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Bioelectromagnetic Field Effects on Cancer Cells and Mice Tumors

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We present possibilities and trends of ELF bioelectromagnetic effects in the mT amplitude range on cancer cells and on mice bearing tumors. In contrast to invasive electrochemotherapy and electrogenetherapy, using mostly needle electrodes and single high-amplitude electropulses for treatment, extremely low-frequency (ELF) pulsating electromagnetic fields (PEMF) and sinusoidal electromagnetic fields (SEMF) induce tumor cell apoptosis, inhibit angiogenesis, impede proliferation of neoplastic cells, and cause necrosis non invasively, whereas human lymphocytes are negligibly affected. Our successful results in killing cancer cells—analyzed by trypan blue staining or by flow cytometry—and of the inhibition of MX-1 tumors in mice by 15–20 mT, 50 Hz treatment in a solenoid coil also in the presence of bleomycin are presented in comparison to similar experimental results from the literature.

In conclusion, the synergistic combinations of PEMF or SEMF with hyperthermia (41.5°C) and/or cancerostatic agents presented in the tables for cells and mice offer a basis for further development of an adjuvant treatment for patients suffering from malignant tumors and metastases pending the near-term development of suitable solenoids of 45–60 cm in diameter, producing >20 mT in their cores.

Keywords Non invasive PEMF; Synergism; Bleomycin; Hyperthermia; Photodynamic effect.

Introduction

Studies of extremely low-frequency pulsating electromagnetic fields (ELF-PEMF) and sinusoidal electromagnetic fields (ELF-SEMF, carried out mostly in vitro) revealed a variety of metabolic effects depending on the choice of electrical and electromagnetic parameters on the one hand, and the physiological states of cells or animals on the other (Markov, 2007; Simko, 2004; Williams et al., 2001; see several examples in Table 1 for comparison). Recently, the application of electric pulses (using electrodes, e.g., Yao

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Table 1
Typical examples of different PEMF and SEMF treatments of cancer cells and tumor animals also in combination with cytostatic agents from literature

Cells					
HL-60, ML-1, lymphocytes	45 mT, 50 Hz	–	3.5 h	Cancer cells:100% dead, lymphocytes: only 10% dead	Hisamitsu (1997)
Human sarc.:HT-29, drug resist.	10.8 mT, peak;	Daunorubicin		Cytotoxicity increased	Liang (1997)
HL-60	20 mT, 50 Hz	Na, K ions	4 days	Inhibition of proliferation, induction of apoptosis, depends on osmolarity	Huang (2006)
Osteosarcoma cells (mouse)	0.4–0.8 mT; 10 Hz	Doxorubicin		Decrease of growth rate of the resistant cells	Miyagi (2000)
K-562 ,U-937	8.5 mT, 50 Hz + vis. light	Daunorubicin actinomycin-C	12 min + vis. light	Exp.: 37–47% dead, more than in control	Pang (2001)
K-562	8 mT, 50 Hz	–	2 days	80% apoptosis, 19.5 necrosis, 0.5% vital	Pang (2002)
K-562, U-937	10 mT, 50 Hz	Actinomycin-C	6 h	Exp.: 35, 4% dead, control: 19% dead	Traitcheva (2003)
Chron.myel.leukem.	250 mT, 25 Hz	Lmatinib	80 s/day	62% cells death	Yamaguchi (2007)

K-562, DG-75, HL-60, lymphocytes	35 mT, 50 Hz	–	4 h	Exp.: % K : 6.1, DG: 5.5, HL:16 lymphoc.: 1.6; Contr. % K: 1.5, DG: 2.6, HL: 4.4 lymphocytes : 0	Radeva (2004)
Animals					
nude mice + KB-CH- 8-5-11	10.8 mT (peak)	Daunorubicin		Decrease of tumor volume	Liang (1997)
Mice + adenocarcinom	20 mT, 120 Hz (half sine wave)		10 min/day 11 days	Apoptosis, inhibition of angiogenesis,	Williams (2001)
Mice + B16-BL6 cells Intratumoral injection, Melanoma model	200 mT, 25 Hz pulses, width 0.24 ms 1000/d.		80 s /d during 17 days	Exp.: 60% survival at 25 day tumor weight decreases to 54%, necrosis; increase of TNF-α and IL-2; Contr.: only 20% survival at 25 d	Yamaguchi (2006)
Buffalo rats + Morris hepatoma	Biocom 15 LF- stimulator		14 days	69% total regression of tumor	Fedorowsky (2004)
Mice + S-180 Sarcome	> 600 mT, static		15 min/day, 28 days	Exp.: only 20% tumor mass of the control	Zhang (1995)

