Cell Biology International 2002, Vol. 26, No. 7, 599–603
doi:10.1006/cbir.2002.0883, available online at http://www.idealibrary.com on IDEAL®

EXTREMELY LOW FREQUENCY (ELF) PULSED-GRADIENT MAGNETIC FIELDS INHIBIT MALIGNANT TUMOUR GROWTH AT DIFFERENT BIOLOGICAL LEVELS

XINCHEN ZHANG[1], HUSHENG ZHANG[1]*, CONGYI ZHENG[2], CHAOYANG LI[2], XINSONG ZHANG[3] and WEI XIONG[4]

[1]*Biomedical Physics Unit, Department of Physics, Wuhan University, Wuhan 430072, China;* [2]*College of Life Science, Wuhan University, Wuhan 430072, China;* [3]*Business School, Wuhan University, Wuhan 430072 China;* [4]*Institute of System Engineering, Huazhong University of Science & Technology, Wuhan 430074, China*

Received 19 April 2001; accepted 17 January 2002

Extremely low frequency (ELF) pulsed-gradient magnetic field (with the maximum intensity of 0.6–2.0 T, gradient of 10–100 $T \cdot M^{-1}$, pulse width of 20–200 ms and frequency of 0.16–1.34 Hz treatment of mice can inhibit murine malignant tumour growth, as seen from analyses at different hierarchical levels, from organism, organ, to tissue, and down to cell and macromolecules. Such magnetic fields induce apoptosis of cancer cells, and arrest neoangiogenesis, preventing a supply developing to the tumour. The growth of sarcomas might be amenable to such new method of treatment.

KEYWORDS: extremely low frequency (ELF) pulsed-gradient magnetic field; malignant tumour; inhibitory; biological levels.

INTRODUCTION

Interactions between a malignant tumour and the host can be affected by bioelectromangetic phenomena. Many experiments have been carried out to investigate the effect of magnetic fields on tumour cell growth. It is reported that the incidence of leukaemia in people residing near to power transmission lines is higher (Poole, 1991; Chen, 1997). The frequency of fluctuating electromagnetic fields around power transmission cables is ~50 Hz, whereas the varying frequency of pulse magnetic fields approaches human physiology frequency of ~1 Hz, or is a static magnetic field.

Two kinds of frequency range give completely different biological effects. For example, Chang *et al*. (1985) reported that pulsed magnetic field (0.8 T, 22 ms, 1 Hz) inhibited the growth of S-180 sarcoma in mice, and have since used the same magnetic field strength to treat patients with middle and late-stage disease. Nine of 18 cases showed good improvement, and nine were less well inhibited (Chang *et al*., 1990). Since 1987, Wollin (see Zhou, 1994) has treated effectively 50 cancer cases with a Nd-Fe-B permanent magnet (0.4 T). And we have then reported from electron microscopic evidence the effects of ELF pulsed-gradient magnetic field can not only inhibit the growth of S-180 sarcoma in mice, but promote their oncolytic ability of host immune cells (Zhang *et al*., 1995, 1997). We used the same magnetic field to treat six patients with middle and late stage cancer in the Tumour Department, Wuhan Center Hospital of Guang-Zhou Military Region. Four cases were highly effective, and two were moderately effective (Zhang *et al*., 1994).

Apoptosis is a genetically regulated process. Since the loss of balance between cell death and division can be one of the factors leading to carcinogenesis, much interest is shown in the regulation of apoptosis (Fang *et al*., 1998; Silva *et al*., 1996). We show here that an ELF Pulsed-gradient magnetic field can induce apoptosis in cancer cells, and that it may also block the development of neovascularization required for tumour supply.

