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Treatment of Experimental Tumors with a Combination of a Pulsing Magnetic Field and an Antitumor Drug

Yoshiharu Omote,[1,2,3] Masuo Hosokawa,[1] Masashi Komatsumoto,[1] Tsutomu Namieno,[1] Susumu Nakajima,[2] Yoshihiko Kubo[2] and Hiroshi Kobayashi[1]

[1]*Laboratory of Pathology, Cancer Institute, Hokkaido University School of Medicine, Kita-15, Nishi-7, Kita-ku, Sapporo 060 and* [2]*First Department of Surgery, Asahikawa Medical College, Nishikagura 4-5, Asahikawa 078-11*

We investigated the effects of a combination treatment involving a pulsing magnetic field (PMF) and an antitumor drug, mitomycin C (MMC), on two experimental tumors (fibrosarcoma KMT-17 and hepatocellular carcinoma KDH-8) in WKA rats, paying attention to possible potentiation of the therapeutic effect of the antitumor drug. PMF was obtained using a system generating a pulsed current in a solenoid coil. On day 7 after tumor implantation into the right thighs of rats, the region of the tumor was exposed to PMF (frequency 200 Hz, mean magnetic flux density 40 gauss) for 1 h immediately after iv injection of MMC at a dose of 1 mg/kg. Survival rates at day 90 of KMT-17 implanted rats were 0% (0/10) in the non-treated group, 34% (4/12) in the MMC-treated group, 47% (6/13) in the PMF-treated group and 77% (10/13) in the MMC/PMF combination group. The increase of life span (ILS) of KDH-8-implanted rats in the combination therapy group was significantly prolonged (%ILS 17.6%) compared with that in the MMC-treated (%ILS 3.4%) and PMF-treated (%ILS 7.6%) groups. By using cultured cells of the above two lines of tumor, the therapeutic effects of MMC and PMF were also determined from the cell colony-forming efficiency in soft agar. The colony-forming efficiencies of both cell lines were significantly suppressed in the combination therapy group compared with those in the other single therapy groups. The present results indicate that PMF exhibited a potentiation of the antitumor effect of mitomycin C.

Key words: Pulsing magnetic field — Mitomycin C — Tumor growth curve — Increase of life span — Colony formation

In hyperthermia or thermo-chemotherapy[1] using electromagnetic heating, possible nonthermal effects of an electromagnetic field on cancer cells have been taken into consideration, but most studies evaluating them have found no such effects.[2–5] However, Batkin *et al.*[6] reported that neuroblastoma cells implanted into A/J mice exposed to an alternating magnetic field (60 Hz, 12 gauss) for 16 days showed initial growth suppression and partial necrotic change. We have designed and constructed a pulsing magnetic field (PMF)[4] generator, and used it to investigate combination treatment employing an intense pulsing magnetic field (which causes virtually no heating) together with antitumor drugs on experimental tumors implanted into rats, thus concentrating on effects other than the heating effect of an electromagnetic field on cancer cells.

[3] To whom reprint requests should be addressed, at the First Department of Surgery, Asahikawa Medical College, Nishikagura 4-5, Asahikawa 078-11.

[4] The abbreviations used are: PMF, pulsing magnetic field; MMC, mitomycin C; WKA rat, Wistar King Aptekman/HMK rat; FCS, fetal calf serum; PBS, phosphate-buffered saline; sc, subcutaneous; ip, intraperitoneal.

MATERIALS AND METHODS

Rats Inbred male Wistar King Aptekman/HMK (WKA) rats, 8 to 12 weeks old, were obtained from the Experimental Animal Institute, Hokkaido University School of Medicine, Sapporo.

Tumors KMT-17 is a transplantable fibrosarcoma induced by 3-methylcholanthrene in WKA rats.[7,8] KDH-8 is a transplantable hepatoma induced by dimethylaminoazobenzene in WKA rats.[9] Each tumor is maintained in an ascites form.

Cell culture A cultured KMT-17 cell line (c-KMT-17) and a cultured KDH-8 cell line (c-KDH-8) were maintained in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS) and L-glutamine (2 m*M*); the cells were incubated at 37°C in a humidified atmosphere of 5% CO_2.

Anesthesia All rats were anesthetized with pentobarbital sodium (Nembutal; Abbott Laboratories, North Chicago, IL) ip at a dose of 25 mg/kg.

