, as anti-cancer drugs bound to different polymeric carriers represent an advanced approach for anti-cancer treatment. Such derivatives have long-term peripheral blood circulation, increased tumor accumulation, decrease of side-toxicity (Kopecek 2010; Kopecek & Kopeckova, 2010) and those based on N-(2-hydroxypropyl)methacrylate (HPMA) carrier repeatedly documented therapy-dependent activation of the immune system (Rihova & Koval’, 2010).

We used a suboptimal dose (15 mg of Dox eq.ikg) of doxorubicin bound to N-hydroxypropyl)methacrylamide (HPMA) carrier through a hydrazone bond (DOXH-HPMA). It is a formulation which was repeatedly shown to have an exceptional anticancer effect based on the direct cytotoxicity and therapy-activated anticancer-immune response (Siroya et al., 2010; Rihova & Koval’; 2010). The decreased growth of tumor was recorded in all experimental groups. Only 60% of cured mice, when treated with DOXHYD-HPMA only, correspond to the fact that a suboptimal dose of the drug derivative was used. Similar percentage of lo11g-t1,-rm survivors and thus no effect of EMF - BEMER was seen when mice were simultaneously exposed to EMF of the intensity of 10.5 µT (30 minutes every four hours; 60% of LTS) or permanently to 3.5 ,LT (70% of LTS). Considerably better results were obtained in mice exposed to EMF - BEMER of the intensity of 21 µT (30 minutes every four hours; 80% of LTS) or 35 µT (30 min every four hours; 80% of LTS) (Fig. 4). The survival of athymic nude (nu/nu) mice was also prolonged when the animals were treated with the same d,)SC of DOXHYD-HPMA at conventional animals (15 mg of Dox eq.ikg) and exposed to the EMF - BEMER. Also here, the higher intensities (21 µT and 35 µT) were more efficient (FIG.5). Unexpectedly, one mouse survived more than four months. It could not be recorded as a long-term survivor as tumor, even if considerably shrunk, was still there (see "The case report").

The observation that EMF intensity of 21 µT is optimal in all so far tested systems is worthy of additional research. Barbault et al. (2009) suggest that tumor-specific frequencies have to be used for the treatment of patients with advanced tumors. Such studies could be the basis for the design of strategic and clinical application of selected EMF sources for the treatment of different diseases.

Immunocompetent cells involved in the defense mechanisms are those preferentially acting in native (natural) immunity such as macrophages and NK cells and those effective in acquired (specific) immunity such as NKT and different subpopulations of T and B cells. NK cells have an important, though not decisive role in anticancer response where CTL cells are the major player in the game. The possibility of the effects of EMF on activity of the immune functions in living organisms has already been hypothesized and tested (Arafa et al., 2003; Tuschl, Novak & Molla-Djafari, 2006; Di Giampaolo et al., 2006; Boscolo et al., 2007; Akan et al.. 2010; Kleijn et al. 2011) but never directly demonstrated in vivo. For instance, Rossi et al. (2007) report that ELF-EMFs (so rce SEQEX) reduce the oxidative stress and reduce the side effects of chemotherapy, and specifically myelodepression (myelotoxicity), in patients with Hodgkin's lymphoma. As oxidative stress may be, at least in part, responsible for secondary malignancies they conclude that SEQEX with its ability to reduce an oxidative stress induced by treatment with chemo-radiotherapy may reduce the risk of late toxicities. The EMF was reported as both increasing or decreasing the activity/number of circulating
natural killer cells (NK) cells or no effect at al. (Gobba, 2009a; 2009b). However, it has to be stressed, that serious scientific data are so far still extremely limited.

In all our experimental systems we routinely proved the activation of the immune system during anticancer therapy by re-transplantation of LTS with a lethal dose of cancer cells. As no therapy is provided after such a re-transplantation, the only explanation for the eventual eradication of re-injected cancer cells is the activation of defense mechanisms of the cancer-bearing host during the primary treatment (Rihova & Kovar, 2010). Figure 6 documents a high cancer resistance in experimental groups exposed to EMF - BEMER. While only 20% of re-transplanted LTS survived when treated with DOXHvo_HPMA, up to 100% of them survived if simultaneously exposed to EMF - BEMER (86% when pennanently exposed to 3.5 µT; 84% when exposed to 10.5 µT and 100%, when exposed either to 21 r1T or to 35 µT given 30 min every four hours). To our knowledge it is the first direct in vivo documentation of the immune-stimulating effect of EMF.

The case report.

One mouse treated with DOXHY^0-HPMA and exposed to EMF-BEMER of an intensity of 21 µT survived more than four months, which is quite exceptional (Fig. 5). As a rule for conventional or nude mice, immediately after the treatment with polymeric drugs the growth of experimental cancer stops. In a week or so the cancer shrinks. After another few days the tumors disappear (mice are cured) or their aggressive growth starts again. However, in that one mouse the size of the tumor (about 15 mm^3) and health condition stayed unchanged for more than four months. One hundred forty days from the beginning of the experiment and 122 days after "stabilization" of the cancer size it was decided to re-transplant the mice with a lethal dose of cancer cells similarly as we have routinely done for conventional mice to test the mechanisms responsible for the control of cancer growth. Here, mainly innate immunity could be involved in cancer eradication as the number of T cells responsible for adaptive anticancer immunity in nude mice is very limited. Rather surprisingly, no cancer growth was observed at the site of secondary re-injection, i.e. on the left side on the back of mice. However, immediately after such a second cancer cell attack" we have detected aggressive growth of previously stabilized primary cancer (solid EL4 thymoma) on the right side of the mouse back. The growth was almost exponential until day 38 (Fig. 7). Then, from day to day, a substantial decrease in the size of tumor was observed which was usual in tumor-exhausted experimental models. We decided to end the experiment and to test a) the sensitivity/resistance of EL4 cells isolated from the tumor to original DOXHYD_HPMA conjugate, b) the ability of spleen cells to respond to activation with Con A (T cell response), LPS (B cell response) and anti•CD3 plus IL-2, c) different immU11c cell subpopulations in blood and finally d) to perform histopathological examination of different organs (tumor, liver, spleen, lung, heart and bone marrow). The drug sensitivity of EL4 cancer cells isolated from the tumor was comparable with that of original cancer cell line EIA (IC^50 = 0.44 µg/ml vs 0.53 µg/ml) and so was the ability of spleen cells to respond to different activation stimuli. Histopathological analyses did not reveal substantial metastatic cancer cell infiltration. Unfortunately, there was not enough material to precisely determine the immune cell subpopulations in the blood. However, we consider the case interesting enough to share it with others as hypothetical documentation of "immunocediting" (Dunn et al., 2004: Prestwich ct al., 2008).
Acknowledgements

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Legends to figures

Fig. 1: Typical form of electromagnetic impulse generated by the BEMER device.

Fig. 2A: Effect of pulsed EMF on the growth of EL4 mouse T cell lymphoma in conventional C57BL/6 mice exposed to EMF for 30 minutes every four hours (intensity of 10.5 µT, 21 µT or 35 µT) or permanently (intensity of 3.5 µT). The exposure to EMF started four days before cancer cell transplantation.

Fig. 2B: Effect of pulsed EMF on the survival of C57BL/6 mice bearing EL4 mouse T cell lymphoma and exposed to EMF for 30 minutes every four hours (intensity of 10.5 µT, 21 µT or 35 µT) or permanently (intensity of 3.5 µT). The exposure to EMF started four days before cancer cell transplantation.

Fig. 3A: Effect of pulsed EMF on the survival of nu/nu CD-1 mice bearing EL4 mouse T cell lymphoma and exposed to EMF for 30 minutes every four hours (intensity of 10.5 µT, 21 µT or 35 µT) or permanently (intensity of 3.5 µT). The exposure to EMF started four days before cancer cell transplantation.

Fig. 3B: Effect of pulsed EMF on the survival of nu/nu CD-1 mice bearing EL4 mouse T cell lymphoma and exposed to EMF for 30 minutes every four hours (intensity of 10.5 µT, 21 µT or 35 µT) or permanently (intensity of 3.5 µT). The exposure to EMF started four days before cancer cell transplantation.

Fig. 4: The combinatory effect of pulsed EMF and DOXHYu.HPMA (15 mg DOX eq./kg) in conventional C57BL/6 mice exposed to EMF for 30 minutes every four hours (intensity of 10.5 µT, 21 µT or 35 µT) or permanently (intensity of 3.5 µT). The exposure to EMF started four days before cancer cell transplantation.

Fig. 5: The combinatory effect of pulsed EMF and DOXHYu.HPMA (15 mg DOX eq./kg) in nu/nu CD-1 mice exposed to EMF for 30 minutes every four hours (intensity of 10.5 µT, 21 µT or 35 µT) or permanently (intensity of 3.5 µT). The exposure to EMF started four days before cancer cell transplantation.

Fig. 6: Re-transplantation of long-term survivors (sec Fig. 4) with a lethal dose (I x 10^5 pf EL4 T cell lymphoma cells.

Fig. 7: The growth of primary solid EL 4 T cell lymphoma injected s.c. on the right back of experimental mouse after its s.c. re-transplantation with a lethal dose of the same cancer cells on the left back.
Effect of BEMER magnetic field therapy on the level of fatigue in patients with multiple sclerosis: a randomized, double-blind controlled trial.

Piatkowski J, Simeon T.

Abstract

OBJECTIVES: Electromagnetic field therapy has been reported to be beneficial in patients with multiple sclerosis (MS) with significant fatigue. This study was designed to evaluate the long-term effects of Bio-Electro-Magnetic-Energy-Regulation (BEMER) on MS-related fatigue.

DESIGN: This was a monocenter, patient- and rater-blinded, placebo-controlled trial.

PATIENTS: There were 37 relapsing-remitting patients with MS with significant fatigue in the study.

INTERVENTION: The intervention consisted of BEMER magnetic field treatment for 8 minutes twice daily in comparison to placebo for 12 weeks.

OUTCOME MEASURES: The primary outcome criterion was change in the Modified Fatigue Impact Scale (MFIS) between baseline and 12 weeks. The secondary outcome criteria were changes of the Fatigue Severity Scale (FSS), a general depression scale-long version (ADS-L), Multiple Sclerosis Functional Scale (MSFC), and the Expanded Disability Status Scale (EDSS).

RESULTS: There was evidence of a significant difference of MFIS value (primary outcome criterion) after 12 weeks in favor of the verum group (26.84 versus 36.67; p = 0.024). In addition, FSS values were significantly lower in the verum group after 12 weeks (3.5 versus 4.7; p = 0.016). After 6 weeks' follow-up, verum and placebo groups did not differ in experienced fatigue (MFIS, FSS). Regarding the subscales of the MFIS, there was a significant decrease in physical (p = 0.018) and cognitive (p = 0.041), but not in psychologic subscales only in the verum group regarding the timepoints baseline and 12 weeks. BEMER therapy was well tolerated.

DISCUSSION: In this pilot study, we were able to demonstrate a beneficial effect of BEMER intervention on MS fatigue. As this was only a pilot study, trials with more patients and longer duration are mandatory to describe long-term effects.

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Effect of BEMER Magnetic Field Therapy on the Level of Fatigue in Patients with Multiple Sclerosis: A Randomized, Double-Blind Controlled Trial

Joachim Piatkowski, M.D., 1 Simone Kem, Ph.D., 2 and Tjalf Ziemssen, M.D. 2

Abstract

Objectives: Electromagnetic field therapy has been reported to be beneficial in patients with multiple sclerosis (MS) with significant fatigue. This study was designed to evaluate the long-term effects of Bio-Electro-Magnetic-Energy-Regulation (BEMER) on MS-related fatigue.