*To whom correspondence should be addressed: E-mail: hushengzhang@eLong.com

1065–6995/02/$-see front matter

Table 1.
Comparison of weights of mice and sarcomas of treated and control groups

Groups	*Weight of the mice (X ± SD)/g*					*Weight sarcomas (± SD)/g*
	0 day	*5 day*	*10 day*	*15 day*	*20 day*	
Treated	38.10 ± 3.50	42.09 ± 3.24	43.70 ± 3.57	46.11 ± 5.66	50.38 ± 8.59[a]	1.40 ± 0.81[b]
Control	37.53 ± 2.69	41.04 ± 2.62	38.68 ± 3.37	38.04 ± 4.67	36.79 ± 5.18	2.45 ± 0.95

[a]$P<0.05$; [b]$P<0.01$. X, Mean value; SD, standard difference.

EXPERIMENTAL PROCEDURES

Treatment of mice in magnetic fields

Twenty Kunming mice , ~2 months of age and weighing (36–40 g), were kept in a single cage, thus living conditions (food, water, temperature, light, air, etc) were the same for all. They were randomly divided into two groups, one being treated and the other acting as its control. The mice were inoculated in their right front legs with 1×10^6 S-180 sarcoma ascites cells. Sarcomas formed 4–5 days after inoculation.

The treated mice were put in plexiglass boxes with air holes, and located on the magnetic coil (10 cm in diameter) of a customized (ELF) pulsed-gradient magnetic field generator. When pulsed electric current passed through the coil, the magnetic field had a maximum intensity of 0.6–2.0 T, with a gradient of 10–100 T per metre, and the pulse width was 20–200 ms with a frequency of 0.16–1.34 Hz being generated. The treated animals were exposed to this magnetic field for 15 min per day at room temperature 18 ~ 26°C, while no treatment was applied to the control samples. Twenty-eight days later, the mice were killed by cervical dislocation, and the sarcomas cut out and weighed. Sarcoma specimens were prepared for the following examinations.

Optical sections and observation

Sarcomas with the volume of 1 cm^3 were fixed in 10% formalin, dehydrated through graded alcohols, brightened with ditoluene and soaked in paraffin wax in an incubator at 60°C. They were finally embedded in paraffin wax at room temperature. Sections cut at 3–4 μm were stained with H & E. They were examined microscopically at 100 and 300 magnifications.

Transmission electron microscope (TEM) sections and observation

Specimens of sarcomas of ~1 mm^3 were fixed, washed, post-fixed, dehydrated, and embedded conventionally before being cut on an ultramicrotome at ~50 nm. The sections were double-stained with lead citrate and uranyl acetone before being observed in a HU-11A TEM at 50 kV accelerating voltage.

Detecting apoptosis through in situ *nick-end-labelling*

Terminal deoxynucleotidyl transferase (TdT)-mediated nick-end-labelling (TUNEL) was performed with the ApopAlert DNA Fragmentation Assay Kit, as instructed by its user manual (CLONTECH, U.S.A.). Briefly, formalin-fixed, paraffin-embedded cancer tissue sections were mounted on glass slides (Ben-Sasson *et al.*, 1995). The paraffin wax was removed by immersing slides in fresh xylene followed by 100% ethanol. Slides were rehydrated through descending concentrations of ethanol (100%, 95%, 85%, 70%, 50%), fixed in 4% formaldehyde/PBS, washed with PBS, and treated with proteinase K. Subsequently, the slides were incubated with buffer containing fluorescein-dUTP and TdT enzyme, and the cells stained with propidium iodide (PI), followed by a final wash in PBS. The slides were examined as soon as possible thereafter. Apoptotic cells exhibited strong green nuclear fluorescence using a standard fluorescein filter of 520 ± 20 nm. All cells staining with PI exhibited strong red cytoplasmic fluorescence when viewed at ~620 nm (Fang, 1998).

