Anticancer effects on leiomyosarcoma–bearing Wistar rats after electromagnetic radiation of resonant radiofrequencies

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Abstract

In the present study, the effects of a resonant low intensity static electromagnetic field (EMF), causing no thermal effects, on Wistar rats have been investigated. Sarcoma cell lines were isolated from leiomyosarcoma tumors induced in Wistar rats by the subcutaneous (s.c) injection of 3,4-benzopyrene. Furthermore, smooth muscle cells (SMC) were isolated from the aorta of Wistar rats and cultivated. Either leiomyosarcoma cells (LSC) or SMC were used to record a number of characteristic resonant radiofrequencies, in order to determine the specific electromagnetic fingerprint spectrum for each cell line. These spectra were used to compose an appropriate algorithm, which transforms the recorded radiofrequencies to emitted ones. The isolated LSC were cultured and then exposed to a resonant low intensity radiofrequency EMF (RF-EMF), at frequencies between 10 kHz to 120 kHz of the radiowave spectrum. The exposure lasted 45 consecutive minutes daily, for two consecutive days. Three months old female Wistar rats were inoculated with exposed and non-exposed to EMF LSC (4 x 10^6 LCS for animal). Inoculated with non-exposed to EMF cells animals were then randomly separated into three Groups. The first Group was sham exposed to the resonant EMF (control Group-CG), the second Group after the inoculation of LSC and appearance of a palpable tumor mass, was exposed to a non-resonant EMF radiation pattern, for 5 h per day till death of all animals (experimental control Group-ECG). The third Group of animals after inoculation of LSC and the appearance of a palpable tumor mass, was exposed to the resonant EMF radiation for 5 h per day, for a maximum of 60 days (experimental Group-I, EG-I). A fourth Group of animals was inoculated with LSC exposed to EMF irradiation and were not further exposed to irradiation (experimental Group-II, EG-II). Tumor induction was 100% in all Groups studied and all tumors were histologically identified as leiomyosarcomas. In the case of the EG-I, a number of tumors were completely regretted (final tumor induction: 66%). Both Groups of animals inoculated with exposed or non-exposed to the EMF LSC, (EG-I and EG-II, respectively) demonstrated a significant prolongation of the survival time and a lower tumor growth rate, in comparison to the control Group (CG) and the experimental control Group (ECG). However, the survival time of EG-I animals was found to be significantly longer and tumor growth rate significantly lower compared to EG-II animals. *In conclusion,* our results indicate a specific anticancer effect of resonant EMF irradiation. These results may possibly be attributed to (a) the duration of exposure of LSC and (b) the exposure of the entire animal to this irradiation.

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Introduction

Radiofrequency electromagnetic fields (REMF) exert a variety of effects on cells, experimental animals and humans, some of these effects referring to applying electromagnetic resonance principles [1-3].

Linked to malignancy and depending on the intensity, frequency and duration of application of the electromagnetic waves (EMW), the following main concepts have been expressed, so far: i) The EMF may act as co-carcinogens in combination with the initiating carcinogen, especially in experimental animals and ii) the EMF can exert anticarcinogenic effects, inhibiting the proliferation of malignant cells *in vitro* as well as decreasing the size of the experimental tumors *in vivo* [4-6]. The studies on EMF pro-carcinogenic or carcinogenic effects in experimental animals are however, not numerous and it seems that the described methods have a lot of uncertainty [4]. In comparison, the studies on high frequency, radiofrequency included, EMF anticancer effects are abundant and their methodology is well documented [7]. It has also been shown, that the cytostatic effects of the EMF on cancer cells are not related to their thermal effects but are exerted via temperature-independent actions [8-10]. There are also reports that EMF induces apoptosis in human leukemia cells [11], as well as on human squamous carcinoma cell lines [12].

We have recently shown that the exposure of sarcoma cell lines (SCL) to resonant static

electromagnetic radiation (RSEMR) exerts significant antiproliferating effects, arresting cell cycle and causing apoptosis [13].

In the present study the anticancer effects on SCL exposed to low intensity RSEMR in Wistar rats has been investigated.