Chemicals Mitomycin C (MMC), supplied by Kyowa Hakko Co., Tokyo, was dissolved in distilled water just before use.

PMF generating system A solenoid coil was connected to a DC power supply, and at the half-way point of its

circuit, we interposed a switching circuit using a transistor, diode and solid-state relay with a photo-coupler driven by a digital stimulator (SEN-3201, Nihon Koden, Tokyo). By setting the DC power and digital stimulator at specified values, a PMF was obtained around the solenoidal coil. The mean magnetic flux density was measured by a Gauss meter (Yokogawa Electric Inc., Tokyo) using a Hall effect probe. Increase in temperature around the solenoid coil and in the tumor was monitored by using a thermometer (Yokogawa Electric Inc.). A block diagram of this system is shown in Fig. 1. The wave-form of the PMF was considered to be similar to that of the current in the solenoid coil. An example of the current is shown in Fig. 2. We prepared two kinds of solenoid coil, one for *in vitro* experiments (diameter $\phi=$ 16.5 cm, length $l=6.0$ cm, $N=100$ turns, inductance $L=2.28$ mH, resistance $r=0.33\ \Omega$), and the other for *in vivo* experiments ($\phi=3.4$ cm, $l=3.0$ cm, $N=24$ turns, $L=0.01$ mH, $r=0.02\ \Omega$).[10]

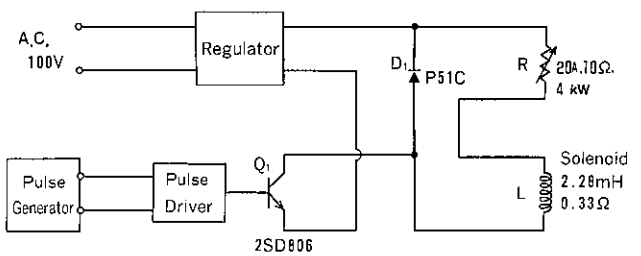

Fig. 1. Block diagram of the system used for generating a PMF.

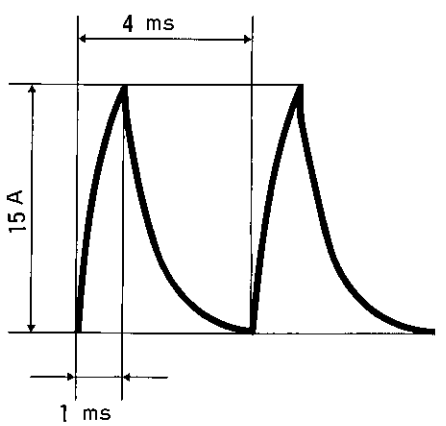

Fig. 2. An example of the current wave-form in the solenoid coil used in our experiments. The wave-form of the PMF was considered to be similar to that of the current, so the peak magnetic flux density was considered to be about 4 times the mean magnetic flux density in our experiments.

Combination treatment with a PMF and antitumor drug on rat tumors On day 0, 1×10^5 KMT-17 tumor cells or 1×10^6 KDH-8 tumor cells were implanted sc into the right hind limb of WKA rats. MMC was administered iv at 1 mg/kg on day 7 and PMF was applied to the local tumor for 1 h just after MMC administration. PMF conditions were: frequency 200 Hz, pulse width 2.0 ms and mean magnetic flux density 40 gauss. Figure 3 shows a diagram of the set-up used for the combination MMC/PMF therapy. In order to eliminate small perfusion differences reflecting anesthesia and restraint, control rats and rats treated with MMC alone were all anesthetized, restrained and sham-treated with the PMF.

Observation of tumor growth and evaluation of antitumor effects After the combination treatment with PMF and MMC, 2 tumor diameters were measured with a caliper every third day. Observation was continued until the rats died or for 3 months. Rats that survived for more than 3 months are referred to as survivors in the results section. Mean survival time (MST) was calculated for each group and percentage increase of life span (% ILS) was also calculated using the following formula.

$$\%\mathrm{ILS}=\left(\frac{\text{MST in treated group}}{\text{MST in control group}}-1\right)\times100.$$

Measurement of growth rate *in vitro* c-KMT-17 cells or c-KDH-8 cells were seeded at a density of 2×10^5 cells/60 mm plastic dish with 5 ml of RPMI-1640 medium containing 10% FCS in triplicate on day -1 and cultured in a CO_2 incubator. PMF was applied to the cultured cells for 2 h on day 0. PMF conditions were; frequency 250 Hz, pulse width 1.0 ms and mean magnetic flux density 40 gauss. Five days after the treatment, the cells were harvested and cell numbers were counted with a hemocytometer, and growth curves of control and PMF-

