

The Effect of Alternating Magnetic Field Exposure and Vitamin C on Cancer Cells

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The authors have previously shown the potential therapeutic effect of very high-dose intravenous vitamin C (ascorbic acid, ascorbate, AA) in the treatment of cancer.¹⁻⁶ We showed 50 percent inhibition of tumor cells with vitamin C concentration at 200 mg/dL in dense monolayer cell culture and 400 mg/dL for the hollow fiber culture model.¹ Since it is difficult to maintain this high level of vitamin C in blood for long periods of time, we were interested to see if cytotoxicity of cancer cells could be obtained by using lower concentrations of vitamin C in combination with alternating magnetic fields. Low frequency pulsed magnetic fields (LFMF) and rotating magnetic fields (RMF) were used. The biological effect of electromagnetic field at different levels of intensity and frequencies have been reported previously.^{7-9,12} It is thought that alternative magnetic fields and induced electric fields alter cell functions by changing cell membranes. Magnetic fields are thought to interfere with cytoplasm enzyme functions by modifying membrane permeability, modifying ion transport and/or ligand-receptor interactions at the cell membrane surface.^{1,10,11} Normal cells and cancer cells have different electrical status. Experimental data has shown that growing cells are electrically negative and cancer cells are the most negative of all.¹³ When LFMF was combined with adriamycin (doxorubicin), cisplatin, mitomycin C, and other chemotherapeutic drugs, LFMF improved the transport of drugs into cells and reduced the therapeutic dose of these drugs.¹⁴⁻¹⁶

In treated rats, the smallest mean tumor size was found in the drug plus

pulsed magnetic field (PMF) treatment group.

The purpose of our study was to examine the anti-tumor effect of vitamin C combined with magnetic field treatments. The inhibitory effect of vitamin C in cancer cells involves its interaction with several compounds: glutathione (GSH), hydrogen peroxide and the enzyme catalase.^{17,18} In the blood, vitamin C is oxidized to dehydroascorbate (DHA). DHA is easily transported across cell membranes where it is then reduced by GSH back to vitamin C. Cancer cells have a high level of GSH compared to normal cells. The higher level of GSH for the same level of vitamin C produces more hydrogen peroxide. In normal cells, catalase inactivates hydrogen peroxide by converting it to water and oxygen. Cancer cells have a reduced (10 to 100 fold) intracellular level of catalase. This results in very high levels of hydrogen peroxide and oxidative by-products in the cancer cell.¹⁸ Hydrogen peroxide is toxic and destroys the cancer cells.

The cells used in our experiment were human pancreatic carcinoma (MiaPaCa); human breast carcinoma (MFC-7); human osteogenic sarcoma (U2-OS); adenocarcinoma colon (SW620); human fibrosarcoma (HT1080); prostate carcinoma (PC-3); and normal cell line colon fibroblast (CCD18CO). The monolayer cells were cultured in 96-well plates with a density of 24,000 cells per well. After cell attachment, vitamin C (sodium ascorbate) was added in concentrations of 100, 200, 300, 400 and 500 mg/dL. The procedure for using the hollow fiber model (three-dimensional cell structure) was that of Casciari, et al.¹⁹ Growing cells were exposed to different intensities of LFMF fre-

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quencies (30 Hz, 60 Hz, 120 Hz, 200 Hz, 500 Hz and 1000 Hz) in the center of a coil at 37C with 100 percent humidity and 5 percent CO₂ concentration. The experiments were carried out in a radio free cage. A control plate for each concentration was treated the same as the cancer cell plate without the magnetic field. The exposure system consisted of a coil (R= 14 ohm, L=0.16 mH, 1200 turns) connected to a DS 345 generator and a Techron 7540 audio amplifier.²⁰⁻²¹ All plates were treated for two days, 2 to 3 hours per day. Figure 1 (below) shows an example of magnitudes versus voltage on coil and the voltage induced on probe for 60 Hz frequency. The rotating magnetic field device consisted of two pairs of permanent magnets rotating at a speed of 1000 rpm. The peak intensity of magnetic field at the center of the rotating field was 40 gauss. At the end of the procedure, the number of viable cells were determined and the Hill equation²² was used (concentration-effect curve for description of drug induced growth inhibition of cancer cells) to determine the IL50 (the drug concentration inducing a 50 percent decrease in the maximum effect).

Figure 2 (p.3) shows the result of the human fibrosarcoma cell culture treated with LFMF at 60 Hz and peak intensity of field at 25 gauss. The control group was vitamin C alone, the treatment group was vitamin C combined with LFMF. Both were exposed two

hours per day.