Design: This was a monocenter, patient- and rater-blinded, placebo-controlled trial.

Patients: There were 37 patients, 19 ps-remitting patients with MS with significant fatigue in the study.

Objective: The intervention consisted of BEMER magnetic field treatment for 8 minutes twice daily in comparison to placebo for 12 weeks.

Outcome measures: The primary outcome criterion was change in the Modified Fatigue Impact Scale (MFIS) between baseline and 12 weeks. The secondary outcome criteria were changes of the Fatigue Severity Scale (FSS), a genetical depression scale, a long version (ADS-L), Mobility, Sclerosis Functional Scale (MSFC), and the Expanded Disability Status Scale (EDSS).

Results: There was evidence of a significant difference of MFIS value (primary outcome criterion) after 12 weeks in favor of the verum group (26.84 versus 36.67; p = 0.024). In addition, FSS values were significantly lower in the verum group after 12 weeks (3.5 versus 4.7; p = 0.016). After 6 weeks' follow-up, verum and placebo groups did not differ in experienced fatigue (MFIS, FSS). Regarding the subscales of the MFIS, there was a significant decrease in physical (p = 0.018) and cognitive (p = 0.041), but not in psychologic subscales. Only in the verum group, fatigue was lower at the timepoints baseline and 12 weeks. BEMER therapy was well tolerated.

Discussion: In this pilot study, we were able to demonstrate a beneficial effect of BEMER intervention on MS fatigue. As this was only a pilot study, trials with more patients and longer duration are mandatory to describe long-term effects.

Introduction

Fatigue is among the most common symptoms of multiple sclerosis (MS), affecting at least 75% of patients, for many of whom it constitutes one of the worst and most distressing features. 2 Fatigue is not a specific symptom in all clinical phenotypes of MS and can be patients of all ages. 3 This symptom is an integral part of the disease process that is usually present at the time of diagnosis and in some cases represents one of the reasons for which patients originally consult a neurologist. Fatigue is not c/o; it is related to physical signs of disability or with magnetic resonance imaging markers of disease activity, although it does seem to increase when the patient is chronically ill.

Different components of fatigue have been described such as:

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motor and cognitive fatigue and lassitude. Management strategies include medications, exercise, and behavioral therapy.1 There have been reports on positive effects of immunomodulatory drugs on fatigue.11 However, the efficacy of treatment remains quite disappointing.10

In addition to pharacological interventions, 11on-prutr111111acological treatments includ.iyoga, oe.obole exercises, cooling therapy, and enery; and V own typically issued have been su=fully. A rec, meta-or<13;lsyc summarized promising data.> on elect<9>llagnetic field dov1cos15 Ri,d,a,ards es al. and Lappm ctat. demol111111 tilted a positive effect of low-level pulsed, electromagnetic field devices worn by th: patients--;14 Unfortunatelv, there were no long-te tm data available. Although Mostert and Kessdring showed that typical relapsing/ronnitting and also motor and cognitive fatigue and lassitude.

In preparation of a multimodal rehabilitation project on fatigue, San'dyk documented improved physi-oical and cognitive fatigue in case studies of patients with MS after a course of treatment.13,14 It is only hypothetical why th:ere is a positive impact of magnetic field therapy on MS fatigue. Faciots such as energy metabolism, oxygen supply, and microcirculation are discussed.12 The tende,ru,y for positive: NSull's warrants further investigation using a double-blinded, OOL'lltrolled prot<o solic. There are different patterns of pulsed magnetic field th:-,rapiies available. Bio-EJetcto-Magnetic-Enen,Y-Regulation (BEMER, Linomed Intmm- tional AC, Lichtenstein) therapy utilizes broad, extremely weak, low frequent pulsed electromagnetic fields indu,cd by flexible, flat electric coils.18 Although there've been several uncontrolled positive reports, with this device, no placebo-controlled, double-blinded study is currently available in the literature.

Our study was doog:nced to evaluate the long-term effect of BEMER therapy in patients with MS with significan:nt fatiug:whose characteristics were typical outpatient setting: Patients with r< resp;ning MS and significant fatigue were randomized to BEMER or placebo treatment and were evolv:1ted,--< after 6 and 12 weeks. TJSinS di=lei=en I fatigue oo,les. We hypothesize that patients with relapsing-remitting MS who use the BEMER for 5 minut,:s twice a day for 12 weeks, will experience improvement in fatigue, compared to patients who use a placebo-de-vice.

Methods

The pre,lent study was a randomi::ied, patient- and rater-blurled, placebo-controlled trial conducted in a neurological o'lpatient effite in Dresden. The study lasted 3 months and was performed between 2006 and. 2007. The study protocol was approved by the international ethical committee Frei burg, Germany (EC 02/TS/06). It was conducted according to the Declaration of Helsinki (Hong.J=ong Amendment) and pflr:inent nation I legal and e:glulatory requirements. Prior to study entry, each patient provided written, informed consent and was free to withdraw from the study at any time for any reason without consequences on the care provided.

Forty-one (41) ambulatory patients with dominically definite, relapsing/or,nnitting MS were randomly assigned to treatment with BEMER or to sham therapy twice a day over 3 months. The sample size was calculated before using the software nQuery Advisor 6.0 (Statistical SolutionSLS, Cork, Ireland) with a power of 97% (two-sided test, * = 0.05). A total of 4 patients were lost to follow-up (17). 2 place-
MAGNETIC FIELD THERAPY FOR MS FATIGUE

TAble 1. Diff:MoGIA:HIC CIAA!:ACnm:STICs OP TH1! MULTITFJ! ScLIU-OOSTSp Nett!NTS IN TH1 Vrmum AND P/AHCHIC GROW

<table>
<thead>
<tr>
<th></th>
<th>Verum</th>
<th>Placebo</th>
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<tr>
<td>N</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>Age</td>
<td>44±8.3</td>
<td>47.5±8.6</td>
</tr>
<tr>
<td>% Female</td>
<td>89.5%</td>
<td>78.3%</td>
</tr>
<tr>
<td>Duration of disease (year,;)</td>
<td>10.5±19.5</td>
<td>6.5±1.8</td>
</tr>
<tr>
<td>EDSS</td>
<td>3.7±1.2</td>
<td>3.1±1.3</td>
</tr>
<tr>
<td>MSFC</td>
<td>-0.7±1.8</td>
<td>-0.4±0.8</td>
</tr>
<tr>
<td>% Patients on immunomodulation</td>
<td>53%</td>
<td>89%</td>
</tr>
<tr>
<td>% Patients on CA</td>
<td>16%</td>
<td>33%</td>
</tr>
<tr>
<td>% Patients on IFN</td>
<td>37%</td>
<td>6%</td>
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Oata are: prefOlted as me n=111mndat. dev.ilion. EDSS, c: g
pan,dl-d disibility =rtu. s 1c.: MSFC, Muiltple ScLICs ji'pntmental Scale; GAf glatitamer ac; t;ate; IFN, interoRQTPP.

visual analogue scale scored from 0 (no fatigue) to 10 (maximum possible fatigue), and with the Modified Fatigue Impact Scale (MFIS,2) in its validated G<lm>an translation. This is a 21-item questionaire that yields a total score ranging from 0 (no impact of fatigue) to 84 points (maximum impact of fatigue), n,well as three subscales representing the physical (ac:<rrange 0-36), cognitive (score range 0-40), and psychosocial (score range 0-8) dimensions of fatigue. Depression was evaluated by the long German version of the Center for Epidemiologic Studies Depression Scale, GCS (CES-D) general d, pression scale-long version (ADS-L)."

Group differences in MFIS, FSS, MSFC, ESS, and ADL scores between treatment and placebo groups at three different timepoints were evaluated by Student’s t-test for independent samples. Changes in fatigue scores over time were statistically significant by paired t-tests for the placebo and the verum group, respectively. Differences in gender group composition were assessed with a F rest. All comparisons were two-tailed and a p value of <0.05 was taken as being statistically significant.

The BEMER devices were kindly supplied by Jannom and International AG, Lichtenstein. No additional support was provided.

Results

Study population and/or chsFrance ili$S

Baseline demographic and base characteristics are presented in Tablo1. Vorum and placebo groups did not statistically differ in terms of age or gender group composition.

At b;aniru; both groups did not differ in terms of: ESS (Student’s t: f 1.21; not significant (n.s.)), MSFC (Student’s t<0; t=0.7; n.s.), duration of disease (Student’s t: 1.10; n.s.), and ADS-L (Student’s t: t -1.23; n.s.). Fatigue scores (MFIS, FSS) were significantly higher in the placebo group compared to the verum group, but hihi effect did not reach Statistical significance (Student’s t-test; MFIS: t=-1.36; n.s.; FSS: t=-1.15; n.s.) (Table 2).

Primary outcome criterion: MFIS bislineale visurus

12 weeks’ HIIJUJiment

Regarding the primary endpoint of our study, there was evidence of a significant difference between the verum group (26.84 versus 36.67; Student’s t-test for independent samples: MFIS, t=-2.36; p=0.02). Secondary outcome criteria: Baseline/no versus 6 Wf/llw treatment

After 6 weeks’ treatment, verum and placebo groups did not differ in experience: IR fatigue (Student’s t-test for induced, pet-dcnt sample<; MFIS<ew f 1.38; n.s.; FSS: 3.07; t<0.03; n.s.) (Table 2). However, tool:ins at changes in fa.

In over time, trre was a decrease in fatigue measured by the FSS in the verum but not the placebo group after 6 w""<s compared to b<:seline (paired r bos FSS: ...., t=1.26; p: 0.015; FSS: ...... /p<:bo t=0.95; n.s.) (Table 3). No difference.f for the MFIS or MFIS subscales (physical, cognitive, psychologic) over time were observed for either group (paired t-test: MFIS combination: t=1.14; p: 0.016; MFIS: t=0.98; n.s.).

