Inhibition of Tumor Growth in Mice by Microwave Hyperthermia, Polyriboinosinic-Polyribocytidylic, and Mouse Interferon

STANISLAW SZMIGIELSKI, MARIAN BIELEC, MAREK JANIAK, MIROSLAW KOBUS, MIROSLAW LUCZAK, AND ERIK DE CLERCQ

Abstract—**Mice bearing sarcoma 180 tumors were irradiated by 3000-MHz microwaves at a power density of 40 mW/cm^2 for 2 h daily during 14 consecutive days. The irradiation resulted in an increase of rectal temperature of 3–4°C. Hyperthermal treatment was started on the second day after tumor-cell transplantation. Some of the animals also received daily injections of polyhiboinosinic acid-polyribocytidylecacid (poly I-poly C), 2 μg/g, or of mouse interferon (100 IU/g), or of both.**

Survival of the animals, mass of the tumors, incorporation of tritiated thymidine, uridine and glycine into the tumor tissue, and intracellular levels of cyclic AMP were determined.

Microwave hyperthermia resulted in a prolongation of the survival of tumor-bearing mice, a regression of tumors in 12 out of 24 mice, and a decreased incorporation of thymidine and glycine. The inhibitory effect of microwave hyperthermia was enhanced by simultaneous treatment with poly I-poly C and mouse interferon. Combination of microwave hyperthermia, and poly I-poly C and interferon treatment resulted in a regression of sarcoma 180 tumors in 16 out of 24 animals. No tumor regression was observed in the control group.

I. Introduction

ELEVATION of temperature above 42°C results in the inhibition of normal cell growth and metabolism, including inhibition of synthesis of nucleic acid, and protein [3], [31]. Cancer cells are significantly more sensitive to hyperthermia than normal cells from homologous tissues [16], [17], and general or local increase of temperature above 41°C provides a promising method for the selective killing of cancer cells [4], [5], [24]. Microwave irradiation offers a unique source of thermal energy for the induction of local and general hyperthermia [4], [17], [25], since this radiation penetrates biologic materials and leads to deep body heating [13].

More extensive destruction of cancer cells may be brought about by combining hyperthermia with cytostatics [14], ionizing irradiation [20], [21], or immunostimulating agents [27], [28]. As both interferon [9]–[12] and interferon inducers such as poly I-poly C [1], [2], [8], [15], [18], [19], [29], [32] have been reported to inhibit the growth of transplantable tumors in mice, it seemed worthwhile to combine these substances with microwave hyperthermia. In this report we present the results of our preliminary observations on the antineoplastic effects of microwave hyperthermia, interferon and poly I-poly C in mice bearing sarcoma 180 tumors.

Manuscript received July 12, 1977; revised January 12, 1978. This work was supported by Research Grant PR-6-210-02 from the Polish Governmental Program.

S. Szmigielski, M. Bielec, and M. Janiak are with the Center for Radiobiology and Radioprotection, 00-909 Warsaw, Poland.

M. Kobus and M. Luczak are with the Department of Medical Microbiology, University Medical School, Warsaw, Poland.

E. De Clercq is with the Rega Institute, University of Leuven, Belgium.

II. Material and Methods

Trypsinized sarcoma 180 cells were isolated from tumor-bearing CFW mice and were transplanted subcutaneously to adult CFW inbred mice, at 10^7 cells per mouse.

The tumor-bearing mice were divided into 8 groups of 24 animals each. Mice of the first group served as controls. Mice of the second group were subjected to microwave hyperthermia. The animals were irradiated with microwaves (3000 MHz) in an anechoic chamber in the far field [26]. The mean power density was 40 mW/cm^2. The mice were irradiated two hours daily from the second until the fifteenth day after transplantation. Rectal temperature was monitored by calibrated thermocouple (ELLAB, Copenhagen). Mice of the third group were daily injected intraperitoneally with poly I-poly C (2 μg/g) during the entire period of observation. Mice of the fourth group were daily injected intramuscularly with purified and concentrated mouse interferon [23] (100 IU/g) during the period of observation. For mice of groups 5–8, microwave hyperthermia was combined with poly I-poly C or with interferon treatment, or with both treatments.