Materials and Methods

Leiomyosarcoma and smooth muscle cell lines

Cells were isolated from tumors histologically identified as leiomyosarcoma (LMS), induced by the subcutaneous injection of 1 ml of 3,4-benzopyrene solution (B[a]P) in tricapryline at a final dose of 10.08 mg B[a]P/ml in each Wistar rat, as previously described [13, 14]. The developed tumors were surgically removed and sliced under aseptic conditions into pieces of 0.5 cm^3 each. Each piece was placed immediately in cold Ringer's solution, then sliced down again to smaller, 1 mm^3 pieces and placed into 5 ml Dulbecco's Modified Eagle's Medium (DMEM) solution which contained small quantities of trypsin and incubated at 37 °C for 4 hours, with gentle constant mixing every 15 min. The cell's suspension derived from the previous process, were centrifuged at 900 rpm for 10 min and the supernatant was rejected. The pellet of cells was resuspended in 90% DMEM+10% foetal bovine serum (FBS) solution and seeded in plastic coated dishes of 52 mm size. Subcultures of these cells were prepared. Tumor cells after had been subcultured, were histologically examined and diagnosed as LSC.

SMC were also isolated from the aorta of Wistar rats and subcultured by the methods described above.

Electromagnetic field equipment

The multi channel dynamic Exciter 100 V1 device (MCDE)® used for the RF measurements and the EMF exposure of cells [15], was designed and manufactured by K. Havelas and collaborators [15]. The MCDE has been certified by the National Center of Investigation in Physics, Demokritos, Athens, Greece for its safe use in humans and animals [15]. This device consists of two basic parts: a) a diagnostic part with an electron paramagnetic resonance spectrometer's characteristics, including an electrode which connects with the restoring frequency system and b) an EMF generator of various intensities from 1.1 to 1.11 ± 0.01 V/m for the electric field and 0.0027 to 0.0029 ± 0.00005 A/m for the magnetic field and RF from 1 kHz to 1 MHz conducted by a sophisticated software. The software's application consists of two fundamental steps: a) to record the biological target system's resonant frequencies and b) use a specific algorithm, in order to calculate the appropriate EMF needed for the exposure of living target systems or cells. In another paper accepted for publication, we shall explain briefly the technical parts of this system and its function.

Estimation of LSC and SMC electromagnetic RF

A measurement of the LSC and SMC resonant RF was taken by the MCDE system described above, before their exposure to EMF. Electromagnetic resonant RF measurements were also taken from the survived LSC after their exposure to EMF for two consecutive days. Both resonant frequencies of LSC and SMC were used in order to form a specific algorithm. By using this algorithm, the electromagnetic fingerprint of LSC was transformed into a decreasing frequency succession, in order to adapt gradually the electromagnetic resonant pattern of LSC *in vitro* (or entire leiomyosarcomas *in vivo*), to the resonant pattern of Wistar rats' SMC. SMC were selected for this process because LSC have been originated, by B[a]B induced malignant transformation of these cells.

LSC exposure to EMF

LSC in a concentration of $1x10^5$ were seeded in twelve Petri dishes, which contained 10 ml of growth medium (zero time). The cell cultures were incubated in 37 °C at 95% O_2 + 5% CO_2 for 48 h and then the medium was changed. After 72 h from zero time, six cell cultures (EMF cells) were placed into a Faraday apparatus at room temperature (RT) and exposed for 45 min to EMRF, with the following characteristics: a) the EMRF ranged from 10 kHz to 120 kHz (Fig. 1, diagram A) and b) the intensity of the electric field ranged from 1.1 to 1.11 ± 0.01 V/m, while the intensity of the magnetic field ranged from 0.0027 to 0.0029 ± 0.00005 A/m. The other six cell cultures (control cells) remained at RT for the same time as EMF cells without being exposed to EMF. The control and EMF cells were incubated once again at the same conditions as before for about 7 h. After 79 h from zero time, the cells of each culture were counted, subcultured at about $1x10^5$ cells per plate and incubated at the same conditions as above. The same procedure was repeated again as described above, after 96 h from zero time. At 120 hours the EMF cells were re-exposed to the EMF as before and after 24 h of exposure, both EMF and control cells of each plate were counted and examined microscopically. The cells were then preserved in liquid nitrogen. The preserved in liquid nitrogen EMF and control cells were defrost and subcultured until confluence. Twelve Petri dishes were then seeded with the same number of