Self-ras.:d depressive symptoms by the CES-D did not differ, for between groups after 6 weeks’ treatment (Student’s t: 0.76; n.s.). There was also no change in depn. an symp:ml $expression ov t line in either group (pai. -: d Mes: ADS-1-w,..., t=0.33; n.s. / ADS-1-<,..., p..,bo 0.45; n. -)

Secondary outcome criteria: Baseline/ve/Su$ 12 weeks’ treatment

In addition to significant different fatigue ratings by MPIS between the verum and placebo groups, there was evidence for a significant difference of FSS values after 12 weeks' treatment in favor of the verum group (Student’s t: t=2.53; p=0.016). In the verum group but not in the placebo group, there was a

Table 2. CTUH’s r1 Moomtn FATICTJE J.MI<ACT SCALn (MFIS) Ov:R ALL SCORE AND AS WELL AS 1’miCAL, COCINTIVI, AND PASC. JLOCIC.1C SumsOORIS IN VERUM AND PLAC.HIO GROUP AT BASRUNE, 6 wF/llwKS, AND 12 WEfrS

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Verum Mean SD</th>
<th>Placebo Mean SD</th>
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<tbody>
<tr>
<td>MFIS</td>
<td>31.68</td>
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<tr>
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<td>6.78</td>
<td>17.25</td>
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<tr>
<td>Cognition</td>
<td>6.32</td>
<td>7.84</td>
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<td>Psychologic</td>
<td>6.23</td>
<td>4.83</td>
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<th>6 Weeks</th>
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<tr>
<td>MFIS</td>
<td>13.45</td>
<td>15.25</td>
</tr>
<tr>
<td>Physical</td>
<td>6.70</td>
<td>7.91</td>
</tr>
<tr>
<td>Cognition</td>
<td>6.84</td>
<td>6.86</td>
</tr>
<tr>
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<td>6.23</td>
<td>4.83</td>
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<th>12 Weeks</th>
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</thead>
<tbody>
<tr>
<td>MFIS</td>
<td>13.25</td>
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<tr>
<td>Physical</td>
<td>6.70</td>
<td>7.91</td>
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<td>Psychologic</td>
<td>6.23</td>
<td>4.83</td>
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*NZ of MPIS overnil: ***orcc. Oata arc pret 't'led as mCMT: ±:standard dev:tion (SD).
significant decrease in perceived fatigue over the 12-week period (paired t-test; *p* < 0.001; 12W/Placebo FSS: 3.1 [2.0; 4.2] vs. 1.8 [1.5; 2.1] *p* = 0.004; MFIS: 12W/Placebo: 1.4 [1.0; 1.8] vs. 1.1 [0.8; 1.4] *p* = 0.033; n.s.). Regulating the subscales of the MFIS, there was a significant decrease in physical (paired *t*-test: MFIS-phys: 12W/placebo 1.2 [1.0; 1.5] vs. 0.8 [0.6; 1.0]; n.s.) and Cognitive (paired *t*-test: MFIS-cog: 12W/placebo 1.3 [1.1; 1.5] vs. 1.0 [0.8; 1.2]; n.s.) scales. No other difference was significant.

Self-rated depressive symptoms by CES-D did not differ between groups after 12 weeks' treatment. This was not surprising, given the baseline fatigued baseline for both groups. However, there was a trend for decreased depressive symptoms on the Visual Analog Scale, with a significant difference in the verum group (Wilcoxon signed-rank test: Z = 2.03; *p* = 0.058; n.s.).

There were no significant side-effects during verum and placebo application.

**Ois.:ussion**

Our study was focused on effects of a new type of pulsed low-frequency electromagnetic fields of the BEMER system on MS fatigue after 6 weeks and 12 weeks. The patients were evaluated by a panel of directors of the rehabilitation centers and the project leader. Using a randomized, placebo-controlled protocol, we were able to demonstrate a beneficial effect of the BEMER system on MS fatigue. Although both groups showed a decrease of fatigue over the 12-week period, the BEMER group showed a more significant decrease in fatigue score compared to the placebo group. This effect was statistically significant in the physical and cognitive subscales of the MFIS.

There is growing evidence in the literature of a beneficial effect of magnetic field therapy on different MS symptoms such as fatigue, bladder control, memory, and quality of life. Nielsen and Sinkjaer reported a reduction of spasticity by magnetic stimulation over the thoracic myelon while &mdash; a finding not replicated in our study. Following physical activity by extracranially applied electrotherapy, there may be a beneficial effect on MS fatigue in a significant way.
MAGNETIC FIELD THERAPY FOR MS FATIGUE

patients in each treatment arm. Other studies investigated comparable numbers of patients, only. Lapin et al. investigated more than 55 patients for each group, but only for 4 weeks. Larger trials on this issue are an "sounded in order to confirm the findings from this pilot study. Again, it is not possible to compare the different devices, the physiology of magnetic field therapy is not well known. Magnetic field therapy is used in a Jot of clinical settings. Unpublished, scientific data on mechanism and so on are still missing. We are beginning to investigate physiologic changes induced by mo:ieo fields.

Co"usions

In this pilot study, we were able to demonstrate a beneficial effect of BEMER therapy on MS fatigue. Although we recognized a placebo effect, there was a statistically significant benefit for treated patients after 12 weeks. From our personal experience, MS patients suffering from MS fatigue can benefit from electromagnetic field therapy. Because devices for pulsed electromagnetic therapy like BEMER are quite expensive, we suggest using the same field for several weeks to see whether there is an individual benefit for the MS patient with significant fatigue.

Disclosure Statement

No competing financial interests exist.

References


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BEMER Electromagnetic Field Therapy Reduces Cancer Cell Radioresistance by Enhanced ROS Formation and Induced ONA Damage

Kerstin Borgmann, Editor

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Data curation: KS ED AA.

Formal analysis: KS ED AA.

Funding acquisition: NC.

Inception: KS ED AA.

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Project administration: NC.

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Abstract

Each year more than 450,000 Ganes are expected to be diagnosed with cancer subsequently receiving standard multimodal therapies including surgery, chemotherapy and radiotherapy. On top, molecular-targeted agents are increasingly administered. Owing to intrinsic and acquired resistance to these therapeutic approaches, both the better molecular understanding of tumor biology and the consideration of alternative and complementary approaches are highly desirable.
therapeutic support are warranted and open up broader and novel possibilities for therapy personalization. Particularly the latter is underpinned by the increasing utilization of non-invasive complementary and alternative medicine by the population. One investigated approach is the application of low-dose electromagnetic fields (EMF) to modulate cellular processes. A particular system is the BEMER therapy as a Physical Vascular Therapy for which a normalization of the microcirculation has been demonstrated by a low-frequency, pulsed EMF pattern. Open remains whether this EMF pattern impacts on cancer cell survival upon treatment with radiotherapy, chemotherapy and the molecular-targeted agent Cetuximab inhibiting the epidermal growth factor receptor. Using more physiological, three-dimensional, matrix-based cell culture models and cancer cell lines originating from lung, head and neck, colorectal and pancreas, we show significant changes in distinct intermediates of the glycolysis and tricarboxylic acid cycle pathways and enhanced cancer cell radiosensitization associated with increased DNA double strand break numbers and higher levels of reactive oxygen species upon BEMER treatment relative to controls. Intriguingly, exposure of cells to the BEMER EMF pattern failed to result in sensitization to chemotherapy and Cetuximab. Further studies are necessary to better understand the mechanisms underlying the cellular alterations induced by the BEMER EMF pattern and to clarify the application areas for human disease.

**Introduction**

Modern multimodal anticancer strategies consist of surgery, chemotherapy and radiotherapy. The combination of intrinsic and acquired therapy resistances, normal tissue toxicities and lack of biological personalization remain obstacles to overcome for a significant improvement in cancer patient survival rates [1]. While our increasing understanding of tumor biology by means of various "omics" technologies and molecular biology provides a wealth of possibilities for the development of molecular-targeted agents, therapeutic strategies falling in the field of complementary and alternative medicine gradually enter the conventional cancer therapy field without clear mechanistic insight. Based on the increasing demand by the population and the unexploited potential of such approaches, we investigated the potential of a particular electromagnetic field (EMF) therapy for cancer cell therapy sensitization shown to effectively normalize tissue microcirculation.

Reviewing the literature indicated an impact of cellular functions and response to cancer therapies upon application of EMF [1]. EMF therapies reduced proliferation [2] and induced apoptosis [8.JQ-II] in different cancer cells such as osteosarcoma, breast cancer, gastric cancer, colon cancer, and melanoma. Marchesi and colleagues also showed that autophagy is induced upon EMF exposure in neuroblastoma cells [11]. Interestingly, tumor vascularization was diminished in vitro and in vivo in breast cancer treated with EMF therapy W, [12]. In line, EMF therapy decreased tumor growth in mouse models of malignant melanoma, colon carcinoma and adenocarcinoma [2.11]. Baharara and colleagues showed that extremely low EMF therapy restored the sensitivity of cisplatin resistant human ovarian carcinoma cells by increased apoptosis rates [11]. In combination with radiotherapy, EMF improved survival of mice bearing hepatoma as compared with EMF or radiotherapy alone [1.2]. Similarly, Cameron and colleagues showed this for breast cancer xenografts including decreased lung metastasis [Z.Q]. These studies clearly illustrate the potential of EMF therapy in combination with conventional cancer therapies as a new approach for sensitizing tumors. Importantly, the applied EMF patterns show great differences in intensity, direction and frequency as well as wave forms, ranging from sinusoidal to square-wave to pulsed-wave forms across studies [2.1J]. Mainly pulsed EMFs with low frequency were used.

In this study, we applied the Bio-Electro-Magnetic-Energy-Regulation (BEMER) system, which uses a low-frequency, pulsed magnetic field (max. 35 μT) with a series of half-wave-shaped sinusoidal intensity variations and was shown to increase vasomotion and microcirculation for improved organ blood flow, supply of nutrients and removal of metabolites [UI:U]. In multiple sclerosis (MS) patients, BEMER therapy decreased the levels of fatigue in a randomized, double-blinded pilot study [M]. A follow-up long-term study demonstrated beneficial effect of long-term BEMER therapy on MS fatigue [22]. In the field of cell biology, Walther and colleagues showed altered gene expression of a limited number of gene products asociated with e.g. energy metabolism, cytoskeleton stabilization and vesicle transport in human mesenchymal stem cells and human chondrocytes upon
BEMER therapy [2§]. A second study revealed BEMER therapy to delay EIA mouse T-cell lymphoma growth and prolong survival of mice [n]. Interestingly, simultaneous BEMER therapy and synthetic HPMA copolymer-based doxorubicin showed a synergizing antitumor effect (21).

By focusing on cells from solid tumors, we explored how the BEMER EMF pattern affects the metabolome in terms of glycolysis and tricarboxylic acid (TCA) cycles and the sensitivity to radiotherapy, chemotherapy and Cetuximab. To better address this question, we utilized a more physiological 3D laminin-rich extracellular matrix (lrECM)-based cell culture model. We found a significant radiosensitization of cancer cells by the BEMER therapy mechanistically derived from higher levels of reactive oxygen species and increased numbers of DNA double strand breaks (DSBs).

Materials and Methods

Cell culture and irradiation

Human head and neck squamous carcinoma (HNSCC) cell line UTSCC 15 was kindly provided by R. Grenman (Turku University Central Hospital, Finland), human lung carcinoma cell line A549, human colorectal carcinoma cell line DLDt and human pancreatic ductal adenocarcinoma cell line MiaPaca2 were purchased from American Tissue Culture Collection. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; PAA, Co!be, Germany) containing glutamax-I supplemented with 10% fetal calf serum (FCS; PAA) and 1% non-essential amino acids (PAA) at 37°C in a humidified atmosphere containing 8.5% CO2. In all experiments, asynchronously growing cells were used. Three (3D)-dimensional cell cultures were accomplished by imbedding cells in 0.5 mg/ml lrECM (Matrigel*M: BO, Heidelberg, Germany) [Z.8.2]. Irradiation was performed at room temperature using single doses of 200 kV X-rays (Yxlon Y.TU 320; Yxlon; dose rate 1.3 Gy/min at 20 mA) filtered with O.S mm Cu. The absorbed dose was measured using a Duplex dosimeter (PTW).