RESULTS AND DISCUSSION

Mice

The statistical results show that the mean weights of the treated mice increased 5 d after the experiment, while the mean weights of controls decreased, indicating that the treated samples are in better body conditions than the control ones. A

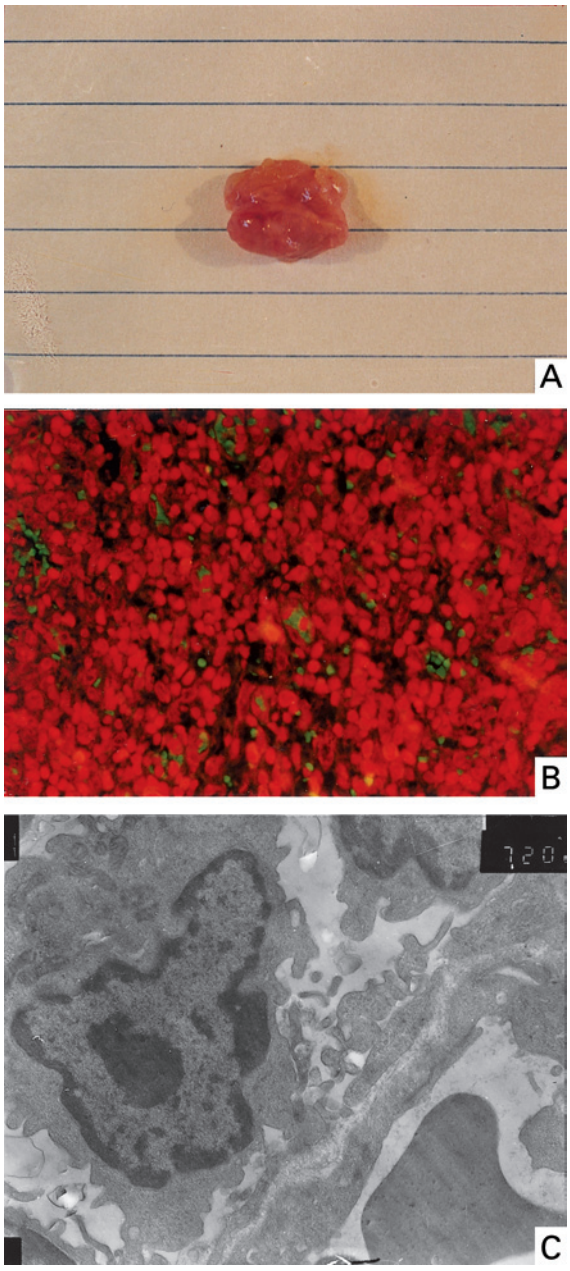

Fig. 1. Treated sample, No. 2, 1.0 g. (A) Hard sarcoma with the surface covered with viscid membrane. × 2.5. (B) Detection of apoptotic cancer cell TUNEL. Green shows apoptotic cells, which make up ~19.3% of the total. × 200. (C) Left: heterochromatin in a nucleus of cancer cell condensed and coagulated together along the nuclear membrane. Right: expanded endoplasmic reticulum. × 7200.