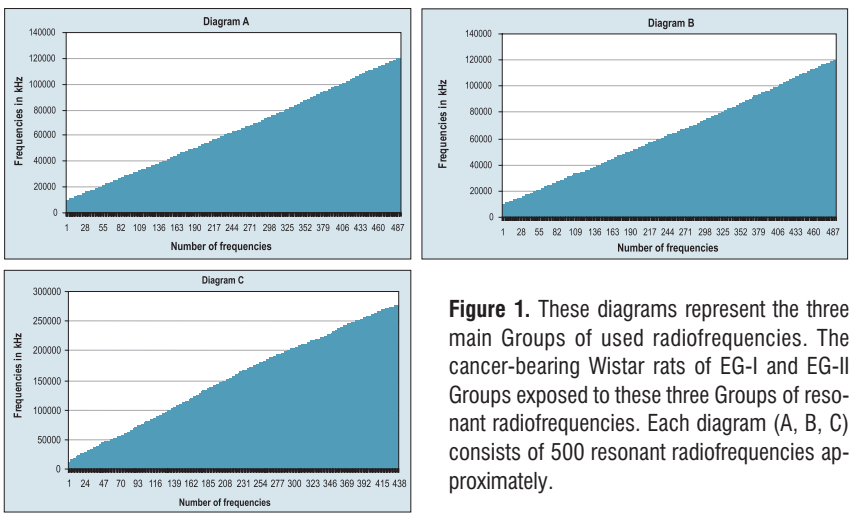

Figure 1. These diagrams represent the three main Groups of used radiofrequencies. The cancer-bearing Wistar rats of EG-I and EG-II Groups exposed to these three Groups of resonant radiofrequencies. Each diagram (A, B, C) consists of 500 resonant radiofrequencies approximately.

LSC and incubated for 24 h. After this procedure, exposed and not exposed to EMF cells were inoculated to Wistar rats.

Animal exposure to the resonant EMF

Animals inoculated with LSC not exposed to EMF. After the appearance of a palpable tumor mass, the inoculated animals were exposed to the same resonant pattern of EMF radiation for 5 h per day in a Faraday cage, till the death of these animals. The distance between the target (animals) and the emitting antenna of the MCDE device was 50 cm. During therapeutic exposure to the resonant EMF frequencies, each cage contained 3 rats and its position was stable. The same procedure was repeated for the experimental control Group of animals to non resonant EMF frequencies.

Further animal studies

A total of 39 female Wistar rats, 3 months old, 250 ± 15 g, b.w, were divided into four Groups. Twenty nine Wistar rats were randomly divided into three Groups, after their inoculation with non-exposed to the resonant EMF LSC: a) a control Group (CG), b) an experimental control Group (ECG) and c) an experimental Group-I (EG-I). The remaining 10 animals belonged to the fourth Group and were inoculated with exposed to the resonant EMF, LSC as follows: a) *Control Group (CG):* This Group consisted of 11 Wistar rats. Each rat was inoculated by $4x10^6$ non-exposed to EMF, LSC. All the animals of the control Group were kept for 5 h per day in the Faraday cage, till the end of the experiment (death of the last animal), while the MCDE system had been turned off (sham exposure). b) *Experimental control Group (ECG):* Consisted of 9 Wistar rats. All animals were inoculated with $4x10^6$ non-exposed LSC, till the appearance of a palpable tumor mass. Afterwards, the animals were exposed to the non-resonant radiofrequency (NRRF) low intensity EMF, for 5 h per day, till the end of the experiment death of the last animal. The frequencies ranged from 10 kHz to 120 kHz, while the range intensities for the electrical field were 1.1 to 1.11 ± 0.01 V/m and for the magnetic field were 0.0027 to 0.0029 ± 0.00005 A/m. c) *Experimental Group I (EG-I):* Leiomyosarcoma-bearing Wistar rats Group: Consisted of 9 Wistar rats. Animals of this Group were exposed to the resonant EMF (10 kHz-120 kHz, electric field: 1,1-1,11 ±0.01 V/m, magnetic field: 0.0027-0.0029 ±0.00005 A/m) after their inoculation by $4x10^6$ non-exposed to EMF, LSC for 5 h daily, for at maximum 60 days [Table 1]. The used resonant radiofrequencies are demonstrated in Figure 1. d) *Experimental Group II (EG-II):* Consisted of 10 Wistar rats. Each rat was inoculated by 4×10^6 LSC exposed to the previously described resonant EMF.

Inoculation of LSC cells in Wistar rats

LSC cells in a concentration of $4x10^6$ exposed or not exposed to EMF, were suspended in 1 ml of Hank's solution. The animals were anesthetized with 3 mg/bw ketamine and 3.5 mg/bw midazolame and a surgical opening was made to their outer skin layer of their dorsal area (by the right scapula) deep up to the muscle layer. The tissue underneath was then traumatized by lancing with a sharp blade, five parallel cuts of 5mm length, till the production of the slight hemorrhage. LSC exposed or not exposed to EMF, at a number of $4x10^6$ cells, were then aseptically infused into the operated area and the operated sites was immediately closed.