BEMER therapy

BEMER (Bio-Electro-Magnetic-Energy-Regulation) therapy uses a low-frequency pulsed magnetic field [22,17] which was applied for 8 min, 1 h or 24 h. The detailed physical properties of this device are reviewed in the following patents: EP0995463 A1, WO2008025731 A1; WO20J1023634 A1 L1l-11). The electromagnetic field (EMF) with a pulse-duration of 30 ms and a pulse-frequency of 30 Hz was generated by a commercially available control unit B.Box Classic (BEMER AG Int.; Fig 1 A) with 10 different levels of magnetic field intensity (from 0.1 µT to 35 µT) and a mattress applicator (fig 1R) with a flat coil system (Bio-Electromagnetic-Energy-Regulation, BEMER International AG, Triscen, Liechtenstein). The pulse generator is fed with a mains voltage of 230 V AC/50 Hz. Based on the commercially available construction, this mattress applicator was specifically designed for cell culture use with a maximum operating voltage of 12 V DC. Additionally, different signal intensities were used at level 1 (~2.7 µT), level 4 (~13 µT), level 7 (~23 µT) and level 10 (~35 µT). The signal is a sequence of individual pulses with a pulse width of approximately 33 milliseconds in the altitude of 3 to 35 µT within a predetermined time period of 18 to 22 seconds. The preferred exponential function described in detail in EP 0995463 A1 is \( y = (x^3 - 3x + 1) \) with \( y \) as amplitude (LU). The amplitudes of the single pulses correspond to an e-function and are then summarized as a group of pulses. As shown in Fig 1, BEMER-treated cells were placed within the labeled area above the flat coil on the mattress, and then stimulated with indicated intensities for 8 min, 1 h or 24 h. BEMER therapy was conducted at 37°C in a humidified atmosphere containing 8.5% CO2 for pH 7.4. Control cells were sham-treated by placing them on the BEMER applicator for the respective time without applying the BEMER signal. BEMER signal intensity was measured using a 30 teslameter (PCE-028, PCB, Germany) and cells were placed in the same area of the BEMER applicator for each treatment.

Eiti

BEMER device and application.
Samples collection for non-targeted metabolomic analysis

For metabolome analysis, A549 cells were cultured for 24 h in 30 ltrECM followed by BEMER therapy (-13 μt, 8 min; sham-treated cells served as control). After 1 h, cells were harvested with 200 μl pre-cooled 80% MeOH containing 4 recovery standards to monitor extraction efficiency. The extraction solvent and cellular material were transferred into a 2 ml microtube (Sarstedt, Numbrecht, Germany). Then, the wells were washed with 200 μl extraction solvent, which was collected in the same microtube. The samples were immediately stored in -80°C until analysis.

Non-targeted metabolomics analysis

Non-targeted metabolomics analysis was conducted at the Genome Analysis Center, Hel01holt.1 Zentrum Munchen. Prior to analysis, all samples were stored at -80°C. Prior to homogenization, 160 mg of 0.5 mm glass beads (Precellys, Berlin, Germany) were placed into the tubes with the cell lysates, which were collected in 80% v/v metanolic extraction solvent spiked with 4 recovery standards. The lysates were then homogenized for 2 times 25 s at 5500 lpm, with a 5 s break. The homogenization was done using a Precellys 24 homogenizer (PEQLAB Biotechnology GmbH, Erlangen, Germany) equipped with an integrated cooling unit to maintain a temperature of 4°C. After homogenization, the cell lysates were centrifuged for 5 min at 11,000 x g at 4°C and the clear extract supernatants were used thereafter. Each sample was loaded onto a 96-well 350-μl PCR plate by splitting it into 2 aliquots, 105 μl each aliquot. The first aliquot was used for LC-MS/MS analysis in positive electrospray ionization mode and the second aliquot was used for that in negative mode.

In addition to the study samples, a pool of all cell homogenates was prepared and aliquoted into the 96-well PCR plate, 105 μl per well, 3 wells for each ionization mode. Furthermore, 100 μl of a pooled human reference plasma sample (Ser.i.lab, West Sussex, United Kingdom) was extracted independently and the extract was loaded into the 96-well PCR plate, a well for each ionization mode, 105 μl in each well. A similar procedure was performed for pure trECM as additional control for measurement and normalization to background. These samples served as control replicates throughout the study to assess process variability. Besides the reference plasma sample, 100 μl water was extracted independently and the extract was aliquoted into a 96-well plate, 3 wells per ionization mode, 105 μl in each well. These samples served as blanks. The samples were then dried in a TurboVap 96 (Zymark, Sotax, Lorrach, Germany).

Before LC-MS/MS in positive ion mode, the samples were reconstituted with 50 μl 0.1% formic acid. Those samples analyzed in negative ion mode were reconstituted with 50 μl 16.5 mM ammonium bicarbonate (pH 8.0). Reconstitution solvents for both ionization modes contain non-titrable standards that allowed monitoring of instrument performance and also served as retention markers. LC-MS/MS analysis was performed on a linear ion trap LTQ XL mass spectrometer (Thermo Fisher Scientific GmbH, Dreieich, Germany) coupled with a Waters Acquity UPLC system (Waters GmbH, Eschborn, Gemany). Two separate columns (2.1 x 100 mm Waters BEH C18, 1.7 mm particle-size) were used for acidic (solvent A: 0.1% formic acid in water, solvent B: 0.1% formic acid in methanol) and for basic (solvent A: 6.5 mM ammonium bicarbonate (pH 8.0), solvent B: 6.5 mM ammonium bicarbonate in 95% methanol) mobile phase conditions, optimized for positive and negative electrospray ionization, respectively. After injection of the sample extracts, the columns were developed with a gradient of 99.5% A to 98% B over an 11 min run time at a flow rate of 0.35 ml/min. The eluent flow was directly routed through the electrospray ionization source of the LTQ XL, mass spectrometer. The full MS scan
was performed from 80 to 1000 m/z and alternated between MS and MS/MS scans using a dynamic exclusion technique, which enables a wide range of metabolite coverage.

Metabolites were annotated by curating of the LC-MS/MS data against proprietary Metabolon's chemical database library (Metabolon, Inc., Durham, NC, USA) based on retention index, precursor mass and MS/MS spectra. In this study, 315 metabolites, 240 compounds of known identity (named biochemicals) and 75 compounds of unknown structural identity (unnamed biochemicals) were identified. The unknown chemicals are indicated by a letter X followed by a number as the compound identifier. The metabolites were assigned to cellular pathways based on PubChem, KEGG, and the Human Metabolome Database.

3D colony formation assay

30 colony formation assays (CFA) were applied for measurement of clonogenic cell survival as published [1/lll]. For 3D CFA cells were imbedded in 0.5 mg/ml 3D IrECM in 96-well plates (BD). After 23 h, cells were treated with BEMER therapy applying different levels and durations. Irradiation occurred at different time points after BEMER therapy. In most experiments, radiotherapy was carried out 1 h after BEMER therapy. After 8-10 days, cell colonies (>50 cells) were counted microscopically. Images of representative colonies were acquired using an Axiovert 40 CFL (Zeiss, Jena, Germany). Each point on the survival curve represents the mean surviving fraction from at least three independent experiments.

3D microtumor assay

3D microtumors originated from single cells embedded in 0.5 mg/ml 3D IrECM in 96-well plates (BD) over a time period of 3 days. After 3 days, cells were treated with BEMER therapy applying different levels and durations. Irradiation occurred at different time points after BEMER therapy. After 8-10 days, cell colonies (>50 cells) were counted microscopically. Each point on the survival curve represents the mean surviving fraction from at least three independent experiments.

Cetuximab, Cisplatin and Gemcitabine treatment

At 24 h after seeding cells were treated with Cetuximab (ErbituxL, Merck, Darmstadt, Germany; 5 µg/ml; IgG as control), Cisplatin (Teva, Ulm, Germany; 0.1 µM) or Gemcitabine (Medac, Wedel, Germany; 10 nM). After 23 h of incubation, cells were treated with BEMER therapy (-13 µT, 8 min) and irradiated 1 h later as described above. Cetuximab remained in the cell culture medium for the entire growth period, Cisplatin and Gemcitabine treated cells were washed with cell culture medium 48 h after treatment.

Foci assay

4x10^5 cells per well were grown in 3D IrECM for 23 h, then treated with different levels of BEMER therapy (-13 µT and -35 µT; 8 min) and irradiated 1 h later with 6 Gy or left unirradiated. After 24 h, cells were isolated using PBS and t.typSin (PAA), fixed with 3% formaldehyde/PBS (Merck, Darmstadt, Germany), permeabilized with 0.25% Triton-X-100/PBS (Roth, Karlsnhe, Germany) and stained with specific antibodies for yH2AX and 53BP1. Samples were spread on a slide and covered with Vectashield/DAPI mounting medium, yH2AX/53BP1-positive foci were counted microscopically with an Axioscope 2 plus fluorescence microscope (Zeiss) and defined as residual DSB [1/lll]. Immunofluorescence images were sustained using LSM 510 meta (Zeiss).

ROS scavenger analysis

Three different scavengers (Thermo Fisher Scientific (Dannstadt, Gennany)), i.e. sodium pyruvate (hydrogen radicals, 10 µM), MnTBAP (superoxide anion) and Carboxy-PTIO (nitric oxid) and (both SO µM), were applied (complete culture medium served as control) and clonogenicity and OSB measured were performed in 3D cell cultures. Cells were treated with scavengers for 10 min, prior to BEMER therapy (-35 µT, 8 min). One hour
later, cells were irradiated with 6 Gy. For foci assays, cells were isolated and fixed 24 h after irradiation, for CFA, cells were grown several days, cell line dependently.

**Data analysis**

Means ± standard deviation (SD) of at least three independent experiments were calculated with reference to non-treated (n.t.) samples defined in total numbers or 1.0. For statistical significance, Student t-test was performed using Microsoft® Excel 2003. P-value of less than 0.05 was considered statistically significant.

**Results**

**BEMER treatment modulates cancer cell metabolism**

Based on previous data, EMF application is likely to influence cell metabolism [I.S.]. Evaluation of A549 cancer cell metabolism by the BEMER system showed metabolites of different pathways (Fig. 2A) and, particularly and of the glycolysis and TCA cycle pathways to be significantly altered relative to non-treated cells (Fig. 2B 20). The levels of pyruvate, succinate, aspartate and adenosindiphosphate (ADP) were significantly downregulated after BEMER therapy whereas serine showed significant upregulation (Fig. 2C). These data demonstrate that the specific low-frequency pulsed BEMER EMF pattern leads to changes in certain part of the cellular metabolism.

**BEMER treatment falls to alter basal tumor cell survival but radiosensitizes tumor cells in a time-dependent manner**

Next, we analyzed basal tumor cell survival of a panel of four cell lines (A549, UTSCC15, MiaPaCa, DLD1) after BEMER treatment. Interestingly, BEMER therapy did not alter basal cell survival of all tested cell lines (Fig. 3A and 3B). In combination with X-ray irradiation, 3D trECM grown cancer cell cultures, however, responded with radiosensitization when BEMER-pretreated for 8 min (Fig. 3C and 3D). Upon longer BEMER exposure times, the radiosensitization was lost (Fig. 3D).

**BEMER therapy mediates radiosensitization of cancer cells.**

Interestingly, the radiosensitizing potential of a pretreatment with the BEMER signal was confirmed in 3D grown microtumors A549, UTSCCIS, MiaPaCa and DLD1 in a time-dependent manner relative to sham-treated microtumors (Fig. 4A and 4B). An 8 minute pretreatment with BEMER therapy radiosensitized all tested cell lines, while longer treatment time of BEMER therapy were less or not effective (Fig. 4C). These observations evidently demonstrate that the cellular radiosensitivity of human cancer cells grown in a physiological environment can be increased by the specific BEMER EMF pattern in a time-dependent manner.
Fig 4
BEMER therapy radiosensitizes microtumors.
BEMER therapy/radiotherapy time interval and BEMER EMF frequency determine BEMER therapy-induced radiosensitizing potential

To further characterize the radiosensitizing effect elicited by a pretreatment with BEMER therapy, we modulated the time interval between BEMER and radiotherapy (Fig 5A) and found that the surviving fraction of 6 Gy-irradiated cells is clearly different between the tested time intervals (Ei, &...fil). With increasing time between BEMER treatment and radiotherapy, the radiosensitizing effect was diminished and completely abolished at the 24 h interval (fig 5B).