et al., 2005), static magnetism by external electrodes (Wang, 2009), and electromagnetic fields (ELF and also HF fields, e.g., Barbault et al., 2009) were extended to medicine, genetics, biochemistry, physiology, biotechnology, etc. (see the reviews of Berg, 1994, 1999; Repacholi and Greenebaum, 1999; Simko, 2004; Markov, 2007; Pilla, 2007; Ueno and Shigemitsu, 2007; and the monographs of Wilson et al., 1990; Lin, 1994; Walz et al., 1995; Stavroulakis, 2003; Rosch and Markov, 2004; Kato, 2006; Barnes and Greenebaum, 2007). Recently, ELF-PEMF and ELF-SEMF treatments directed towards cancer cell suspensions (Table 1), tumors in animals, and some patients show remarkable results: strong differences in necrosis between cancer cells and normal lymphocytes (Hisamitsu et al., 1997); inhibition of proliferation, induction of apoptosis, and increase of necrosis in tumor cells (Glück et al., 2001; Liang et al., 1997; Pang et al., 2001, 2002; Traitchewa et al., 2003); combination of SEMF and anticancer drugs (Miyagi et al., 2000), hyperthermia, or photon irradiation (Radeva et al., 2004); and inhibition of angiogenesis and tumor growth in mice, which yields significant increase in survival time (Tofani et al., 2002; Williams et al., 2001).

Although pilot studies showed successful results, applications in humans are still in the very early stages (Ronchetto et al., 2004). The following experimental studies try to give further answers to five questions.

- What is the effect of long time SEMF-treatment by 15 mT on cancer cells under various conditions?
- Is the SEMF response of malignant cells different from the response of lymphocytes from a healthy donor?
- Does the treatment by SEMF combined with cytotoxic agents or hyperthermia have a synergistic effect on the killing rate of cancer cells?
- What are suitable conditions for effective treatment of tumors in mice?
- Are our results helpful for the development of an adjuvant non invasive therapy of cancer patients?

The aim of our contribution is to detect optimal conditions for the induction of tumor remission, e.g., induction of apoptosis and necrosis and even antiangiogenesis, which would be favorable as a starting point for an adjuvant non invasive tumor therapy in combination with hyperthermia and cytostatic drugs.

Material and Methods

SEMF Exposure Systems

For long extended treatment of cells, a solenoid (15 mT, 50 Hz, 4 cm inner diameter 36.8–37 °C, 41.5°C, or 44°C) containing cells in plastic flasks (12.5 ml, Falcon) was placed inside a humidified incubator (5% CO_2at < 37 °C, < 41.5 °C or < 44 °C). These temperatures should compensate for the heat given off by the active solenoid. The control probes were placed in another incubator at 37°C, 41.5°C, or 44°C. For the exposure of mice, a solenoid (20 mT, 50 Hz, 10 cm inner diameter, and 20 cm length) was connected with a thermostat to ensure a constant temperature of 22°C. Exposure conditions were determined based on our experience from previous studies (Radeva et al., 2004; Traitcheva et al., 2003). For the treatment of mice by 200 mT pulse bursts, a commercial generator from Nihon Kohden Co (Tokyo) was used (Yamaguchi et al., 2006).