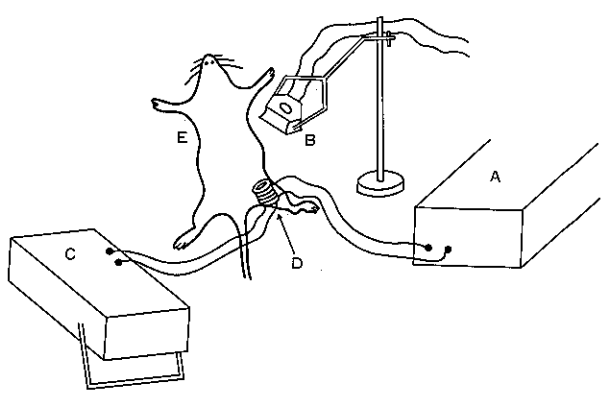

Fig. 3. Set-up for PMF treatment of a local tumor implanted into a WKA rat. A, PMF generator; B, fan; C, thermometer; D, solenoid coil; E, WKA rat.

treated tumor cells were constructed by plotting the cell numbers for each.

Colony-forming assay in soft agar[11] MMC was administered at doses of 0.2, 0.02 and 0.002 μg to 1.25×10^5 c-KMT-17 or c-KDH-8 cells and each cell type was incubated in a CO_2 incubator followed by exposure to PMF for 1 h. PMF conditions were: frequency 250 Hz, pulse width 1.0 ms and mean magnetic flux density 40 gauss. Subsequently, cells of each treated group were washed 3 times with PBS (−), and 1×10^4 c-KMT-17 or 5×10^3 c-KDH-8 cells were plated onto 35-mm Petri dishes containing 1 ml of soft agar consisting of 16.5 ml of RPMI-1640 medium with 10% FCS and 3.5 ml of 3% noble agar (Wako Pure Chemical Indust. Ltd., Osaka). The dishes were incubated at 37°C in a humidified atmosphere of 5% CO_2 for 14 days. Then the colonies (more than 50 cells) of each treated group were counted. The plating efficiency for the control group was calculated as the ratio of the number of colonies observed to the number of cells plated.

Statistical analysis Student's *t* test was used to determine the significance of differences among the means in survival times of rats, tumor sizes and numbers of colonies.

RESULTS

Heating effect Temperature measurements during exposure to PMF are shown in Fig. 4. Temperatures inside the coil and in the tumor were essentially constant at 35.0±0.2°C and 32.8±0.2°C, respectively, so the thermal effects on tumors were apparently negligible.

Enhancement of therapeutic effect of MMC by PMF Therapeutic effects of MMC on KMT-17 tumors in WKA rats with combined use of PMF are shown in Table I. All rats in the untreated group died, and the mean survival time was 21.3 days. MMC-administered rats showed a mortality of 66% (8/12), PMF-applied rats 53% (7/13) and rats given the combination treatment of PMF and MMC only 23% (3/13). These results indicate the effectiveness of PMF and MMC in combination. Therapeutic effects of MMC on KDH-8 tumors in WKA rats through combined use of PMF are shown in Table II and the growth curves for KDH-8 tumor in each treated group are shown in Fig. 5. All rats in each treated group died but the %ILS values of the MMC-treated, PMF-applied and combination-treated groups were 3.4%, 7.6% and 17.6%, respectively. The tumor diameters on day 31 were as follows: control group, 52.4±4.1 mm (n=10); PMF-applied group, 51.1±2.5 mm (n=10); MMC-treated group, 44.7±2.8 mm (n=9, $P<0.001$ compared to the PMF-applied group by Student's *t* test); combination-treated group, 40.0±3.0 mm (n=9, $P<0.01$ compared to the MMC-treated group by Student's *t* test). These results show the potentiated effect of PMF and MMC in combination.