BEMER therapy-mediated radiosensitization depends on treatment intervals and frequency.

Next, we analyzed if the frequency of BEMER treatments influences cancer cell radioresistance (fi: C and D). In general, BEMER application is recommended twice a day every 12 h [U, M]. Consequently, 3D grown cells were treated either once with the BEMER signal (-13 µT, 8 min) at 1 h prior to irradiation or twice where a 12 h time interval was between the two BEMER treatments followed by irradiation after 1 h (E) - Only A549 cells were significantly radiosensitized after one-time and two-time BEMER therapy (fig 5Q). In UTSCC15 and MiaPaCa2 cells, only the one-time BEMER therapy led to radiosensitization (Ei, SQ). DLDL cells remained resistant to BEMER treatment as shown in figure 5G. These data indicate that a one-time BEMER therapy followed by radiotherapy within a short time interval is most effective for radiosensitization of tumor cells with respect to the different treatment schedule tested in this study.

BEMER treatment has no additional effect on radiochemosensitivlty

Due to radiochemotherapy being standard of care for the tumor types investigated in this study, we sought to determine clonogenic survival after respective radiochemotherapy (Figs 1 and 1). According to the treatment schedules (Figs M and Lb), the chemotherapeutics Cisplatin and Gemcitabine or the anti-epidermal growth factor receptor (EGFR) antibody Cetuximab were tested. Cisplatin and Gemcitabine either alone or in combination with BEMER therapy resulted in significantly decreased clonogenic cell survival in all tested cell lines (Figs 6B and 6C). Cetuximab treatment with or without BEMER therapy led to reduced basal survival in UTSCC15 but not A549, MiaPaCa2 or DLDL cells (Fig 6D).

Sensitivity to chemotherapy and Cetuximab is not Influenced by BEMER therapy.

The combination of Cisplatin, radiotherapy and BEMER therapy remained equitoxic to Cisplatin/radiotherapy for clonogenic survival of A549 and UTSCC15 cells (Fig 7B). In MiaPaCa2 cells, the combination of Gemcitabine and radiotherapy showed no effect on cell survival whereas the Gemcitabine/radiotherapy/BEMER combination elicited a significantly decreased survival relative to BEMER sham-treated, irradiated controls (Fig 7C:). Cetuximab plus radiotherapy led to significantly reduced clonogenic survival of A549 and MiaPaCa2 cells with no further enhancement of the effect upon application of BEMER therapy (Fig 7D). In UTSCC15 and DLDL cells, neither Cetuximab plus radiotherapy alone nor in combination with BEMER therapy impacted on clonogenic cell survival (Fig 7G). Thus, the combination of BEMER therapy and radiochemotherapy failed to generally enhance cancer cell sensitization.
BEMER therapy decreases radioresistance and increases DSB numbers dependent on BEMER signal intensity

To elucidate whether the radiosensitizing effect of BEMER therapy is related with increased signal intensity and increased number of radiation-induced DNA double strand breaks (DSBs), we applied the BEMER signal with varying intensities between 2.7 and 35 μT I h after 6-Gy X-ray irradiation (Fig 8A). In A549, UTSCC15 and MiaPaCa2 but not DLD1 I cells, BEMER therapy accomplished radiosensitization in a signal intensity-dependent manner compared with BEMER sham-treated, irradiated controls (Fig 8D). Accordingly, DSB numbers of A549 and UTSCC15 cells were significantly elevated by BEMER EMF exposure intensity-dependently compared to controls (Fig 8C and 8D). These results suggest a connection between BEMER therapy-mediated radiosensitization and DSB induction.

**BEMER signal intensity determines radiosensitization and DSB numbers.**

BEMER therapy increases ROS levels leading to radiosensitization via increased induction of DSBs

Connecting ROS as an essential regulator of metabolomic processes and DNA damaging factor, we tested for different ROS scavengers (here sodium pyruvate, MnTBAP, Carboxy-PTIO) given prior to BEMER therapy (Fig 9A). While sodium pyruvate only abolished the effect of BEMER therapy in UTSCC15 but not in A549 cells (Fig 2B), the ROS scavenger MnTBAP and Carboxy-PTIO abrogated the BEMER-mediated radiosensitization in both cell lines leading to similar clonogenic survival as observed for BEMER sham-treated, irradiated controls (Fig 2C). Next, we tested the effect of MnTBAP and Carboxy-PTIO pretreatment on DSB induction upon BEMER treatment and irradiation and found that both scavengers reduced DSB numbers to a level similar to controls (Fig 8C). These findings indicate that the radiosensitization mediates by the BEMER therapy elicits from increased ROS levels and subsequent generation of DSBs.

**Discussion**

Different studies showed the influence of EMF exposure on various functions of tumor cells, which beneficially impact on therapy response and tumor growth. On this basis, we hypothesized BEMER therapy to exhibit radio- and chemosensitizing potential in tumor cells. Here we show radiosensitization of cancer cell lines upon pretreatment with the particular low-frequency, pulsed EMF pattern of the BEMER system as compared with radiotherapy alone. Mechanistically, this effect is mediated through elevated ROS levels that are critically involved in the generation of DSBs.

Reviewing the literature for effects of EMF therapy in tumor cells, one has to take into consideration large differences in EMF application devices and exposure set-ups. Variations in EMF signal pulsation, strength, amplitude and frequency are highly likely to fundamentally accomplish a differential impact on cell behavior and degree of investigated effects. Using the BEMER system had the clear advantage of reported observations about improved blood flow, vasomotion and microcirculation (22,2,1). Testing the BEMER EMF pattern in conjunction with conventional tumor thempies was conducted to identify the therapy-sensitizing potential of the specific EMF pattern.
As first step, we performed a broad metabolome analysis as EMF exposure is reported to alter physiological and metabolic processes [2,15,16]. Cancer cells exhibit a deregulated metabolism and produce their energy mainly via glycolysis [12.14]. Interestingly, we found decreased levels of metabolites of the glycolysis and the TCA cycle upon BEMER therapy. While the identification of such changes is difficult to test in vitro, it was of utmost importance to demonstrate that the BEMER therapy does not induce cancer cell proliferation and enhanced survival of either Ringle cells as well as microtumors.

Intriguingly, we found cells originating from lung, head and neck and pancreas to be radiosensitized by BEMER EMF exposure. As approximately 60% of cancer patients are receiving radiotherapy alone or as part of a radiochemotherapeutic regimen, this result provides the first basis describing a therapeutic potential for applying the BEMER therapy to cancer patients briefly before radiotherapy. By means of more physiological cell culture models intensively validated to in vivo growth conditions [11,12], our results indicate a differential impact of the BEMER EMF in different tumor types. Why cells from colorectal cancers, taking into account that only one cell line was examined, demonstrated resistance to BEMER therapy warrants further analysis. Moreover, we found the radiosensitization generated by BEMER therapy to depend on (i) the duration of the treatment, (ii) the interval between BEMER therapy and radiotherapy, and (iii) the signal intensity of the EMF. Although highly speculative concerning clinical usage, it becomes obvious that the BEMER therapy is most efficient for radiosensitization when applied 1 h prior to radiotherapy with certain intensity.

Addressing the potential of the BEMER therapy to chemoradiotherapy, we observed no changes in clonogenic cancer cell survival upon chemotherapy alone or upon radiochemotherapy. This result strongly suggests that chemotherapy confers cytotoxicity via molecular mechanisms independent from BEMER therapy-related changes in cell physiology in contrast to X-ray radiation. Moreover, this could be due to our treatment schedule with a 23-h drug pretreatment before BEMER signal application. Ruiz-Gomez and colleagues showed that the EMF therapy is more efficient when cells are simultaneously exposed to ELF and cytostatic agents [1]. In our hands, administering cisplatin on top of BEMER radiotherapy, the radiosensitizing effect caused by BEMER was even abolished. Discussing these observations on a clinical background is highly challenging and speculative. In vivo studies are clearly required administering clinically applied radiochemotherapy regimens to identify the translational bench-to-bedside potential of BEMER EMF exposure for cancer patients.

To further explore the radiation-related mechanisms contextually linked to the BEMER therapy, we measured ROS levels and DSBs as most life-threatening DNA lesions produced by X-ray imidation [11,12]. Interestingly, the application of scavengers for superoxide anions (MnTBAP) and nitric oxides (Carboxy-2,7-TIO) abolished BEMER-related radiosensitization, which strongly proposes that the specific BEMER EMF pattern considerable increases ROS levels by yet to be discovered mechanisms. Despite the fact that our observations are in line with other cancer research studies showing EMF exposure to indirectly provoke DNA strand breaks via free radicals [1,14], the induction of DNA damage by EMF is quite controversially discussed. Other studies reported changes of the redox status and increased DNA damage in EMF-treated neuroblastoma [1] or leukemia cells [1,14,15]. Mechanistically, EMF therapy reduced antioxidant enzyme activity and enhanced nitrogen intermediates in leukemia cells [1] and increased ROS levels in neuroblastoma cells [1]. Kim and colleagues published repetitive EMF exposure of cervical cancer cells and normal lung fibroblasts to result in an increase of γH2AX phosphorylation indicative of DSBs [12]. In accordance, Winker and colleagues found increased chromosomal aberrations and elevated numbers of micronuclei upon exposure to EMF [13]. These studies support our view that BEMER therapy induces higher levels of ROS converted into elevated DSB numbers by X-ray irradiation finally detectable as radiosensitization.

In conclusion, our data suggest that the BEMER therapy radiosensitizes cancer cells via ROS in a time- and intensity-dependent manner. Future studies are required in animal tumor models treated with conventional radiochemotherapy to evaluate the reasonable and safe benefit and bench-to-bedside transferability.

Acknowledgments

Go to:
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Effects of weak, low-frequency pulsed electromagnetic fields (BEMER type) on gene expression of human mesenchymal stem cells and chondrocytes: an in vitro study.

Walther M, Mayer F, Kafka ‘H Schütze N.

Abstract

In vitro effects of electromagnetic fields appear to be related to the type of electromagnetic field applied. Previously, we showed that human osteoblasts display effects of BEMER type electromagnetic field (BTEMF) on gene regulation. Here, we analyze effects of BTEMF on gene expression in human mesenchymal stem cells and chondrocytes. Primary mesenchymal stem cells from bone marrow and the chondrocyte cell line C28I2 were stimulated 5 times at 12-h intervals for 8 min each with BTEMF. RNA from treated and control cells was analyzed for gene expression using the affymetrix chip HG-U133A. A limited number of regulated gene products from both cell types mainly affect cell metabolism and cell matrix structure. There was no increased expression of cancer-related genes. RT-PCR analysis of selected transcripts partly confirmed array data. Results indicate that BTEMF in human mesenchymal stem cells and chondrocytes provide the first indications to understanding therapeutic effects achieved with BTEMF stimulation.

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Abstract

BACKGROUND: Whether and to what extent the complementary use of a biorhythm-defined physical stimulation of insufficient spontaneous arteriolar vasomotion contributes to increasing the therapeutic success of established treatment concepts were examined.