Cultivation of Cells

Human erythroleukemia K-562 cells (ATCC CCL-243) were maintained in RPMI 1640 medium (Sigma) containing 20% fetal calf serum (FCS), 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Sigma) in a humidified atmosphere of 5% CO_2 at 37°C. An asynchronous human breast adenocarcinoma cell line MX-1 (Deutsches Krebsforschungszentrum, Heidelberg, Germany) was cultivated in a monolayer at 37°C, with 5% CO_2 and 95% humidity. The cells were kept in Dulbecco's minimal essential medium containing 10% (vol/vol) fetal calf serum (Life Technologies, Karlsruhe, Germany) (Hilger et al., 2005). Starting cell density was $3–5 \times 10^5$/ml. Human lymphocytes were harvested from a healthy male. His blood was collected by venipuncture into heparinized tubes. The lymphocytes were isolated by centrifugation in a lymphocyte separation medium.

Measurements of Cells

The percentages of necrotic cells were determined by the standard trypan-blue test using an inverted microscope (Olympus).

Four fields of at least 100 cells from each of the experimental and control cuvettes were counted and the SD- values were calculated. The percentage of dead cancer cells before experiments did not exceed 1.5–3% depending on cultivation conditions, values which were always subtracted from the data in the tables.

In contrast to this, all the isolations of normal lymphocytes showed nearly 100% vitality before the beginning of experimental runs. The experiments were repeated 2 or 4 times. The ratio experiment/control was fairly constant, although the absolute values varied during some months because it was not always possible to obtain identical cultivation conditions. In other words, the physiological state of cells in the nutrition or starvation medium and the cultivation time before the field started are also factors influencing the efficacy of the field treatment.

Flow Cytometric Analysis

Apoptotic and necrotic cells were counted using the Annexin-V-FLUOS staining kit (Roche Diagnosis, Nutley, New Jersey, USA). After the SEMF exposure, 1.0×10^6 cells were washed using phosphate buffered saline (PBS, Sigma). Finally, cells were analyzed by the FL1 and FL3 detectors of the flow cytometer (FACScan apparatus from Becton Dickinson, New Jersey, USA) and the data processed by the machine's analysis program. Typical results are included in Table 3.

Preparation of Mice

All investigations were carried out with 6–17 week old male SCID- mice. The animals were bred by the breeding unit of the Institute of Laboratory Animals at the FS- University at Jena (approved by the Animal Ethics Commission) and were kept behind a standardized partial barrier. Water and lab-chow were supplied *ad libitum*. 5×10^5 MX-1 tumor cells were transplanted to every SCID-mouse. The cells were injected subcutaneously in the flank of the mice. About two weeks after the injection solid tumors were visible. Mice were anesthetized by a mixture of narcotics (Medetomidin, Midazolam, Fentanyl 0.175 ml/25 g mouse) intraperitoneally. The fur

of the mice was shaved in the region of the tumor and a small incision was made in the skin over the tumor. Ten microliters solution of Bleomycin (0,1 mg) were injected intratumorally by means of a microliter syringe. After the injection the incision was closed by a suture, then field treatment for 8 days (3 h/day) started. At the end of the experiments, the weights of the tumors were determined and for the calculation of the volumes their sizes were measured using a slide gauge. Student tests were used for calculation of p-values, which are shown in Tables 3 and 4.

Results and Discussion

The Response of Cells

SEMF Treatment of K- 562 and Lymphocytes. The treatment by the 15 mT field started after precultivation of the cells. From Figure 1 it can be seen that the percentage of necrotic K-562 cells increased markedly, whereas the control remained on a comparatively low level. In contrast to the K-562 cells, the normal lymphocytes were quite stable (Hisamitsu et al., 2007; Radeva and Berg, 2004). They were scarcely affected as was shown after a stronger treatment by 35 mT for 4 h according to the FACS can determination 16 h later:

	Apoptosis	Necrosis
Lymphocytes:	about 6%	5%
K-562 cells:	about 35%	14%

Hence, it is clear that normal lymphocytes are more stable than cancer cells, if they are subjected to the same PEMF application. This result is an obvious advantage for a selective cancer therapy producing only low side effects.

Combination of SEMF and Hyperthermia. Combined hyperthermia and SEMF treatment reduced the vitality of tumor cells much more than in the case of heated healthy lymphocytes as it is shown in Table 2 (Berg, 2005). Without the additional field effect the normal lymphocytes are very resistant to heat in contrast to cancer cells (compare the control values in Table 2), as was also known from former hyperthermia results in cancer research (Ardenne, 1997). After some hours of heating at 44°C, most cancer cells died (own data not shown).