Effects of PMF on the growth curves of cultured cells The effects of PMF on the growth curve of c-KMT-17

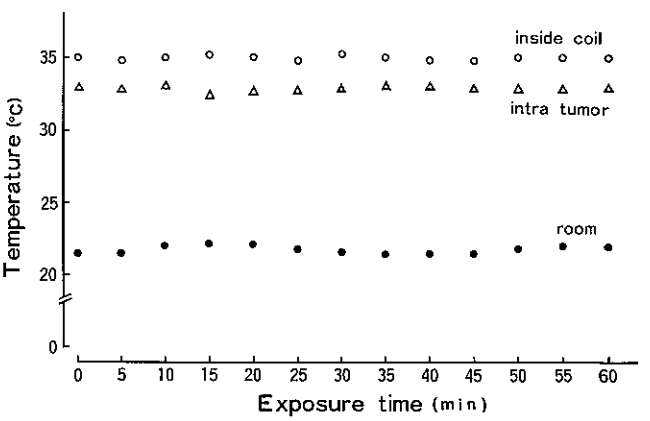

Fig. 4. Temperature records inside the coil and in the tumor during exposure to a PMF.

Table I. Therapeutic Effects of Mitomycin C (MMC) on KMT-17 tumors in WKA Rats by Combined Use of a Pulsing Magnetic Field (PMF)

Treatment group	Therapeutic effect	
	Alive/treated (%)	Survival days of each rat
None	0/10 (0)	16, 17, 18, 19, 19, 19, 23, 23, 28, 31
MMC[a]	4/12 (34)	15, 15, 16, 20, 21, 22, 23, 25, others 90<
PMF	6/13 (47)	17, 17, 21, 22, 28, 30, 61 (lung meta.), others 90<
MMC+PMF[b]	10/13 (77)	19, 25, 37 (liver meta.), others 90<

KMT-17 cells (1×10^5) were implanted sc into WKA rats on day 0.
a) MMC was administered iv 1 mg/kg on day 7.
b) PMF was applied to the local tumor just after MMC administration for 1 h. PMF conditions: frequency 200 Hz, pulse width 2.0 ms, mean magnetic flux density 40 gauss.
Observation period: 90 days.

Table II. Therapeutic Effects of Mitomycin C (MMC) on KDH-8 Tumors in WKA Rats by Combined Use of a Pulsing Magnetic Field (PMF)

Treatment group	Therapeutic effect			
	Died/treated (%)	MST±SD[c] (days)	%ILS[d]	Tumor diameters on day 31 (mm) (mean±SD)
None	10/10 (100)	35.3±2.3	—	52.4±4.1
MMC[a]	9/9 (100)	36.5±2.9	3.4	44.7±2.8
PMF	10/10 (100)	38.0±2.7[e]	7.6	51.1±2.5
MMC+PMF[b]	9/9 (100)	41.5±3.0[f]	17.6	40.0±3.0

KDH-8 cells (1×10^6) were implanted sc into WKA rats on day 0.
a) MMC was administered iv 1 mg/kg on day 7.
b) PMF was applied to the local tumor just after MMC administration for 1 h. PMF conditions: frequency 200 Hz, pulse width 2.0 ms, mean magnetic flux density 40 gauss.
c) MST: Mean survival time and standard deviation.
d) ILS: Increase of life span was calculated using the following formula.

$$\%\text{ILS}=\left(\frac{\text{MST in treated group}}{\text{MST in control group}}-1\right)\times 100.$$

e) Statistically significant compared to non-treated group by Student's *t* test ($P<0.05$).
f) Statistically significant compared to non-treated ($P<0.001$), MMC-treated ($P<0.01$) and PMF-treated ($P<0.02$) groups, respectively, by Student's *t* test.