MATERIALS AND METHODS: In a placebo-controlled study on a biometrically defined sample of older diabetes patients with impaired wound healing, measurements of representative features of the functional status of the microcirculation and the immune system were investigated using high-resolution methods (intravital microscopy, reflective spectrometry, white light spectroscopy combined with laser Doppler microflow measurements). The stimulation signal corresponding to physiological spontaneous arteriolar vasomotion was transmitted using an electromagnetic alternating field of low magnetic flux density.

RESULTS: During the 27-day treatment and observation period, a complementary treatment effect of the applied biorhythm-defined physical vasomotion stimulation could be detected.

PMID: 24271148 DOI: 10.1007/s00391-013-0567-8
BEMER Electromagnetic Field Therapy Reduces Cancer Cell Radioresistance by Enhanced ROS Formation and Induced DNA Damage.

Storch K1,2, Dickreuter E1,2, Artaj A3, Adamski J3,4,5, Cordes N1,2,6,7.

Abstract
Each year more than 450,000 Germans are expected to be diagnosed with cancer subsequently receiving standard multimodal therapies including surgery, chemotherapy and radiotherapy. Owing to intrinsic and acquired resistance to these therapeutic approaches, both the better molecular understanding of tumor biology and the consideration of alternative and complementary therapeutic support are warranted and open up broader and novel possibilities for therapy personalization. Particularly the latter is underpinned by the increasing utilization of non-invasive complementary and alternative medicine by the population. One investigated approach is the application of low-dose electromagnetic fields (EMF) to modulate cellular processes. A particular system is the BEMER therapy as a Physical Vascular Therapy for which a normalization of the microcirculation has been demonstrated by a low-frequency, pulsed EMF pattern. Open remains whether this EMF pattern impacts on cancer cell survival upon treatment with radiotherapy, chemotherapy and the molecular-targeted agent Cetuximab inhibiting the epidermal growth factor receptor. Using motile physiological, three-dimensional, matrix-based cell culture models and cancer cell lines originating from lung, head and neck, colorectal and pancreas, we show significant changes in distinct intermediates of the glycolysis and tricarboxylic acid cycle pathways and enhanced cancer cell radiosensitization associated with increased DNA double strand break numbers and higher levels of reactive oxygen species upon BEMER treatment relative to controls. Intriguingly, exposure of cells to the BEMER EMF pattern failed to result in sensitization to chemotherapy and Cetuximab. Further studies are necessary to better understand the mechanisms underlying the cellular alterations induced by the BEMER EMF pattern and to clarify the application areas for human disease.

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Free PMC Article
Attention deficit hyperactivity disorder (ADHD) and the use of the BEMER Therapy

Attention Deficit Hyperactivity Disorder (also known as Hyperkinetic Syndrome or attention disorder with hyperactivity) is characterized by a complex of symptoms including:

- **Impaired attention**, with reduced concentration and stamina as well as being easily distracted
- **Impulsiveness** in the sense of impulsive behavior (thought about it, did it) and abrupt mood changes
- **Motoric hyperactivity** combined with a strong urge to move about aimlessly.

Today most experts assume that ADHD is an illness with biological causes where genetic factors seem to play a role. Additionally damage from alcohol or nicotin abuse during pregnancy is being discussed as a possible cause. It is probable that children affected by this have brain metabolism disorders. Once the problem has been clearly diagnosed, then as a rule a therapy is prescribed that is a combination of psycho-, movement- and relaxation therapies. Ideally these are coupled with thorough counselling and support for the parents, teachers and educators. Therapy that includes the use of psychopharmical drugs is still controversial, although in thoroughly tested individual cases they have achieved a significant reduction of the symptoms. In addition to a well structured environment and following a planned daily schedule with sufficient sleep, creative and movement intensive activities have a positive effect. At all costs, a stimulus situation through television, computer games or a hectic environment should be avoided.

The BEMER Therapy is an effective complement to the above mentioned therapies, as was to be expected based on the effectiveness of the BEMER. This has also been confirmed by parents' reports. The potential effects on the central, peripheral and vegetative nervous systems are significant. Improvement of blood circulation (including microcirculation) and oxygen partial pressure among other things form the basis for:

- improvement of the brain metabolism
- harmonization of the vegetative nervous system
- anti stress effect and improved psychovegetative regeneration.

**Recommended use of the BEMER Therapy:** It is sensible to set the coil mat at the lower levels, starting at Level 1 and progressing weekly to Level 4 or even 6. Before bedtime Level 1 is recommended.

As a rule, children with ADHD like the BEMER because it feels so „light“ and thus they voluntarily take part in the therapy.

Tips about the hyperactivity syndrome: Many of the afflicted children have significantly damaged intestinal flora. This is frequently accompanied by fungal decay (Candida albicans) in the intestines. This hinders carbohydrate digestion and causes fermentation, leading to small amounts of toxic alcohol being produced that affect the disturbance-prone brain metabolism of children. Thus decontamination of the intestinal flora and a change in diet are important measures. Stress caused by exposure to heavy metals (amalgam!) and other disturbing factors can play an additional role. An individual therapy concept is necessary for each case.
Inflammatory intestinal diseases (Ulcerative colitis/Crohn's Disease) and the BEMER Therapy

Chronic inflammatory intestinal diseases that cause enormous stress are syndromes that are becoming increasingly common. Despite extensive research, the causes of these illnesses have not been clearly established.

Inflammation of the large intestine (colon) is known as colitis; and if this inflammation appears as ulcers (ulceration), it is called Ulcerative colitis. Crohn's Disease on the other hand is a chronic inflammatory and scarring intestinal disease that can spread throughout the entire digestive tract, including the esophagus, and mostly occurs in the form of sudden attacks. It is named after its American discoverer, Dr. B. Crohn. In both cases it is an inflammation of the intestinal wall.

In the case of Ulcerative colitis, only the surface of the intestinal wall is inflamed. The inflammation always starts in the rectum and spreads to the colon in about 50% of those affected. The first and most important symptoms are frequent bouts of diarrhea (up to 30 times a day). This is mixed with mucous and blood, and is accompanied by abdominal and spasm-like pain before and directly after a bowel movement. Further symptoms include weight loss, fever, tiredness and overall exhaustion. If the disease is long term, the risk of developing intestinal cancer increases.

In the case of Crohn's Disease, all layers of the intestines are inflamed. Depending on the course of the disease, the inflammation can become widespread and is accompanied by fistulas and abscesses. The most significant symptoms are watery diarrhea and severe pain, particularly in the right hypogastrium. As in the case of Crohn's disease, weight loss, fever, exhaustion and loss of appetite can occur. Unlike Ulcerative colitis, mucous and blood mixtures are rare.

Particularly in the initial stages, both Ulcerative colitis and Crohn's Disease can very easily be confused with other illnesses. Diarrhea is usually linked to a harmless gastrointestinal influenza. The initial symptoms of Crohn's Disease are often diagnosed as an acute attack of appendicitis. However, there are infectious intestinal diseases that are caused by bacteria or microorganisms, such as salmonella, shigella and amoeba, that have absolutely nothing to do with Ulcerative colitis or Crohn's Disease. This makes comprehensive testing and precise diagnoses all the more necessary.

First, an infection caused by germs must be excluded by means of bacteriological and parasitological tests of the stool. If inflammatory activity is proven in the erythrocyte sedimentation rate tests, and the attacks occur in phases, then an inflammatory intestinal disease is indicated. However, only imaging testing (endoscopy, ultrasonar, etc.) can provide conclusions about the illness. Coloscopy is the most important diagnostic test.

Despite all the research and progress made, Ulcerative colitis and Crohn's Disease cannot be treated medicamentously, Almost 50% of those suffering Crohn's disease ultimately have to have an operation due to threatening complications, such as intestinal obstruction, abscesses and bowel perforation. In the case of Crohn's Disease only the affected area of the intestine are removed, as a cure is not thought possible.
In very severe cases of Ulcerative colitis and when threatening complications exist, such as toxic bowel perforation, the entire large intestine is removed.

Conventional therapy consists of alleviating the symptoms, avoiding complications and extending the periods between individual attacks. The most emphasis is placed on adhering to an individual diet.

General diet principles for:

**Ulcerative colitis**
- High protein with plenty of roughage
- Reduced flatulence
- Avoidance of incompatible foods, possibly a dairy-free diet
- Please note that in acute attacks, only easily digestible food is to be consumed!

**Crohn’s Disease**
- High protein, rich in calories
- Easily absorbed
- Strict avoidance of incompatible foods

Treatment with low intensity, pulsating electromagnetic fields is an additional starting point. Such fields counter the inflammatory processes and expedite the regeneration of the mucosal cells and of the intestine as a whole.

The following effects of the BEMER Therapy are significant for the treatment of chronic inflammatory intestinal diseases:

- improved blood circulation
- increased oxygen concentration in the blood
- improvement of the blood flow
- general metabolic regulation of the cells
- improved microcirculation in the damaged tissue, promoting the excretion of acidic and metabolic end products
- activation of the „repair proteins“ and anti-inflammatory enzymes; these support the best possible regeneration of the damaged tissue

Electromagnetic field therapy, which can be optimally performed with the BEMER 3000, is a complex one that not only improves the blood circulation and oxygen supply, but also has a general regulatory effect on the metabolism. In conjunction with other biological and clinical methods, it frequently succeeds in alleviating symptoms and positively affecting the entire course of the illness.

**Application recommendation of the BEMER Therapy**

Use of the coil mat in accordance with the basic program twice a day; and to optimize and stabilize the immune system once a day, with the coil mat set at Level 10. In addition, the intensive applicator containing P4 can be used to aid local intestinal functions and for the best possible regeneration of the damaged sections. During acute inflammatory periods, the intensive applicator containing P3 or the coil cushion (without reducing cable) should be used.
Synergistic effect of EMF - BEMER type pulsed weak electromagnetic field and HPMA-bound doxorubicin on mouse EL4 T cell lymphoma

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Abstract

We have investigated the effects of low-frequency pulsed electromagnetic field (EMF) produced by BEMER device on experimental mouse T cell lymphoma EL 4 growing on conventional and/or athymic (nude) mice. Exposure to EMF - BEMER slowed down the growth of tumor mass and prolonged the survival of experimental animals. The effect was more pronounced in immunocompromised nude mice compared to conventional ones. Acceleration of tumor growth was never observed. No measurable levels of Hsp 70 or increased levels of specific anti-EL4 antibodies were detected in the serum taken from experimental mice before and at different intervals during the experiment, i.e. before solid tumor appeared, at the time of its aggressive growth and at the terminal stage of the disease. A significant synergizing anti-tumor effect was seen when EL4 tumor-bearing mice were simultaneously exposed to EMF - BEMER and treated with suboptimal dose of synthetic HPMA copolymer-based doxorubicin, \( \text{DOXHYD} \cdot \text{HPMA} \). Such a combination may be especially useful for heavily treated patients suffering from advanced tumor and requiring additional aggressive chemotherapy which, however, at that time could represent almost life-threatening way of medication.