Data from four different experiments are shown. The LFMF induced cell death at a much lower level than just vitamin C alone. There was 80 percent cell death (20 percent survival) at 120 mg/dL of vitamin C and LFMF while the vitamin C group needed 220 mg/dl for the same response.

Figure 3 (p.3) shows the effect of the rotating magnetic field (RMF, 40 gauss, 1000 rpm) on human pancreatic cancer cell lines (MiaPaCA). The control group received treatment with vitamin C only. An 80 percent cell death (20 percent survival) was achieved with 160 mg/dL of vitamin C in the magnetic field treatment group. It required 360 mg/dL to achieve the same effect with vitamin C only treatment group. Table 1 (p.4) lists all the cell line and their response to LFMF and vitamin C group compared to the control plates (vitamin C only). All the cells were treated with LFMF at 60 Hz with an intensity of 25 gauss. The calculated IL50 in the LFMF group ranged from 23 percent for colon adenocarcinoma to 51 percent for pancreatic carcinoma. For normal cell line of colon fibroblast magnetic field did not potentiate inhibition of cell growth. These are all mono-layer cell culture.

It has been shown that the response of tumor cells are less in solid tumors than in cell culture. To more approximate the solid tumor response, we repeated the experi-

Figure 1. Magnitudes of time-varying voltage on coil and induced electric field on probe for 60 Hz.

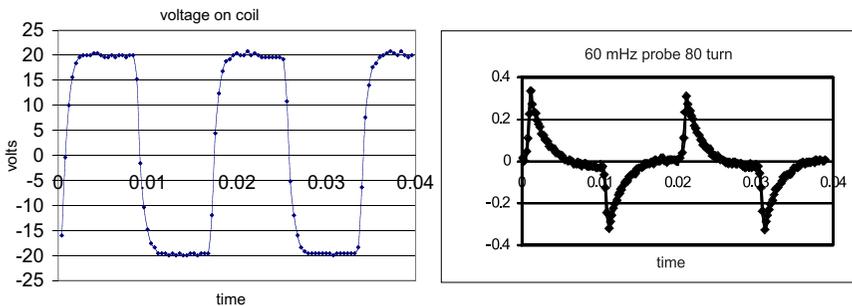


Figure 2. Response of human fibrosarcoma to LFMF + vitamin C and vitamin C only

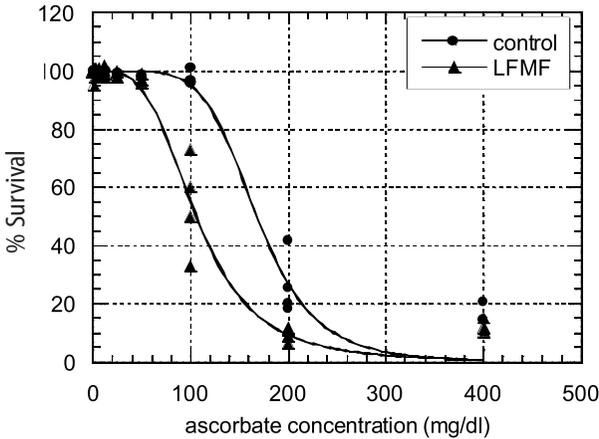
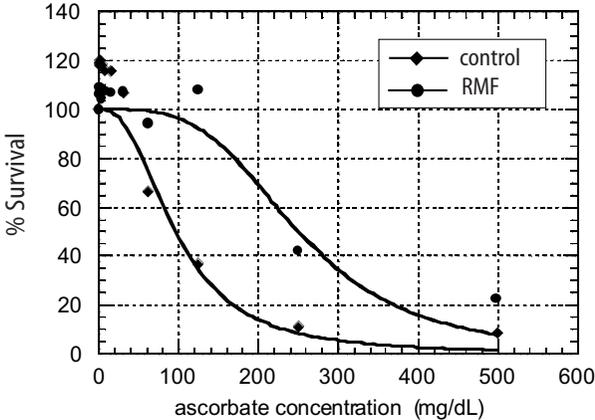


Figure 3. Effect of rotating magnetic field (RMF) on human pancreatic cancer cells (MiaPaCa).



ments using the hollow-fiber model with SW620 cell line (human osteogenic sarcoma). The results are shown in Table 2 and Figure 4 (p. 4,5).