Table 2
Comparison of the stability against the influence of hyperthermia at 41.5°C between K-562 cells and lymphocytes treated by 15 mT, 50 Hz, SEMF; experiment: E + field; control: C without field

	K- 562 cells			Lymphocytes		
Hours	Exp. + field % necrosis	Control % necrosis	E/C	Exp. + field % necrosis	Control % necrosis	E/C
24	9.4 ± 1.1	6.5 ± 1.5	1.4	5.2 ± 1.7	0.2	26 ($p < 0.01$)
48	40.9 ± 2	20.4 ± 6.7	2	22 ± 6.8	1.7 ± 0.9	13

Table 3

Synergism of SEMF and bleomycin on cancer cells. Results of treatments of K-562 cells (7.5×10^5 /ml) by combinations of field (SEMF, 15 mT, 50 Hz) with bleomycin (BLM, 450 μg/ml in the suspension) during 72 h. The difference in percentages of necrotic cells between field + BLM and control: yields $p < 0.01$

	Field	BLM	Field + BLM	Control
% Necrotic cells, dead	7.8 ± 0.6	29 ± 3.1	58 ± 5.8	3 ± 0.4
FACScan: % Apopt. + Necrotic. dead cells	16.7	79.3	87.8	9.5
FACScan: % Vital cells	83.3	20.7	12.2	90.6

Table 4

Synergistic SEMF treatment of tumor mice. SCID-tumor mice, produced by 1×10^{-6} MX-1 cells. Treatments for 3 h/day during 8 days by: (A) 20 mT, 50 Hz at 25°C; (B) + 0,1 mg Bleomycin in 10 μL; (C) 20 mT + 0,1 mg Bleomycin; (D) the control. Ve/Vb differences between the columns (C) and (D) yields for $p < 0.05$; Vb: mean tumor volume in the beginning; Ve: mean tumor volume at the end of treatment; TW: tumor weight; m: number of treated mice

Treatment	(A)	(B)	(C)	(D)
Mice	20 mT, 15 m	Bleomycin, 7 m	20 mT + Bleomycin, 8 m	Control 13 m
Vb/ cmm	124	75	96	65
Ve/ cmm	260	165	134	423
Ve/Vb	2.1 ± 0.4	2.2 ± 0.3	1.4 ± 0.2	6.5 ± 0.8
TW/ mg	–	78 ± 7	64 ± 5	149 ± 6

Combination of SEMF with Bleomycin. The percentage of necrosis of K-562 cells increases synergistically to 58% when the bleomycin was applied in combination with SEMF (Table 3). Whereas the control reaches $>90\%$ vital cells, the percentage of vital cells decreases to only 12% determined after the treatment. Similar synergistic effects have been measured with combinations of the both antibiotics daunorubicin (Table 1) and actinomycin-C, which result is further enhanced by the powerful photodynamic effect caused by visible light irradiation (Pang et al., 2001; Traitcheva et al., 2003).

Principal Results of the SEMF Treatment by B = 15 mT, 50 Hz. In contrast to the high lethality of cancer cells, normal lymphocytes were far more resistant against this field treatment. This result will be essential for an adjuvant cancer therapy in the future without too many deleterious side effects.

Hyperthermia up to 41.5°C or higher was suitable for the increase of the percentage of death cancer cells. Again, the responses of cancer cells differed from normal lymphocytes significantly because membranes of cancer cells become unstable at higher temperatures (Ardenne, 1997).