Key words: EL4 T cell lymphoma; athymic mice; DOXHYD·HPMA; EMF; anti-cancer resistance; stimulation of the immune system
Introduction

Over the past three decades, potential health effects of exposure to electromagnetic fields (EMFs) have been extensively investigated in epidemiologic studies. This awareness has been triggered by the growing body of knowledge on how EMFs interact with cellular systems of living organisms. Electromagnetic field treatment is widely applied in clinical practice for prevention, diagnosis, and treatment of diseases with various etiology. The mechanisms of biological and therapeutic effects of EMFs are still not entirely understood (Gapeyev et al., 2011). It was even suggested that low-frequency EMFs may be a risk for human health (Zheng et al., 2000; Erren, 2001; Scott et al., 2002; Porock and Gentry, 2002; Trosic, Busljeta & Pavicic, 2004; Busljeta, Trosic & Mlko-Kraus, 2004; Chen et al., 2010; de Vocht, 2010).

Widespread concerns about whether EMFs could affect human health have been raised in epidemiologic studies trying to answer the question of their involvement in cancer appearance (Pollan et al., 2001; Weiderpass et al., 2003; Girgert et al., 2005). Low-frequency electromagnetic fields were suspected of being involved in carcinogenesis, acting as co-promoters during neoplastic transformation, modifying cell proliferation and/or signal transduction pathways, (Jin, Blank & Goodman, 2000; Richard et al., 2002). Experimental findings also suggested that exposure to low-frequency EMFs may affect various cell functions via actions exerted on intracellular and membrane proteins, including ion channels, membrane receptors and enzymes and cytoskeleton (Grassi et al., 2004; Lange, Viergutz & Simko, 2004). On the other hand, Scarfi et al., 2005 and Jian et al (2009) report that ELF EMF induces apoptosis only in cancer cell lines which could be even enhanced by low doses of X-ray irradiation. Liteniture in the area of DNA strand breaks as a consequence of EMF exposure is also contradictory (Ruiz-Gomez & Martinez-Morillo, 2009). Some investigators report on DNA damage (Vijayalaxmi & Prihoda, 2009) while others deny it (Phillips, Singh & Lai, 2009). So far, the findings gave no support to the hypothesis that EMF exposure increases the risk of cancer (Forssen et al., 2005; Beniashvilli et al., 2005; Sommer et al., 2007; Chen et al., 2010; de Vocht, 2010).

It is the reality that the data from scientific literature as well as from epidemiologic studies are still controversial. While some researches associate ELF-EMF exposure with carcinogenesis, other studies suggest that treatment with selected frequencies is feasible and well tolerated and may have biological efficacy in diseased patients (Lacy-Hubert et al., 1998; Ronchetto et al., 2004; Lange et al., 2004; Ronchetto et al., 2004; Chen, 2010).

Here, we aim to study the biological effects of a low-frequency pulsed electromagnetic field produced by the BEMER device (EMF - BEMER) (Katka, 1998) on an experimental cancer model, EL4 T-cell lymphoma (H-l', Thy-1,21 growing on normal immunocompetent mice of inbred strain C57BL/6 (B/6) and/or on immunodeficient athymic nu/nu CD-1 mice. It was demonstrated that EMF - BEMER influences microcirculation and the activity of antioxidant enzymes (Kafka & Sporadyk, 2003), especially after chemo- and radiotherapeutic cancer treatment (Gabrys, 2004) and wound healing (Kafka et al., 2005).

A new generation of polymeric anticancer drugs based on N-(2-hydroxy,op3ylmethacrylamide (HPMA) with improved therapeutic potential, considerably decreased nonspecific side effects and the ability to stimulate anticancer immunity is already well documented (Kopecek & Kopeckova, 201 O; Rihova & Koval', 2010; Lammers & Ulbrich, 2010).

The main purpose of this study was to test a) the effect of an exposure to EMF - BEMER on growth an experimental cancer model (El4 T cell lymphoma) and b) a possible synergic effect of suboptimal treatment with HPMA capolymer-based doxorubicin (DOX -HPMA) as an anti-cancer agent and EMF - BEMER.
Material and methods

Polymer conjugate noJ111'D_HP./ld4.

was prepared according to Etrych et al. (2008). It is a doxorubicin bound to N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer carrier through a hydrazone bond with a MW-34000.

EMF source and exposure

The BEMER device is a certified medical instrument. The control unit works with an operating voltage from 12-1SV. In the connected coil mat the special multidimensional pulsating current generates a weak, pulsating electromagnetic field. The basic BEMER impulse starts at a frequency of 0 Hz, is constantly increasing and within 30ms reaches its maximum of 2 kHz. From there it falls back to 0 Hz and the impulse starts again. Parallel the magnetic flux intensity begins at 0 µT and pulses upwards until it reaches its maximum, according to the chosen level. From there, like the frequency, it falls back to 0 µT and the impulse starts again (Fig. 1). For the experiment were chosen maximum levels of 3.5 µT; 10.5 µT; 21 µT and 35 µT. The BEMER device neither offers the choice of only one constant frequency nor only one constant intensity.

Cancer cell line

Mouse T-cell lymphoma EL4 cell were obtained from American Type Culture Collection (ATTC).

Culture conditions

EL4 cells were grown at 37° C with 5% CO2 in RPMI 1640 medium (Gibco BRL) supplemented with heat-inactivated 1% v/v fetal calf serum (FCS) selected for low mitogenicity, 4 mM L-glutaminic (Gibco BRL), 1 mM Na-pyruvate, 50 mM 2-mercaptoethanol, 4.5 g/l glucose, 100 U/mL penicillin and 100 µg/mL streptomycin (Sigma).

Animals

All experiments were done either on conventional eight-week-old female mice of inbred strain CS7BL/6 (H-2b) purchased from the Animal Center of the Institute of Physiology, Academy of Sciences of the Czech Republic, v.v.i. or on eight-week-old female immunodeficient athymic nu/nu CD-1 mice obtained from AnLab Ltd., Prague. The mice were randomly assigned to either experimental or control groups and housed in accordance with approved guidelines. Food and water were given ad libitum. The animal room was maintained at 22° C. The experimental designs were in accordance with the Czech Republic Act for Experimental Work with Animals (Decrees No.311/97, I J 7/87, and Act No. 246/96), which is fully compatible with the corresponding European Community Acts.

In vivo tumor growth

a) Exposure to EMF only

On day zero, 1 x 10^6 EL 4 T-cell lymphoma cells in 0.1 mL RPM! 1640 medium were injected s.c. (subcutaneously) on right back of C57BL/6 or nu/nu CD-1 mice. The experimental animals were exposed to low-energy EMF - BEMER. Controls were
transplanted with cancer cells but were not exposed to EMF. At least ten mice were used for each experimental group. The animals were observed daily for signs of tumor progression. The survival time, size of tumor and the number of long-term survivors (LTS) were determined.

b) Exposure to EMF and DOXHYD-HPMA conjugate

Mice were exposed to EMF similarly as described in paragraph a). The mice that developed palpable tumors reaching 5 - 8 mm in diameter within 8 to 9 days after the implantation of cancer cells were intravenously treated with DOXHYD-HPMA (15 mg of DOX eq./kg) diluted in PBS. Those surviving at least 60 days without any signs of a tumor were considered as long-term survivors (LTS), and they were re-transplanted with a lethal dose (1 x 10^5) of the same tumor cells and left without treatment to determine the therapy-induced tumor resistance.

Quantitative heat shock protein analysis was performed in serum samples using a mouse heat shock protein 70 (HSP70) ELISA kit according to the manufacturer's manual.

The statistical significance (P < 0.05) of the differences between volumes of tumors in the various groups was assessed by applying a two-sided Student's t-test. For each approach,
Results and discussion

Conventional C57BL/6 mice were first exposed to pulsed EMF - BEMER (30 min every four hours with the 10.5 μT, 21 μTor 35 μT intensity or permanently with the intensity 3.5 μT) on day four before s.c. transplantation of cancer cells and then every day until the end of the experiment. Figure 2A illustrates mean tumor volume change for each of the four treatment groups and Figure 2B documents survival of experimental animals. Both figures show significant retardation of tumor growth and prolongation of lifespan in mice exposed to EMF - BEMER with an intensity of 21 μT. The experiment was repeated three times with similar results.

The slight anti-tumor activity demonstrated in the experimental group exposed to EMF-BEMER 3000 of the intensity of 21 μT could be, among others, related to the activation of the immune system. To elucidate a tentative involvement of innate (natural or native) and/or adaptive (specific) immunity in the mechanism of action of EMF - BEMER on tumor growth, we used for further experiments immunocompromised athymic nude (nu/nu) mice. Athymic mice suffered from an extremely limited number of T cells, which is the reason why they have only marginal specific immunity and are routinely used to define the role of T/B lymphocytes in immunity and disease.

Similarly as in conventional mice, we have repeatedly observed in nude mice that EMF - BEMER to which the animals were exposed slowed down the growth of experimental EU T-cell lymphoma (Fig. 3A) and significantly extended their average lifespan (Fig. 3B). Interestingly enough, the exposure to EMF BEMER gave a better result in terms of the tumor growth retardation and prolongation of survival time in immunocompromised nude mice, where the effect was more pronounced than in conventional animals. This suggests that either innate immunity, that is strong in athymic mice, or absence of T suppressive activity may contribute to the protective effect of EMF - BEMER.

Taken together, the results point to slight but clear-cut anti-tumor effects of low-power EMF - BEMER on EU mouse T cell lymphoma or at least could be taken as a proof that exposure to EMF - BEMER is not a risk factor intensifying the development of experimental mouse T-cell lymphoma EL4.

There are numerous data confirming not only the safety but a certain antiproliferative effects of EMF treatment (Beneducci et al., 2005; Jimenez-Garcia et al., 2010). Williams et al. (2001) were the first to report the reduction of tumor angiogenesis after exposure of mice with experimental cancer to pulsating electromagnetic fields. As a result, tumor growth was significantly reduced in female C57H/HeJ mice bearing mammary adenocarcinoma. Tofani et al. (2002) documented that the treatment of tumor-bearing nude mice with daily exposure to extremely low-frequency magnetic fields for 4 weeks caused significant tumor growth inhibition. Mice suffering from cancer xenograft had significantly fewer lung metastatic sites, slower tumor growth and reduced vascularization, which together resulted in an increased survival time compared to untreated controls. Similar data were obtained with AK.R/J mice suffering from spontaneous lymphoblastic lymphoma. EMF exposure did not alter malignancy or the progression of the disease and lymphatic infiltration did not occur more often in EMF exposed than in control mice (Sommer et al., 2004; 2007). Cameron et al. (2005) report a decreased growth and reduced vascularization of human breast cancer xenografts in female athymic (nude) mice exposed to EMF either alone or in combination with gamma radiation. Similarly, a slight inhibition of the formation of chemically induced neoplastic foci in rat livers was observed when the animals were exposed to the EMF (Rannug et al., 1993).
The anti-cancer effects of EMF could result from inhibition of cell proliferation, targeted apoptosis induction, regulation of cellular homeostasis, affecting pathways associated with heat stress and/or activation of the immune stem.

Plotnikov et al. (2004) demonstrated the expression of heat shock genes in particular HSP70, 90 and 60 in tumor cells in response to extremely low frequency electromagnetic fields (EMF) alone or in combination with the heat stress. Since electromagnetic fields interact with biological tissue they can induce temperature change by interacting directly with moving electrons. DNA (Blank & Goodman, 1999), an essential mediator in the electromagnetic field-stimulated stress response appears to be similar to these reported for other physiological stresses (e.g. hyperthermia, heavy metals, oxidative stress) and could well constitute the general mechanism of cell response to electromagnetic field (Li, Blank & Goodman, 1999). Detailed mechanisms of the processes of modulating the electromagnetic signals into biological responses, especially changes in biochemistry, are poorly understood.