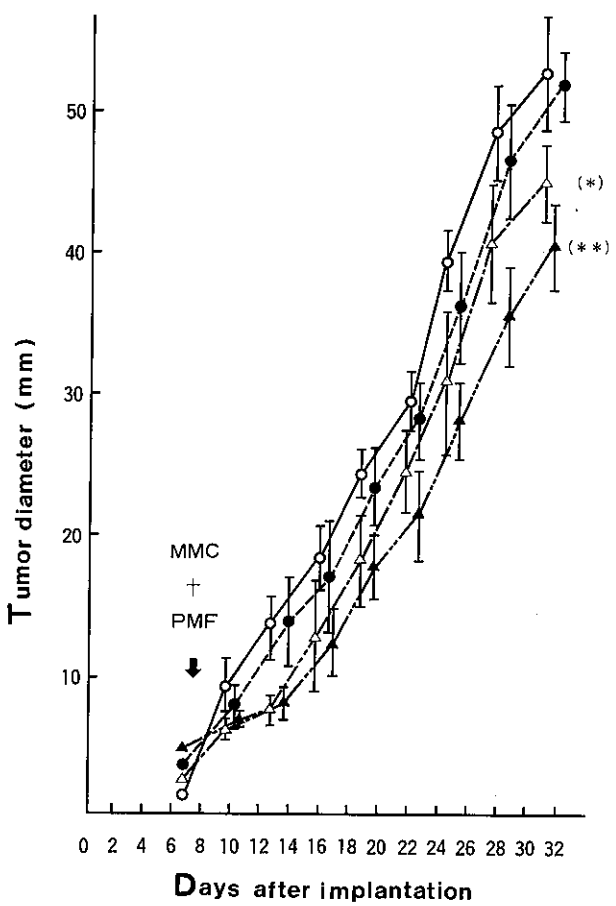

Fig. 5. Growth curve of KDH-8 tumors implanted in WKA rats. (*) $P<0.001$ compared to the PMF-exposed group by Student's *t* test. (**) $P<0.01$ compared to the MMC-administered group by Student's *t* test. ○ Non-treated (n=10), △ MMC-administered (n=9), ● PMF-exposed (n=10), ▲ MMC+PMF (n=9). Each point and vertical bar represent the mean±SD of these experiments.

cells or c-KDH-8 cells are shown in Fig. 6 and Fig. 7, respectively. Exposure to PMF did not affect the growth of either cell line. Doubling times of c-KMT-17 and c-KDH-8 cells were 14.4 h and 25.5 h, respectively.

Decrease in colony formation of cultured cells after treatment with MMC and PMF The numbers of colonies of c-KMT-17 cells after treatment with MMC and PMF are shown in Table III. Cells exposed to the combined treatment gave significantly fewer colonies as compared with MMC-administered cells at all concentrations of MMC. The numbers of colonies of c-KDH-8 cells after treatment with MMC and PMF are also shown in Table IV. At a concentration of 2×10^{-2} μg/1.25×10^5 cells MMC, the number of colonies of combined-treatment cells was significantly decreased compared with MMC-administered cells. These results demonstrated the enhanced antitumor effects of MMC and PMF in combination.

DISCUSSION

We investigated the effects of treatment with a combination of PMF and an antitumor drug, MMC, in two

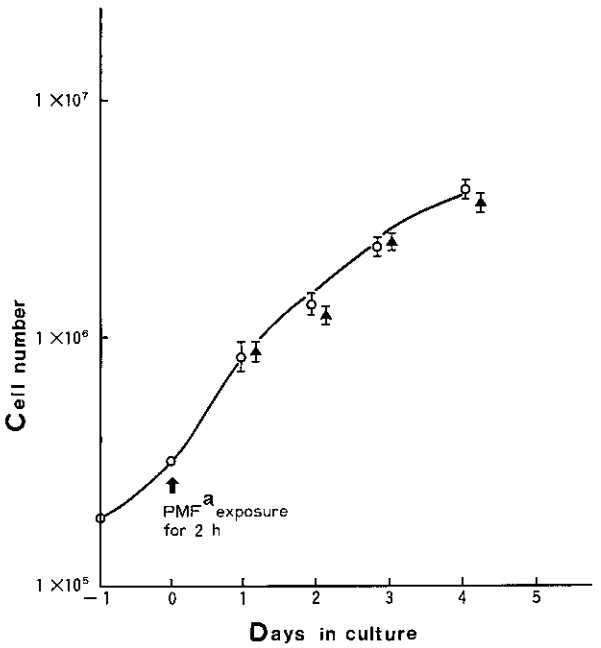

Fig. 6. Effects of a PMF on the growth curve of cKMT-17 cells after exposure to PMF. a. PMF conditions: frequency 250 Hz, pulse width 1.0 ms, mean magnetic flux density 40 gauss. ○, Control; ▲, PMF-exposed. Each point and vertical bar represent the mean±SD of three experiments.