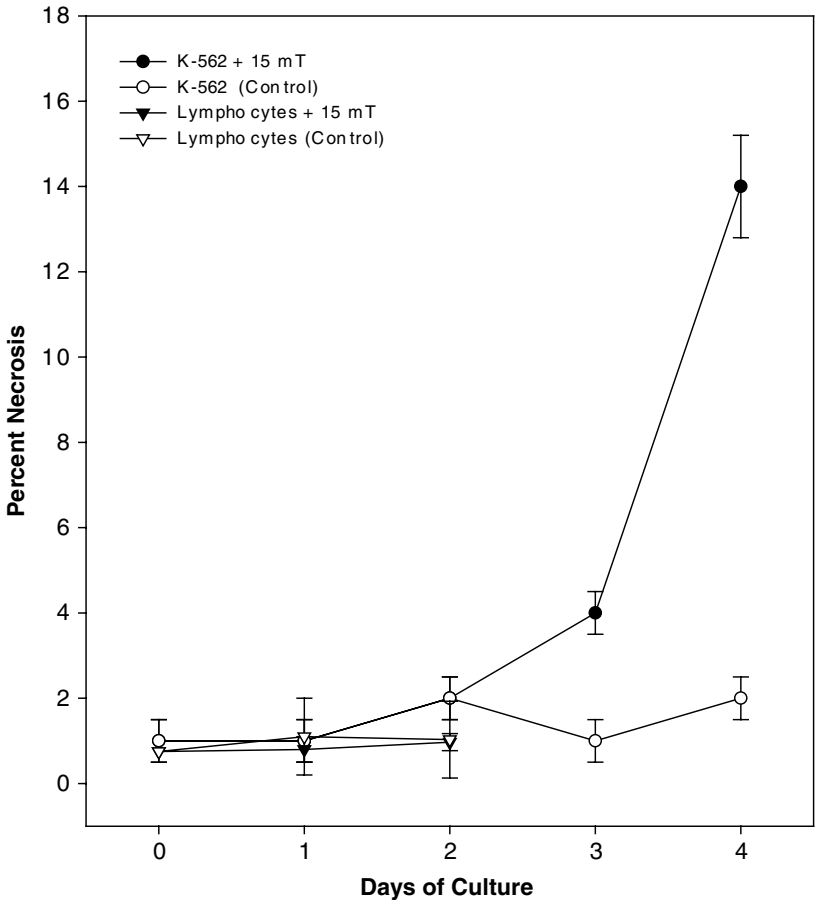

Figure 1. Long-term treatment of K-562 cells and lymphocytes by 15 mT, 50 Hz, and control without field.

In this respect, some promising improvements were described in the literature for cells and for animals (see Table 1). Nevertheless, negative results were also published (McLean et al., 2003) mostly because of too weak B-fields or too short application time of too low fields.

In Tables 3 and 4, our results with tumor mice confirm successfully the outcomes using the novel combination of SEMF with bleomycin. Moreover, they represent a further basic contribution to the establishment of a non invasive tumor therapy hopefully realized in the near future. In some respects, our results can be compared to bleomycin treatment supported by 70 V/cm, 500 impulses for 12 min/day using needle electrodes (electrochemotherapy) fixed into CT 16 colon carcinoma of BALB/c mice (Plotnikov et al., 2004). However, because of this rather short pulsation time per day complete cure of 51% of these animals required a rather long treatment time of 120 days. Depending on origin, age, size, and position of particular tumors, special modification of treatment devices will be necessary and should be developed as soon as possible. Much stronger PEMF-field densities were used on patients with brain diseases, e.g., by a "figure-eight" coil emitting 1.5 T (Ueno, 1994; Ueno and Shigemitsu, 2007). This procedure, by local application of such strong flat coils, will be effective for cancer patients as well.

In general, the efficacy of SEMF or PEMF treatment will be enhanced by combinations with:

(a) cytotoxic agents doxorubicin, daunorubicin, actinomycin-C, Imatinib (Liang et al., 1997; Miyagi et al., 2000; Traitcheva et al., 2003; Yamaguchi, 2007), and also with cisplatin, cyclophosphamide, bleomycin inhibitors, tyrosin-kinase antibodies, anti-sense oligonucleotides (Elez et al., 2002), etc.;
(b) hyperthermia (41.5–43°C; Ardenne, 1997);
(c) hyperacidity (pH < 6.5; Ardenne, 1997);
(d) photodynamic treatment (Pang et al., 2001; Traitcheva et al., 2003; Radewa et al., 2004; Radeva and Berg, 2004);
(e) static magnetism (Zhang et al., 1995; Pengfei et al., 2009).