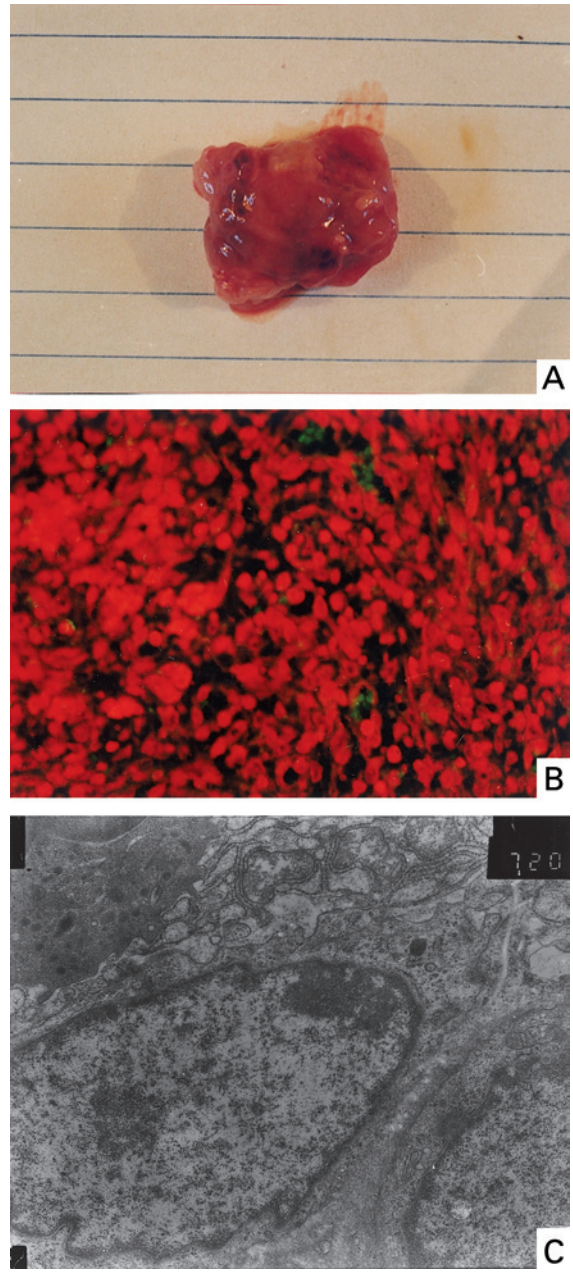

Fig. 2. Control sample, No. 16, 3.3 g. (A) Softer sarcoma joined tightly to its surroundings. × 2.5. (B) Apoptotic cells which make up 4.2% of the total. × 200. (C) Left: large nucleus of a cancer cell. Right: well-developed surface endoplasmic reticulum. × 7200.

statistical difference appeared within 20 days (Table 1).

Organs and tissues

Sarcomas from treated mice were smaller and harder than those from controls (Table 1). They were more easily cut off from the surrounding tissues. Their surfaces were covered with a viscid capsular membrane (Fig. 1(a)). Sarcomas in the control group were much larger and softer. Lacking an intact viscid membrane, they seemed to be joined tightly with the surrounding tissues (Fig. 2(a)).

Endothelial cells of the blood vessel were swollen in the treated mice tumours (Fig. 3), which seemed

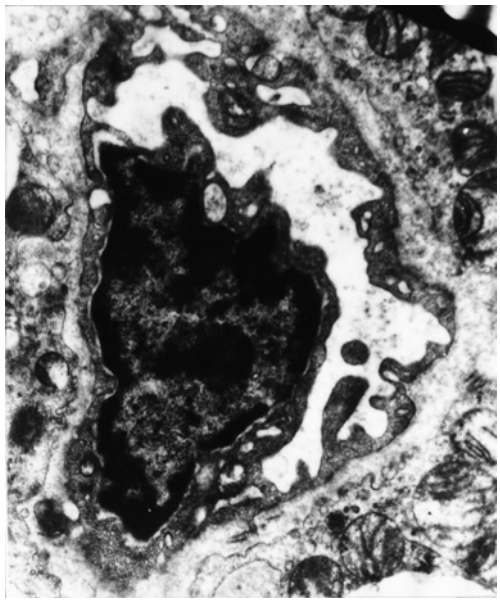

Fig. 3. Treated sample, No. 1, 0.5 g. The endothelium cell of blood vessel are swelled by magnetic field, thus the blood vessel is blocked. × 5000.

to occlude the blood vessels, and therefore might have shut off oxygen and nutrition supplies (Zhang *et al*., 2000)

Cells

Compared with controls, it is found that the mitochondria and RER expanded in response to the ELF treatment, indicating that the magnetic field may have affected cell metabolism. It is normally accepted that poorly differentiated cancer cells have high N/P ratios, more heteromorphic nuclei, are often multinucleolate, and these features relate to rapid growth. N/P ratios were an indicator of the malignancy of the cancer cells. Our microscopic data indicate that the exposed sarcoma cells had rounder nuclei, and that the volume fraction of nucleus and N/P decreased (cf. Zhang *et al*., 1997). These indicate that the magnetic field lower the 'degree of malignancy' of sarcoma cells and inhibits their rapid and heteromorphic growth.

Cancer cells in the treated group showed some of the morphological properties associated with programmed cell death (PCD). Cancer cell contacts were looser and more divorced from adjacent cells. Their heterochromatin condensed and coagulated together along the nuclear membrane (Fig. 1(c)). The endoplasmic reticulum seemed to be dilated and fused with the cellular membrane. Many apoptotic bodies, packed with cellular membrane debris, appeared and were probably being digested by lymphocytes and macrophages.