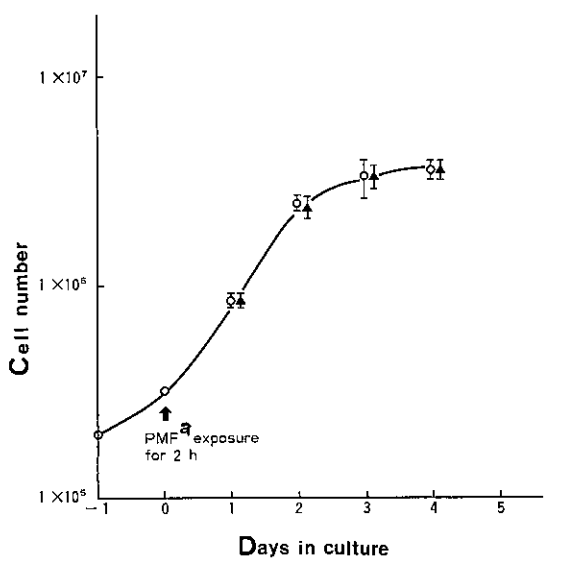

Fig. 7. Effects of a PMF on the growth curve of cKDH-8 cells after exposure to PMF. a. PMF conditions: frequency 250 Hz, pulse width 1.0 ms, mean magnetic flux density 40 gauss. ○, Control; ▲, PMF-exposed. Each point and vertical bar represent the mean±SD of three experiments.

Table III. Colony-forming Efficiency of cKMT-17 Cells after Treatment with Mitomycin C (MMC) and a Pulsing Magnetic Field (PMF)

MMC concentration (μg/1.25×10^5 cells)	Number of colonies surviving (mean±SD n=6)	
	PMF-unexposed	PMF-exposed[a]
0	333±57.7	261±62.0
2×10^{-3}	275±52.3[b]	195±58.7[c]
2×10^{-2}	175±41.7[d]	104±42.8[e]
2×10^{-1}	8.5±2.2[f]	3.3±1.8[g]

MMC- and PMF-treated (for 1 h) cells (1×10^4) were plated on soft agar. After 14 days of incubation, colonies (more than 50 cells) on each plate were counted. Plating efficiency of the untreated control sample was 3.33±0.57%.
a) PMF conditions: frequency 250 Hz, pulse width 1.0 ms, mean magnetic flux density 40 gauss. $P<0.05$ *c* vs. *b* and *e* vs. *d*, $P<0.01$ *g* vs. *f*, respectively, by Student's *t* test.

Table IV. Colony-forming Efficiency of cKDH-8 Cells after Treatment with Mitomycin C (MMC) and a Pulsing Magnetic Field (PMF)

MMC concentration (μg/1.25×10^5 cells)	Number of colonies surviving (mean±SD n=3)	
	PMF-unexposed	PMF-exposed[a]
0	194±11.6	192±12.0
2×10^{-3}	178±13.6	164±12.8
2×10^{-2}	127±9.4[b]	92±9.4[c]
2×10^{-1}	33±11.0	32±16.6

MMC- and PMF-treated (for 1 h) cells (5×10^3) were plated on soft agar. After 14 days of incubation, colonies (more than 50 cells) on each plate were counted. Plating efficiency of untreated control sample was 3.89±0.23%.
a) PMF conditions: frequency 250 Hz, pulse width 1.0 ms, mean magnetic flux density 40 gauss. $P<0.01$ *c* vs. *b* by Student's *t* test.

lines of tumor cells syngeneic with WKA rats. PMF caused no significant change in the growth curve or colony-forming efficiency of either cell line (Figs. 6 and 7, Tables III and IV). In relation to these results, Liboff *et al.*[12] and Takahashi *et al.*[13] reported that incorporation of thymidine into cells was promoted by exposure to PMF, but they did not observe cell growth; their results are consistent with our current findings, because the phenomenon that incorporation of thymidine is promoted by exposure to PMF, which we also confirmed, is believed not to be associated with natural cell growth. Nevertheless, PMF potentiated the antitumor effect of MMC on both cell lines according to the results of the colony-forming efficiency study. In c-KMT-17 cells, the potentiation effect of the MMC/PMF combination was

appreciable at all MMC concentrations used; on the other hand, in c-KDH-8 cells the potentiation effect was appreciable at only one concentration (0.02 μg/1.25$\times$ 10^5 cells). Thus, in both tumors implanted into WKA rats the potentiation effect of the MMC/PMF combination was appreciable, but the degree of the effect on the two tumors was markedly different. This is considered to be due to the difference in antigenicity of the two tumors in the host rat.[7-9, 14, 15] The KMT-17 tumor is highly antigenic, so that residual live cells after treatment were destroyed by the host's immune response. On the other hand, the KDH-8 tumor has low antigenicity, so that residual live cells after treatment could survive and grow.

