Supplementary data

1. Materials and methods

Drug

Bleomycin, a membrane impermeable chemotherapeutic drug, was used in preliminary experiments to test the application of induced electroporation mediated with magnetic field. The stock solution of the bleomycin (3 mg/mL, BLM, Bleomedac, Medac GmbH, Hamburg, Germany) was dissolved in aqua pro injection and frozen in aliquots of 1 mL. In order that each animal received a dose of 100 μg of bleomycin, a fresh solution at the appropriate concentration of BLM (1.25 mg/mL) was prepared in 0.9% sodium chloride solution daily before each experiment.

In vivo electrochemotherapy protocol using noninvasive electroporation induced by PEMF

In the experiments were used the same strain of mice and tumor model described above in the section of Materials and methods. Seven days after subcutaneously induction of B16F10 melanoma tumors (40 mm^3) mice were randomly divided into the experimental groups as follows: intravenously injection of saline solution alone (Control) or combined with electroporation induced pulsed electromagnetic field (PEMF), intravenously injection of bleomycin (BLM) or combined with electroporation induced by PEMF (PEMF + BLM). Noninvasive electroporation was performed 3 minutes after intravenously injection of BLM by magnetic field pulse generator connected to an applicator consisted of round coil with 72 turns as precisely described above in the section of Materials and methods.

Four different sequences of bipolar pulses of electric current were delivered to the applicator. All sequences had t_p = 340 μs long bipolar pulses with a peak of I_p = 400 A (Figure 2) whereas repetition frequency (f_p) and duration of each sequence (t_s) was varied (Supplementary Table 1).

During the noninvasive PEMF treatment animals were anaesthetized as is described in the section of materials and methods.

SUPPLEMENTARY TABLE 1. Four different sequences of bipolar pulses of electric current applied for electroporation induced by PEMF.

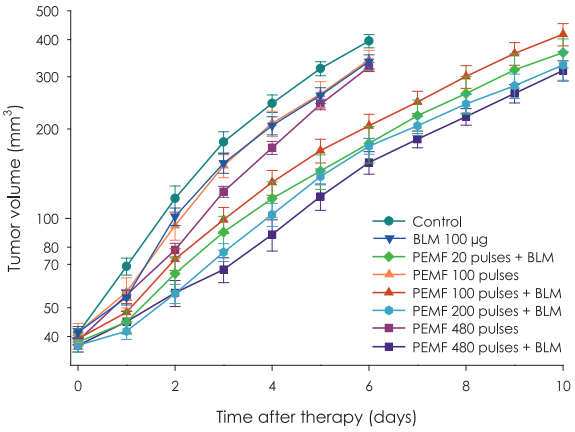
Sequence number	Duration of sequence (ts [s])	Bipolar pulse repetition frequency (fp [Hz])	Number of applied pulses (Np = ts × fp)
1	20	1	20
2	10	10	100
3	20	10	200
4	6	80	480

2. Results

SUPPLEMENTARY TABLE 2 Tumor doubling times of melanoma B16F10 tumors after treatment with bleomycin or combined with electroporation induced by PEMF.

Group	n	DT (Mean ± SE)	GD	P (<0.05)
Control*	18	1.5 ± 0.1		
BLM* 100 μg	17	1.7 ± 0.1	0.2	
PEMF seq. 2	8	1.8 ± 0.2	0.3	0.030 (PEMF seq. 2 vs combination)
PEMF seq. 4 (480 pulses)	9	1.9 ± 0.1	0.4	
PEMF seq. 1 + BLM (20 pulses)	9	2.8 ± 0.4	1.3	
PEMF* seq. 2 + BLM (100 pulses)	17	2.7 ± 0.3	1.2	0.027 (PEMF seq. 2 or at seq. 4 vs combination)
PEMF seq. 3 + BLM (200 pulses)	9	3.0± 0.3	1.5	
PEMF seq 4 + BLM (480 pulses)	9	3.6 ± 0.2	2.1	<0.001 (PEMF seq. 4 vs combination)

BLM = intravenously injection of bleomycin (100 μg/mouse); PEMF = pulsed electromagnetic field treatment; PEMF + BLM = PEMF after intravenously injection of BLM; DT = tumor doubling time; GD = tumor growth delay; p < 0.05 statistically significant difference; * Data pooled from two individual experiments.



SUPPLEMENTARY FIGURE 1. Antitumor effectiveness of electrochemotherapy with bleomycin mediated by PEMF in mouse melanoma B16F10 tumors. Data were collected from two individual experiments and each point on graph represent mean and standard error of the mean (AM ± SE). Each group consisted at least of 8 animals.

BLM = intravenously injection of bleomycin (100 μg/mouse); PEMF = pulsed electromagnetic field treatment; PEMF + BLM = PEMF after intravenously injection of BLM