Table 1. *Time of exposure (hours) to the resonant EMF, for each animal of the EG-I Group*.*

Number of animals	1	2	3	4	5	6	7	8	9	Mean
Time of exposure	300	300	215	250	295	300	90	100	300	238.9

* time of exposure less than 300 h (<60 days) is due to the death of the animals

Animals of all Groups were then placed into cages of Plexiglas (2 animals in each cage) and kept at temperature of 19°C ± 1.2°C, for 12 h at light and 12 h at dark environment. Animals feeding and water drinking was *ad libidum*. Palpable tumors dimensions were grossly calculated by the use of caliper bow compass. Dead animals of all Groups were autopsied, tumors were carefully excised, weighed and tumor or muscles at the site of cell inoculation removed for histological examination. Histological study for possible metastases was also performed in the lungs, the stomach, the intestine and the kidneys in all animals studied.

For all Groups, the mean survival time of the animals (MST), the mean tumor weight (MTW) and mean tumor growth rate (MTGR) were calculated. The tumor growth rate (TGR) for each animal was calculated by the following equation:

TGR (g/d) = tumor weight (grams)/survival time (days)

Student's t-test (unpaired) was used for statistical evaluation of the results and $P<0.05$ was considered statistically significant.

Results

Estimation of cell EM-RF

RF for not exposed to EMF, LSC were ranging from 10.5 to 120.5 kHz, for the EMF-exposed LSC, from 10 to 120 kHz and for the SMC, between 10 to 120 kHz. Spectrum analysis of the above estimations revealed that the RF of LSC, exposed to EMF showed significant differences as compared to those of the non-exposed LSC (control cells), reaching almost 70% similarity to the RF recorded from SMC. Similar results have also been recorded in our previous study [13].

Tumor induction in Wistar rats

All animals (100%) of the CG, inoculated with non–exposed to EMF LSC, developed at the site of the inoculation by the 14th day, palpable tumors with an average dimension of 22.8×24.3 mm. These tumors were histologically identified as leiomyosarcomas (Fig. 2). MST of the tumor bearing animals of this Group was 20.36 ± 3.23 days after inoculation, while MTW was 86.7 ± 27.6 g and MTGR was 4.22 ± 0.9 g/day.

In animals of the EG-II inoculated with exposed to EMF LSC, tumor induction was also 100%. Tumors were also palpable the

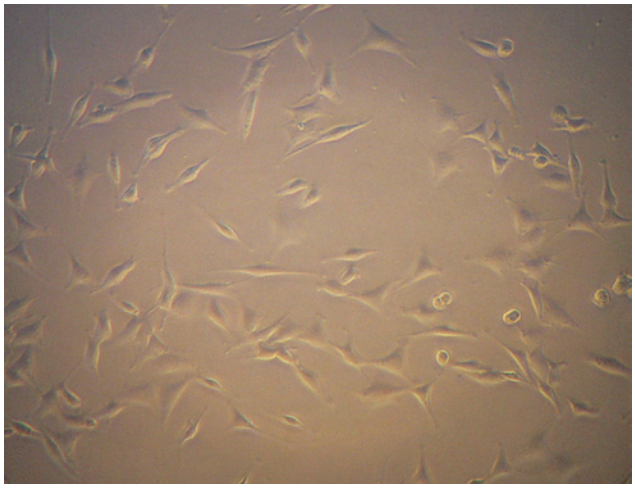

Figure 2. Wistar rat LMC isolated from tumors developed after carcinogenesis with 3, 4-benzopyrene. A certain number (4×10^{-6}) of LSC were inoculated into syngenic Wistar rats for cancer development. In this figure the LSC are of wild type without any exposure to EMF.

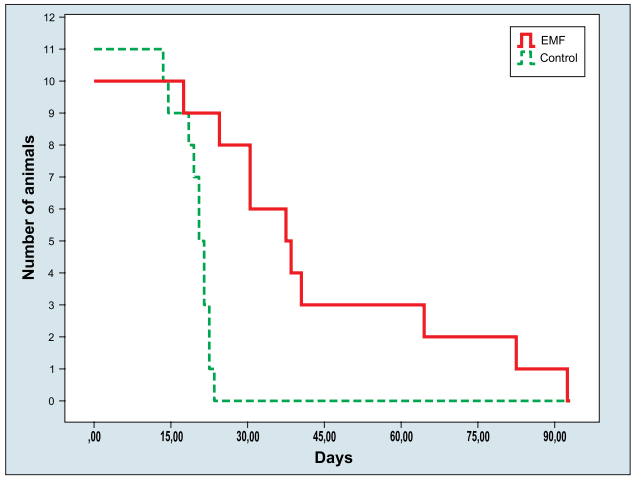

Figure 3. Death rate curves of tumor-bearing animals of CG and EG-II Groups. There is a significant difference between the two Groups of animals in life prolongation reflecting the different rates of LMC cells proliferation. Animals of CG-Group (dotted line), showed a double survival time in comparison to EG-II Group (continuous bold line). This *in vivo* experiment reflects the *in vitro* observed phenomenon of different cell proliferation rate of exposed and non-exposed LSC to EMF under culture conditions.