We had previously investigated the effect of a magnetic field on the incorporation of precursors into DNA and protein in human leukemia cells, and had found that a static magnetic field was not effective, but incorporation of the precursors was increased by PMF at more than 200 Hz. The magnetic conditions in our present experiment were based on these data. It is known that a time-variable magnetic field can induce eddy currents in the tissue according to Faraday's law of electromagnetic induction.[16] We need to examine basic aspects of PMF, including quantifying the eddy current, in order to find the most effective conditions for anti-tumor combination therapy.

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REFERENCES

1) Harn, G. M., Braun, J. and Har-Kedar, I. Thermochemotherapy: synergism between hyperthermia (42°C~43°C) and adriamycin (or bleomycin) mammalian cell inactivation. *Proc. Natl. Acad. Sci. USA*, **72**, 937–940 (1975).
2) Chang, B. K., Joines, W. T. and Gregory, J. A. Experimental method for the hyperthermic treatment of cells in tissue culture. *Bioelectromagnetics*, **8**, 195–202 (1987).
3) Lin, J. C. and Peterson, W. D. Cytological effects of 2450 MHz CW microwave radiation. *J. Bioeng.*, **1**, 471–478 (1977).
4) Livingston, G. K., Johnson, C. C. and Dethlefsen, L. A. Comparative effects of water-bath and microwave-induced hyperthermia on survival of Chinese hamster ovary (CHO) cells. *Radiol. Sci.*, **14**, 117–123 (1979).
5) Sapareto, S. A., Li, G. C., White, K. A., Harn, G. M., Vaguine, V. A., Giebeler, R. H. and Tanabe, E. Microwave cytotoxicity: lack of *in vitro* evidence for nonthermal effects at high power levels. *Radiat. Res.*, **89**, 124–133 (1982).
6) Batkin, S. and Tabrah, F. L. Effects of alternating magnetic fields (12 gauss) on transplanted neuroblastoma. *Res. Commun. Pathol. Pharmacol.*, **16**, 351–362 (1977).
7) Gotoda, E., Sendo, F., Hosokawa, M., Kodama, T. and Kobayashi, H. Combination of active and passive immunization and chemotherapy to transplantation of methylcholanthrene-induced tumor in WKA rats. *Cancer Res.*, **34**, 1947–1951 (1974).
8) Chiba, I., Oikawa, T., Naiki, M., Takimoto, M., Miyoshi, I., Mizuno, S., Yamashina, K., Yamagiwa, S. and Kobayashi, H. Enhanced immunogenicity of the cultured rat fibrosarcoma KMT-17 by cultivation in a low concentration of fetal calf serum. *Cancer Res.*, **47**, 1815–1819 (1987).
9) Kato, H. Immunological regression of metastatic tumor cells after surgical excision of primary tumor. *Hokkaido J. Med. Sci.*, **57**, 37–46 (1982) (in Japanese).
10) Omote, Y. An experimental attempt to potentiate therapeutic effects of combined use of pulsing magnetic fields and antitumor agents. *J. Jpn. Surg. Soc.*, **89**, 1155–1166 (1988) (in Japanese).
11) Hamburger, A. and Salmon, S. E. Primary bioassay of human myeloma stem cells. *J. Clin. Invest.*, **60**, 846–854 (1977).
12) Liboff, A. R., Williams, T., Strong, D. M. and Wister, R. Time-varying magnetic fields: effect on DNA synthesis. *Science*, **223**, 818–819 (1984).
13) Takahashi, K., Kaneko, I., Date, M. and Fukada, E. Effect of pulsing electromagnetic fields on DNA synthesis in mammalian cells in culture. *Experientia*, **42**, 185–186 (1986).
14) Yamaguchi, H., Moriuchi, T., Hosokawa, M. and Kobayashi, H. Increased or decreased immunogenicity of tumor-associated antigen according to the amount of virus-associated antigen in rat tumor cells infected with Friend virus. *Cancer Immunol. Immunother.*, **12**, 119–123 (1982).
15) Hosokawa, M., Okayasu, T., Ikeda, K., Kato, H., Suzuki, Y. and Kobayashi, H. Alteration of immunogenicity of xenogenized tumor cells in syngeneic rats by the immune responses to virus-associated antigens produced on immunizing cells. *Cancer Res.*, **43**, 2301–2305 (1983).
16) Ueno, S., Lövsund, P. and Öberg, P. A. Effects of alternating magnetic fields and low-frequency electric currents on human skin blood flow. *Med. Biol. Eng. Comput.*, **24**, 57–61 (1986).