We used ELISA test to examine the release of HSP70 into the serum of treated tumors to determine whether heat shock proteins are involved in the protective anti-cancer response of mice exposed to EMF-BEMER. The level of HSP70 in the serum after the heat treatment represented a control. Serum samples were then taken from individual mice on day 20, i.e. before transplantation, at the time when solid cancer is not yet palpable, on day 30, i.e. at the time of regressive growth of the tumor and on day 39, i.e. in the terminal state of the disease. Using sensitivity of ELISA test we repeatedly failed to determine measurable levels of HSP70 in serum samples. The reason could be quantitative as the positive effect of EMF on the expression of the heat shock protein genes HSP70, HSPA6 and HSP70 was documented in tissue culture of human cells, malignant as well as normal, exposed to a wide range of environmental stimuli, including electromagnetic fields alone or in combination with thermal stress (Blank & Goodman, 1999; Dressler & Gunther, 1998).

A single combination of low frequency fields and chemotherapy with 5FU, anthracyclines (Liang et al., 1997; Orel et al., 2005) or methotrexate (Laquer-Ruperez et al., 2003) Liang et al. (1997) report the enhancement of direct in vitro cytotoxicity of daunomycin by a pulsed magnetic field using multidrug resistant subline KB-ChR-8.5.11 while no such effects were seen by Laquer-Ruperez et al. (2003) in MCF-7 breast cancer cells treated with methotrexate. The rare animal studies explain a
positive effect of EMF given simultaneously with anti-cancer drugs by enhancing the drug delivery across biological barriers (Murthy, 1999).

The original reason for the conduction of this study was to document the effect, if any, of EMF - BEMER on the growth of cancer cell line EIA in vitro and on experimental EIA cancer model in vivo. The data presented in Figs. 2 - 3 which document slight but undoubted anti-cancer effect of EMF, substantiated the study of a hypothetical combinatorial effect of EMF - BEMER and a cytostatic drug. We decided to use its polymeric form, as anti-cancer drugs bound to different polymeric carriers represent an advanced approach for anti-cancer treatment. Such derivatives have long-term peripheral blood circulation, increased tumor accumulation, decrease of side-toxicity (Kopecek 2010; Kopeces & Kopeckova, 2010) and those based on N-(2-hydroxypropyl)methacrylate (HPMA) carrier repeatedly documented therapy-dependent activation of the immune system (Rihova & Koval', 2010).

We used a suboptimal dose (15 mg of Dox eq./kg) of doxorubicin bound to N-hydroxypropyl)methacrylamide (HPMA) carrier through a hydrazone bond (DOX • HPMA). It is a formulation which was repeatedly shown to have an exceptional anticancer effect based on the direct cytotoxicity and therapy-activated anticancer-immune response (Sirova et al., 2010; Rihova & Kovar, 2010). The decreased growth of tumor was recorded in all experimental groups. Only 60% of cured mice, when treated with DOXHYO_HMPA only, correspond to the fact that a suboptimal dose of the drug derivative was used. Similar percentage of long-term survivors and thus no effect of EMF • BEMER was seen when mice were simultaneously exposed to EMF of the intensity of 10.5 μT (30 minutes every four hours; 60% ofLTS) or permanently to 3.5 μT (70% ofLTS). Considerably better results were obtained in mice exposed to EMF - BEMER of the intensity of 21 μT (30 minutes every four hours; 80% ofLTS) or 35 μT (30 min every four hours; 80% ofLTS) (Fig. 4). The survival of athymic nude u/nu mice was also prolonged when the animals were treated with the same dose of DOXH -HPMA as conventional animals (15 mg of Dax eq./kg) and exposed to the EMF - BEMER. Also here, the higher intensities (21 μT and 35 μT) were more efficient (FIO.5). Unexpectedly, one mouse survived more than four months. It could not be recorded as a long-term survivor as tumor, even if considerably shrunken, was still there (see "The case report").

The observation that EMF intensity of 21 μT is optimal in all so far tested systems is worthy of additional research. Barbault et al. (2009) suggest that tumor-specific frequencies have to be used for the treatment of patients with advanced tumors. Such studies could be the basis for the design of strategic and clinical application of selected EMF sources for the treatment of different diseases.

Humunocompetent cells involved in the defense mechanisms are those preferentially acting in native (natural) immunity such as macrophages and NK cells and those effective in acquired (specific) immunity such as NKT and different subpopulations of T and B cells. NK cells have an important, though not decisive role in anticancer response where CTL cells are the major player in the game. The possibility of the effects of EMF on activity of the immune functions in living organisms has already been hypothesized and tested (Arafa et al., 2003; Tuschl, Novak & Molla-Djafari, 2006; Di Giampaolo et al., 2006; Boscolo et al., 2007; Akan et al., 2010; Kleijn et al., 2011) but never directly demonstrated in vivo. For instance, Rossi et al. (2007) report that ELF-EMFs (source SEQEX) reduce oxidative stress and reduce the side effects of chemotherapy, and specifically myelodepression (myelotoxicity), in patients with Hodgkin's lymphoma. As oxidative stress may be, at least in part, responsible for secondary malignancies they conclude that SEQEX with its ability to reduce an oxidative stress induced by treatment with chemo-radiotherapy may reduce the risk of late toxicities. The EMF was reported as both increasing or decreasing the activity/number of circulating...
natural killer cells (NK) cells or no effect at al. (Gobba, 2009a; 2009b). However, it has to be stressed, that serious scientific data are so far still extremely limited.

In all our experimental systems we routinely proved the activation of the immune system during anticancer therapy by re-transplantation of LTS with a lethal dose of cancer cells. As no therapy is provided after such a re-transplantation, the only explanation for the eventual eradication of re-injected cancer cells is the activation of defense mechanisms of the cancer-bearing host during the primary treatment (Rihova & Koval', 2010). Figure 6 documents a high cancer resistance in experimental groups exposed to EMF - BEMER. While only 20% of re-transplanted LTS survived when treated with ooxHYD_HPMA, up to 100% of them survived if simultaneously exposed to EMF - BEMER (86% when permanently exposed to 3.5 $\mu$T; 84% when exposed to 10.5 $\mu$T and 100% when exposed to 21 $\mu$T or 35 $\mu$T.

The case report.

One mouse treated with DOXHYD-HPMA and exposed to EMF-BEMER of an intensity of 21 $\mu$T survived more than four months, which is quite exceptional (Fig. 5). As a rule for conventional or nude mice, immediately after the treatment with polymeric drugs the growth of experimental cancer stops. In a week or so the cancer shrinks. After another few days the tumors disappear (mice are cured) or their aggressive growth starts again. However, in that one mouse the size of the tumor (about 15 mm$^3$) and healthy condition stayed unchanged for more than four months. One hundred forty days from the beginning of the experiment and 122 days after "stabilization" of the cancer size it was decided to re-transplant the mice with a lethal dose of cancer cells similarly as we have routinely done for conventional mice to test the mechanisms responsible for the control of cancer growth. Here, mainly innate immunity could be involved in cancer eradication as the number of T cells responsible for adaptive anticancer immunity in nude mice is very limited. Rather surprisingly, no cancer growth was observed at the site of secondary re-injection, i.e. on the left side on the back of mice. However, immediately after such "a second cancer cell attack" we have detected aggressive growth of previously stabilized primary cancer (solid EL4 thymoma) on the right side of the mouse back. The growth was almost exponential until day 38 (Fig. 7). Then, from day to day, a substantial decrease in the size of tumor was observed which is usual in tumor-exhausted experimental models. We decided to end the experiment and to test a) the sensitivity/resistance of EL4 cells isolated from the tumor to original DOXHYD-HPMA conjugate, b) the ability of spleen cells to respond to activation with Con A (T cell responder), LPS (B cell response) and anti-CD3 plus IL-2, c) different immune cell subpopulations in blood and finally d) to perform histopathological examination of different organs (tumor, liver, spleen, lung, heart and bone marrow). The drug sensitivity of EL4 cancer cells isolated from the tumor was comparable with that of original cancer cell line EL4 (IC$_{50}$ = 0.44 $\mu$g/ml vs 0.53 $\mu$g/ml) and so was the ability of spleen cells to respond to different stimulation stimuli. Histopathological analyses did not reveal substantial metastatic cancer cell infiltration. Unfortunately, there was not enough material to precisely determine the immune cell subpopulations in the blood. However, we consider the case interesting enough to share it with others as hypothetical documentation of "immunoeediting" (Dunn et al., 2004; Prestwich et al., 2008).
Acknowledgements

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Legends to figures

Fig. 1: Typical form of electromagnetic impulse generated by the BEMER device.

Fig. 2A: Effect of pulsed EMF on the growth of EL4 mouse T cell lymphoma in conventional C57BL/6 mice exposed to EMF for 30 minutes every four hours (intensity of 10.5 μT, 21 μT or 35 μT) or permanently (intensity of 3.5 μT). The exposure to EMF started four days before cancer cell transplantation.

Fig. 2B: Effect of pulsed EMF on the survival of C57BL/6 mice bearing EL.4 mouse T cell lymphoma and exposed to EMF for 30 minutes every four hours (intensity of 10.5 μT, 21 μT or 35 μT) or permanently (intensity of 3.5 μT). The exposure to EMF started four days before cancer cell transplantation.

Fig. 3A: Effect of pulsed EMF on the survival of nu/nu CD-1 mice bearing EL4 mouse T cell lymphoma and exposed to EMF for 30 minutes every four hours hours (intensity of 10.5 μT, 21 μT or 35 μT) or permanently (intensity of 3.5 μT). The exposure to EMF started four days before cancer cell transplantation.

Fig. 3B: Effect of pulsed EMF on the survival of nu/nu CD-1 mice bearing EL4 mouse T cell lymphoma and exposed to EMF for 30 minutes every four hours hours (intensity of 10.5 μT, 21 μT or 35 μT) or permanently (intensity of 3.5 μT). The exposure to EMF started four days before cancer cell transplantation.

Fig. 4: The combinatory effect of pulsed EMF and DOXHYD-HPMA (15 mg DOX eq./kg) in conventional C57BU6 mice exposed to EMF for 30 minutes every four hours (intensity of 10.5 μT, 21 μT or 35 μT) or permanently (intensity of 3.5 μT). The exposure to EMF started four days before cancer cell transplantation.

Fig. 5: The combinatory effect of pulsed EMF and DOXHYD-HPMA (15 mg DOX eq./kg) in nu/nu CD-1 mice exposed to EMF for 30 minutes every four hours (intensity of 10.5 μT, 21 μT or 35 μT) or permanently (intensity of 3.5 μT). The exposure to EMF started four days before cancer cell transplantation.

Fig. 6: Re-transplantation of long-term survivors (see Fig. 4) with a lethal dose (1 x 10^5) of EL4 T cell lymphoma cells.

Fig. 7: The growth of primary solid EL 4 T cell lymphoma injected s.c. on the right back of experimental mouse after its s.c. re-transplantation with a lethal dose of the same cancer cells on the left back.
### TABLE 1. Serum Level of Anti-EU Antibodies; Antigen EL4 Cells

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- The numbers represent log₁₀ of serum dilution
- Positive control > 24, negative control (natural antibodies) 4.5 - 6.0
TABLE 2, Serum Level of Anti-EU Antibodies; Antigen= EL4 Cell Lysate

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<th>Day</th>
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* The numbers represent log2 of serum dilution

b Positive control > 22, negative control (natural antibodies) 8.5 - 10.5