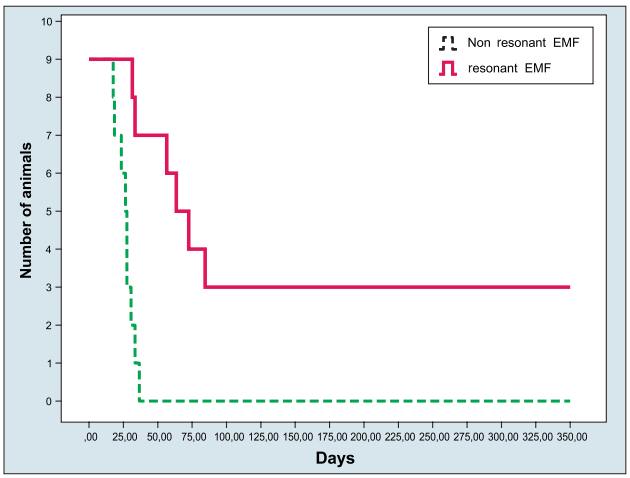

Figure 4. Death rate curves of: a) tumor-bearing animals in EG-I Group (continuous bold line) and b) tumor-bearing animals in ECG Group (dotted line). The difference in the survival time and death rate between the two Groups is very obvious. 33% of the animals of the EG-I Group survived without sings of tumor, compared to the ECG animals.

14^{th} day after inoculation and their average dimensions were calculated at 12.5×13.7 mm. MST was 46.4±25.27 days, MTW was 89.8±36.8 g and MTGR 2.17±0.73 g/day. Seven of 10 animals (70%) of the CG manifested lung metastases in contrast to 4 out of 10 animals (40%) of the EG-II, which possessed lung metastases ($P< 0.03$) (Table 2). The histological type of all tumors developed in both Groups was leiomyosarcoma (Fig. 2).

Moreover at the 30^{th} day after cell inoculation none of the animals of the CG was surviving (the first animal died at the 15^{th} day and the last in the 24^{th} day), while 8 animals out of 10 of EG-II were still alive and in good condition in the 30^{th} day, while the last one died in the 93^{rd} day after cell inoculation (Fig. 3).

Statistical evaluation between CG and EG-II revealed a statistically significant difference in MST and MTGR and the number of animals possessing lung metastases between the two Groups and a not significant difference in MTW (Table 2). Animals of EG-II inoculated with EMF-exposed LSC lived remarkably longer (approximately twice compared to CG), developed tumors with slower TGR and had lung metastases at a lower number, than the animals inoculated with non-exposed LSC (CG).

All animals (100%) of the ECG, which were inoculated with non-exposed to EMF LSC and exposed for 5 h/day to the non-resonant randomly selected low intensity EMF, immediately after their inoculation, developed tumors (leiomyosarcomas) at the site of the inoculation at the 14^{th} day with an average dimension of 21.9±23.2 mm. MST of the tumor bearing animals of this Group was 27.28±7.29 days after inoculation, while MTG was 90.3±11.24 g and the MTGR was 3.42±0.68 g/day. Six out of 9 (66.6%) of these animals had lung metastases. Our data revealed that there was not any significant statistical difference of the measured values of this Group compared to the CG Group. This indicates that the exposure of rats inoculated with non-exposed LSC, to a randomly selected non resonant RF low intensity EMF, did not affect the oncogenicity and the carcinogenic potency of the in-

Table 2. *Comparison of mean survival time (MST), mean tumor weight (MTW), mean tumor growth rate (MTGR) and the percentage of animal with lung metastases (LM) between the control Group (CG) and the experimental Group II (EG-II).*

Parameters	CG	EG II
MST (days)	20.36 ± 3.23*	46.4 ± 25.27*
MTW (g)	86.7 ± 27.6	89.8 ± 36.8
MTGR (g /day)	4.22 ± 0.9**	2.17 ± 0.73**
LM (number of animals)	7/11 (72.3 %)	4/10 (40%)***

* Results statistically significant (* $P<0.005$, ** $P<0.001$ and *** $P<0.09$)

oculated LSC and the course of the malignant disease.