Data from Table 2 tends to support the theory that solid or three-dimensional tumor models are more resistant to treatment than cell culture or mono-layer models. The monolayer required only 1.48 mg/

mL of vitamin C for an IL50 with SW620 cell line. The hollow-fiber model required 9.69 mg/mL of vitamin C, (approximately six times as much) for the same effect. Again, the LFMF treatment reduced the amount of vitamin C to achieve IL50 in both models. The amount of vitamin C decreased from 9.69 mg/mL (R or correlation coefficient = 0.88) to 4.52 mg/mL (R=0.91) for

Table 1. IL50 of 8 cell lines exposed to LFMF + vitamin C and vitamin C only.

Cell line	Υ_{50} mg/dL	$^{\circ}$ -slope parameter	IL ₅₀ mg/dL	Υ -slope parameter	LFMF IL ₅₀ reduction
	control plates		treated by LFMF		
MiaPaca (pancreatic carcinoma)	454.06 ±3.90	4.20 ±0.22	223.04 ±2.38	6.42 ±0.55	51%
SW620 (colon adenocarcinoma)	148.41 ±1.79	6.42 ±0.40	120.0 ±2.2	6.8 ±2.3	23%
HT1080 (human fibrosarcoma)	168.2 ± 12.4	5.72 ±0.52	106.2 ±5.1	3.55 ±0.82	37%
PC-3 (prostate cancer)	227.8 ± 7.8	6.27 ±1.77	136.7 ±3.5	4.39 ±0.57	40%
U2-OS (human osteogenic sarcoma)	310.7 ± 6.9	4.45 ±0.32	167.59 ±3.87	4.03 ±0.33	46%
MCF-7 (breast carcinoma)	143.34 ±3.49	8.47 ±0.63	96.4 ±7.9	12.1 ±2.7	33%
CCD-18co (normal cell line colon fibroblast)	279.3 ± 3.9	3.47 ±0.16	298.56 ±5.17	4.31 ±0.28	-7%

Table 2. LFMF + Vitamin C vs, Vitamin C only in a hollow-fiber model with SW620 cell line.

Control samples	Hollow fiber tumors (SW620)			Mono-layer (SW620)	
		Value (mg/mL)	SD	Value (mg/mL)	SD
ascorbate	Υ_{50}	9.69	1.27	1.48	0.02
	g-slope	1.44	0.28	6.42	0.40
	R	0.88		0.98	
LFMF exposure + ascorbate		Value	SD	Value	SD
	Υ_{50}	4.52	0.88	1.18	0.01
	g-slope	1.19	0.28	4.61	0.32
	R	0.91		0.94	

hollow-fiber and 1.48 mg/mL (R=0.98) to 1.18 mg/mL (R=0.94) with the mono-layer model.

Figure 4 (below) shows the effect of combined treatment by LFMF plus ascorbate or ascorbate alone on survival of human colon carcinoma cells SW620 grown inside the hollow fiber as a three-dimensional tumor cell structure.

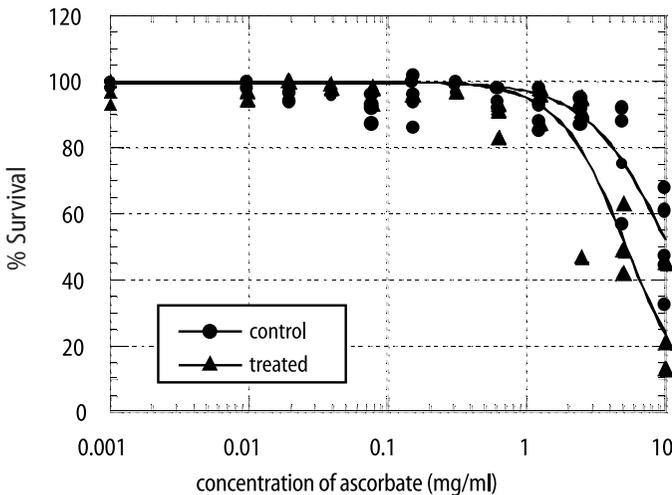
To investigate the possibility that vitamin C or LFMF could induce cell growth, tumor cell lines and normal fibroblasts were exposed to 30-100 Hz for three to four hours over a three day period. There was no increase in the growth rate of tumor cells or normal cells. It appears from our data that vitamin C combined with low frequency magnetic field or rotating magnetic field reduces the amount of vitamin C to induce 50 percent inhibition of tumor cells. This widens the therapeutic window of vitamin C and does not harm normal cells.

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Figure 4. Response of human colon carcinoma cells SW620 grown as hollow-fiber in vitro solid tumor model to LFMF + vitamin C and vitamin C only.



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