Extensive DNA degradation is characteristic in the early stages of apoptosis. Cleavage of the DNA may yield double-stranded fragments with 3′-OH termini as well as single strands. The free 3′-ends, in DNA can be labelled with DIG-dUTP by terminal deoxynucleotidyl transferase (TdT), with the incorporated nucleotides being detected in a second incubation step with an anti-DIG antibody conjugated with fluorescein. The immuno-complex has an emission wavelength of 523 nm (green light) when excited at 494 nm. Labelled apoptotic cells, counted under fluorescence microscopy, in treated sarcomas were greater on number than in the controls (Fig. 1(b) and 2(b)).

The phenomenon of immune cells, including lymphocytes and phagocytes, accumulating around cancer cells to destroy and digest them was seen more prominently in sarcomas exposed to the magnetic field.

Macromolecules

We observed a decrease of DNA content by Feulgen staining which indicated that magnetic field can block DNA replication and mitosis of sarcoma cells (Zhang *et al*., 1997). It is found that a decrease of the mitotic phases of carcoma cells is due to exposure (Zhang *et al*., 1995).

ELF pulsed-gradient magnetic field may be able to inhibit the growth and division of cancer cell and enhance the host cellular immune response. How the low frequency pulsed-gradient magnetic field induces apoptosis of cancer cell and blocks new blood vessel development remains unknown, but it nevertheless has been found that this could be a new method for the treatment of cancer, although clearly further research on the mechanism is much needed.

ACKNOWLEDGEMENTS

The authors thank Prof. Li Tongping of the Pathological Unit of Fundamental Medical School, Tongi Medical University, for his kind instruction and help. This work was supported by research grants from the Natural Science Foundation of China and the Ministry of Science and Technology of China.

REFERENCES

Chang H, Li G, Pan Y, *et al.*, 1985. Experimental observation on the effect of magnetic field on S-180 sarcomas in mice. *Chinese Journal of Physics Medicine* **7:** 169–170 (in Chinese).

CHANG H, LI G, WANG G, *et al.*, 1990. Primary report of 18 cases on magnetic field therapy for malignant tumour, in Biomedical Physics Study. Wuhan: Wuhan University Press, 74–75 (in Chinese).

CHEN J, 1997. Electromagnetic field and organism. *Biomagnetism (in Chinese)* **11:** 6–8.

FANG F, 1998. *Forward position technology of molecular biology*. Beijing, United Press of Beijing Medical University and Chinese Xiehe Medical University. 164–167 (in Chinese).

FANG M, ZHANG HQ, XUE SB, 1998. Role of Calcium in apoptosis of HL-60 cells induced by harring tonine. *Science in China, Ser. C* **41:** 600–607.

POOLE C, 1991. Extremely low frequency electric and magnetic fields and cancer. *Cancer Causes* **2:** 267–270.

SILVA CP, OLIVEIRA CR, LIMA MCP, 1996. Apoptosis as a mechanism of cell death induced by different chemotherapeutic drugs in human leukemic T-lymphocytes. *Biochem Pharmacol* **51:** 1331–1340.

ZHANG H, JIN X, YE H, *et al.*, 1994. Extremely low frequency pulsed magnetic field restrains cancer cells in animal and human, in Biomagnetism Study and Application. Beijing, Atomic Energy Press. 18–21 (in Chinese).

ZHANG H, YE H, ZHANG Q, *et al.*, 1995. SEM and TEM observations on inhibitory effect of ultralow frequency pulse magnetic field on mice's S-180 Sarcomas. *Chinese Science Bulletin,* **40:** 512–516.

ZHANG H, YE H, ZHANG Q, *et al.*, 1997. Experimental studies on extremely low frequency pulsed magnetic field inhibiting sarcoma and enhancing cellular immune functions. *Science in China, Ser. C* **40:** 392–397.

ZHOU W, 1994. Applied advance of magnetic field therapy for tumour. *Chinese Biomagnetism* **8:** 1–6 (in Chinese).