All animals of the EG-I Group, manifested tumors which were palpable at the 14th day of inoculation, with an average dimension of 26.6 x 24.4 mm. In this Group, the exposure to the resonant EMF resulted in complete regression of tumors for the 3 out of 9 rats (33.3 %) after 40 days and after 200 h of total exposure. Then, these three animals were exposed for 20 more days to the resonant EMF for a total sum of 60 days -300 hours of exposure- and are still alive. These rats have survived till now, for more than 365 days, free of tumor and symptoms and their survival time is expected to reach that of healthy Wistar rats (850±50 days). If the days of survival of the three free tumor animals, still alive, are calculated in total survival of this Group then the MST of the EG-I Group animals exceeded the 160 days. From the rest 6 animals of this Group, EG-I, the first died on the 32nd day and the last one on the 85th day after inoculation (Fig. 4). MST of these six tumor-bearing animals was: 57.5 ± 21.17 days, MTW: 85.5 ± 23.9 g and MTGR: 1.74 ± 1.12 g/d. Autopsy of these 6 tumor bearing animals revealed no lung metastases. Tumors were histologically identified as LMS. The main interior parts of the tumors were then liquefied and their necrotic material was drained by means of a syringe. The injection of more than 3 ml of the liquefied necrotic material caused the animals' death, due to disseminated intravascular coagulation, as shown by necropsy and histology (Fig. 5). On the other hand, after visual estimation, the necrotic areas of the CG tumor were significantly restricted (Fig. 6) in comparison to the EG-I tumors, which were exposed to the EMF therapeutic resonant radiation (Fig. 5). Statistical evaluation between the CG and EG-I Groups showed: $P<0.01$ for MST, $P< 0.0007$ for MTGR and $P<0.001$ for lung metastases (Table 3). Statistical evaluation of the above parameters between the CG and EG-II Groups showed: $P<0.001$ for MST, $P<0.005$ for MTGR and $P<0.09$ for lung metastasis (Table 2). Between the EG-I and EG-II Groups there was no statistical significant for MTGR ($P<0.41$) a marginal difference for MST ($P<0.05$) and for lung metastases ($P<0.05$).

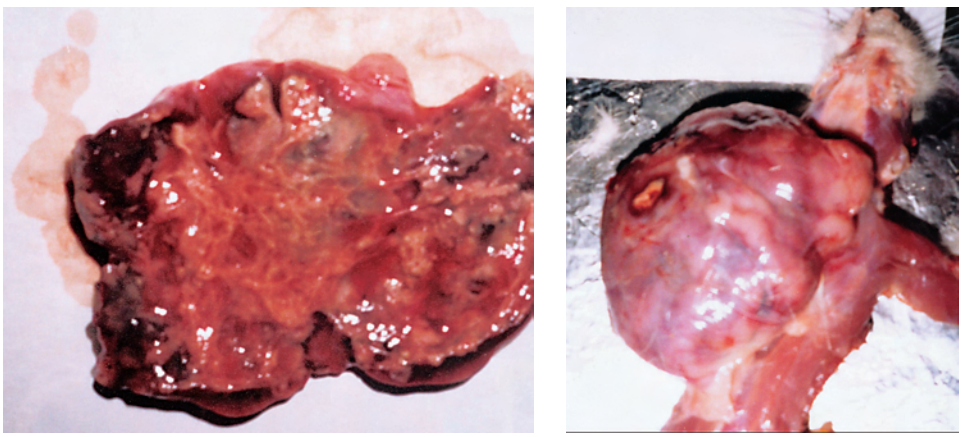

Figure 5. Therapy Group. Characteristic aspect of extensive necrosis of a malignant tumor after a long lasting exposure of a cancer-bearing rat (Group EG-I) to resonant EMF. The tumor was liquefied in its internal part and the release of this necrotic material in blood circulation, caused animal's death, because of disseminated intravascular coagulation.