The synergism of SEMF or PEMF with photodynamic therapy (compare d) has the advantage that the light (Laser at wavelengths > 600 nm) can be focused directly into the tumor by light conductors focused on porphyrin photosensitizers (e.g., the Protoporphyrin derivatives: Photophrin, Foscan, Foslip from Biolitec, Jena, Germany) accumulated in the tumor region.

The great advantage of SEMF or PEMF combinations with agents (a)–(e) is the prospective synergism for an advanced tumor therapy.

The Response of Tumor-Bearing Mice

SEMF Treatment by 20 mT, 50 Hz. These results are shown in Table 4 as differences in tumor volumes (V) and tumor weights (TW). The relative tumor volumes Ve/Vb (e: end, b: beginning) of the controls in column D were about three times higher than the relative volumes of tumors exposed by the field only (column A) or by the addition of bleomycin only (column B). The combination of 20 mT + bleomycin in column C yields a statistically significant, synergistic effect. Moreover, the density of blood vessels was lower in treated tumors as compared to control tumors, which diminishes the nutrition of the tumor by the inhibition of angiogenesis. Similar results were found by M. Markov (Williams et al., 2001). However, under his conditions the mice were exposed briefly for only 10 min/day by an half-wave- sinusoidal 120 Hz field (Table 1).

Further experiments will be necessary to ascertain the optimal combinations of magnetic flux density (B), frequency, pulse type, and exposure time.

PEMF Treatment by 200 mT for Comparison to 1. As was found recently by Yamaguchi et al., (2006), the increase of the magnetic flux density B shortens the required exposure time markedly to minutes. Using a commercial flat coil device producing about B $= 200$ mT in 1 cm distance from the tumor inside the mouse, the tumor weight was decreased to 50%. The survival of the 14 treated mice bearing melanoma B16-BL6 cells (treatment: 80 s/day for 17 days) increased in the experiments to 60% vs. control of 20% ($p < 0.05$) (Yamaguchi et al., 2006). Such high B-values generated by commercial flat coils may be promising for the inhibition of such localized tumors, which are grown about 6–10 cm deep inside the body of cancer patients.

Successful cancer therapy requires not only significant lethal and apoptotic SEMF or PEMF effects inside tumors, but at the same time also a minimal

influence on surrounding healthy cells. This is the case because, in comparison to healthy cells, the responses of tumor cells are more intensive according to their physiological conditions, their density, the nutritional situation, electromagnetic field characteristics, and the duration of exposure.

As to the molecular mechanisms acting simultaneously on different targets, there are several hypotheses (Astumian, 1991; Binhi, 2002; Kato, 2006; Pilla, 2007; Liboff, 2007) taking into account disturbance of ion distribution at surfaces of enzymes (Binhi, 2002), the equilibria of protein conformations of different magnetic moments (Astumian, 1991), ion resonance (Liboff, 2007), free-radical reactions (Simko, 2004), etc., which were all evaluated in the comprehensive monograph by Binhi (2002). However, a general theory for the mechanism for all targets is still not available.

Conclusions

Pulsating (PEMF) or sinusiodal (SEMF) electromagnetic field effects can be enhanced by the combination with hyperthermia, hyperacidification, specific cytostatic drugs, static magnetism, or/and photodynamic action. The simplest possibility for additional treatment will be the hyperthermia because the rise of temperature may occur either by heating from of the solenoid itself or by microwave or infrared irradiations.

For the treatment of patients there are very powerful commercially flat coils (Magstim Comp., Wales, England) available, generating up to 3.5 Tesla at their surface, which could generate > 20 mT locally at a distance of about 10–20 cm inside the human body. However, the energy required for their operation is rather high. Therefore, a solenoid of ca 30 cm for children and ca 45 cm in diameter for adults producing > 20 mT of a fairly uniform field distribution in the core for a systemic treatment of tumors and metastases inside of all body regions will be more suitable energetically (construction still in preparation) than by a flat coil only locally applicable. From our experiences with tumor mice and the results of Yamaguchi et al. (2006), one can draw the conclusion that the higher the magnetic amplitude of magnetic flux density in the tumor region, the shorter the necessary treatment time will be. In any case, the possibilities of using electromagnetic field applications in modern oncology show a great promise for a successful, non invasive, adjuvant therapy of cancer patients.

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