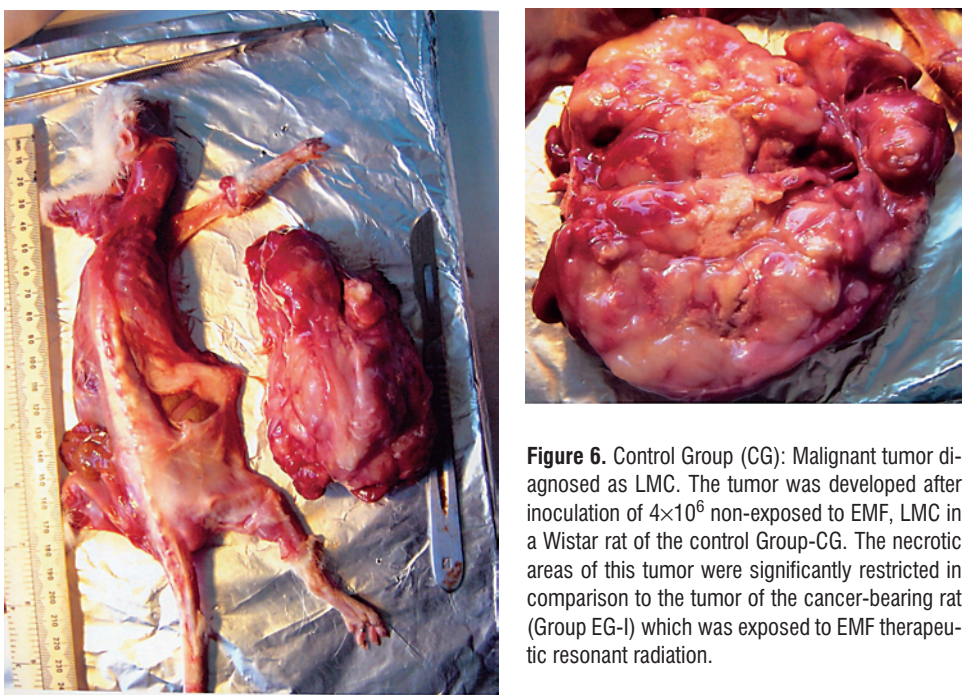

Figure 6. Control Group (CG): Malignant tumor diagnosed as LMC. The tumor was developed after inoculation of 4×10^6 non-exposed to EMF, LMC in a Wistar rat of the control Group-CG. The necrotic areas of this tumor were significantly restricted in comparison to the tumor of the cancer-bearing rat (Group EG-I) which was exposed to EMF therapeutic resonant radiation.

Discussion

Our results indicate that there is a significant prolongation of survival and also metastases diminution but not a remarkably lower TGR for tumor-bearing animals in EG-I Groups as compared to the EG-II Group. This can probably be attributed to the longer duration of exposure to resonant EMF (Table 1) and to the radiation administrated to total rat body, in EG-I Group rather than to the exposed to EMF inoculated LSC, in the animals of the EG-II Group. Our results also indicate that the used EMF, exert a significant anticancer effect. Wistar rats developing LMS tumors, induced by the inoculation of LSC previously exposed to the EMF, manifest statistically significant prolongation of their survival time, significant lower tumor growth rate and significant lower number of

Table 3. *Comparison of mean survival time (MST), mean tumor weight (MTW), mean tumor growth rate (MTGR) and the percentage of animal with lung metastases (LM) between the control Group (CG) and the experimental Group I (EG-I)*

Parameters	CG	EG I
MST (days)	20.36 ± 3.23*	160*
MTW(g)	86.7 ± 27.6	85.5 ± 23.98
MTGR (g/day)	4.2 ± 0.99**	1.22 ± 1.07**
LM (number of animals)	8/11 (72.3%)	0/6 (0%) ***

* Results statistically significant (* P<0.01, **P<0.0007 and ***P<0.003) [Note that there was no statistically significant difference between the parameters of non-exposed Group CG and exposed to a not-resonant EMF rats Group EGI. Note also that there was a 33% complete regression of tumors in Group–I].

lung metastases compared to rats of the CG Group.

This effect is in agreement to the fact that in 3 out of 9 (33%) tumor-bearing animals of EG-I Group, the tumors completely disappeared. These animals are still alive and healthy. The rest 6 tumor-bearing animals of the EG-I Group manifested a lower extend of life prolongation compared to the above 3 animals. In comparison to CG and ECG Groups, these 6 animals demonstrated statistically significant prolongation of their survival time, significant lower tumor growth rate and no lung metastases.

Our data indicate that LSC exposed to EMF may manifest a 100% induction of LMS tumors when inoculated to Wistar rats. However, these animals bare tumors with lower malignancy, and diminished metastatic potential in comparison to animals inoculated with non-exposed LSC. Results also indicate that a randomly selected non-resonant EMF pattern, does not exert any effect on the LMS inoculated rats. The latter reveals a specific effect of EM resonance principles in biological processes [2, 3]. There is also some evidence that resonant RF of a biological active substance may induce similar to the substance effects when electronically transmitted to target cell lines [16-18]. Thomas et al (2000) have suggested that signals derived from phorbol-myristate acetate and transmitted electronically induce a similar activation of human neutrophils to that of the biological active substance [18].

The potency of the anticarcinogenic effects of this resonant radiofrequency EMF seems to depend on the duration of exposure. EG-I Group animals (Table 1), showed a 33% total tumor reduction, normal survival and a significantly longer survival time than EG-II ones. These potent beneficial effects, resulting in complete tumor remission in 33% of tumor bearing animals, may be also attributed to the systemic actions of RF-EMF and mainly to an immunologic modification induced by resonant RF of low intensity EMF on living organisms [19]. It has also been shown that cancer cells have significantly lower transmembrane potentials than normal cells [20, 21]. Low transmembrane potential is related to rapid cell proliferation rate, changes in membrane structure and malignancy [21, 22]. These neoplastic cells potentials in intact animals may also alter normal physiological function of macrophages, causing an immunological collapse at a distance of the tumor [21]. Static as well as pulsing EMF may restore transmembrane potentials, by regulating the opening of voltage gated channels and influencing the ions influx and efflux in cancer cells [23]. Karkabounas et al (2006) found that resonance RF pattern of LSC after exposure to characteristic EMF, manifests similarities to SMC, RF pattern [13]. The above mentioned phenomenon is possibly derived from a normalization of the LSC transmembrane potential. Furthermore, it has been recently shown that the increase of malignant hepatocytes transmembrane potential results into inhibition of cell growth rate [24]. Preliminary data derived from our running studies indicate that EMF normalize the transmembrane potential of cancer cells (data not shown).

We have previously shown that the exposure of LSC lines to the same resonant EMF of low energy waves and frequencies ranging from 10 kHz to 120 kHz, as those used in the present study, can cause potent growth inhibition of more than 95% of LSC, causing cycle arrest and cell apoptosis in 45 % [13]. Apoptotic effects of EMF have also been reported [7, 11, 12]. Significant lower TGRs as recorded in the Groups of our study exposed to these resonant RF-EMF, could be attributed to similar effects like the above. We have also demonstrated that the EMF of resonant frequencies strongly decreased DNA synthesis, and inhibited mitosis [13]. In the same study, the low percentage of cells found in synthesis (face S) and in mitosis (face M) (9% and 2% respectively), as compared to the controls (38% and 19% respectively), indicated that RF of the EMF can act as an inhibitor to cell's cycle, with effects similar to those of magnetic and EMF on DNA synthesis [25, 26]. The inhibition of the growth rate of Jurkat cells, due to decreased DNA synthesis, was also induced by EMF [27]. Although, immunoreactive p53 expression in BaP-induced LMS in Wistar rats was found to be increased [28], there is evidence that the exposure to EMF inhibits tumor growth and reduces immunoreactive p53 expression in tumor bearing mice [7, 29]. Effects, as above, of RF on LSC, may be attributed to energy transfer. It has been shown that the average specific absorption rate of RF in living organisms can be substantially higher at resonant frequencies [30]. EMF may also enhance electron spin of free radicals, leading possibly to their neutralization, especially to those produced by the activation of arachidonic cascades [10, 31, 32]. It is also known that the EMF induces free radicals production that may act as activators of signal transduction pathways [33, 34]. Frequencies of time selection and exposure seem to be important factors in order to obtain an optimum biological effect either on *in vitro* or on in *vivo* models [13, 25, 35].

The fact that the EMF pattern of the LSC changed after their exposure to EMF and resembled to that of SMC [13] may possibly indicate that some type of LSC differentiation could have taken place. There is also evidence that the RF-EMF, as well as the generalized ERF, is able to induce differentiation in cancer cells and other types of undifferentiated cells [16, 36, 37].

Anticarcinogenic effects of EMF on animal models have also been investigated [7], but according to the relevant literature, this is the first experiment investigating the *in vivo* malignant properties, after resonant EMF-treated malignant LSC, and in LMS-bearing animals.

It has to be pointed out, that the intensity of the electric and magnetic field, used in the present study was 75 times and more than 1800 times lower respectively, than the average of the international safety standards according to the Greek Atomic Energy Agency (Demokritos, Athens, Greece, Agia Paraskevi, 15310) [15]. Because of that, the use of the MCDE device as an electronic apparatus for the cancer treatment is considered safe. This present work continues and results are to be soon published.

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