In the first set of experiments, survival time was observed. In the second set of experiments, tumors were harvested during the sixteenth day after transplantation, and 6 h after an intraperitoneal injection of either ^{3}H-thymidine, ^{3}H-uridine, or ^{3}H-glycine at 1 μCi/g (6 mice per group). Mass of the tumors was determined and incorporation of the labeled precursors was measured with a liquid scintillation counter (ICN, Corumat 2700). The

0018-9480/78/0800-0520$00.75

levels of cyclic AMP in tumor tissues were analyzed by radioimmunoassay (Amersham cyclic AMP assay kit TRK 432) [7]. For measurement of the incorporation of labeled precursors the samples of tumorous tissue were homogenized and extracted with 6 percent trichloroacetic acid (TCA).

All experiments were performed in duplicate; a total of 384 animals were used. Mean value and standard deviation were calculated for each group; the significance of differences between means was evaluated by using t-student's test or chi^2 analysis (number of regressed tumors).

III. Results

The results are summarized in Figs. 1 and 2. Irradiation of tumor-bearing mice by microwaves at 40 mW/cm^2 (increase in rectal temperature, 3–4°C) resulted in prolonged survival (Fig. 1). On the twenty-eighth day after transplantation 8 of the 24 mice were still alive, while all control animals died before the twenty-sixth day. When microwave hyperthermia was combined with poly I-poly C treatment, 12 of the 24 animals were alive on the twenty-eighth day.

While no regression was noted in the control group, tumors regressed in 12 of the 24 animals submitted to microwave irradiation in 8 out of the 24 animals treated with poly I-poly C and in 6 out of the 24 animals treated with interferon (Fig. 1). The combination of microwave irradiation, poly I-poly C, and interferon resulted in regression of tumors in 16 out of the 24 animals. Tumors harvested on the sixteenth day after transplantation were significantly larger in control mice (mean = 1.36 g) than in mice exposed to hyperthermia (mean = 0.96 g) or to hyperthermia combined with poly I-poly C (mean = 0.81 g) or to hyperthermia combined with interferon (mean = 0.79 g) (Fig. 1). Again, combination of all three factors (microwave hyperthermia, poly I-poly C, and interferon treatment) resulted in the most pronounced reduction of tumor mass (mean = 0.57 g).

Surprisingly, the rate of incorporation of ^{3}H-thymidine, ^{3}H-uridine, or ^{3}H-glycine and the intracellular levels of cyclic AMP did not show a strict correlation with tumor mass or percentage of regressed tumors (Fig. 2). In general, a decreased incorporation of ^{3}H-thymidine and ^{3}H-glycine was observed in animals exposed to microwave hyperthermia alone or in combination with poly I-poly C and/or interferon treatment.

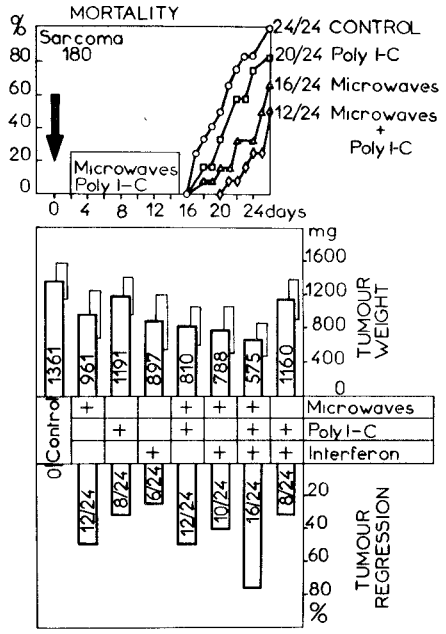

Fig. 1. Mortality, tumor mass (mean ±2 SD), and tumor regression in mice bearing sarcoma 180 tumors. The mice were treated with microwave hyperthermia, poly I-poly C and/or mouse interferon.

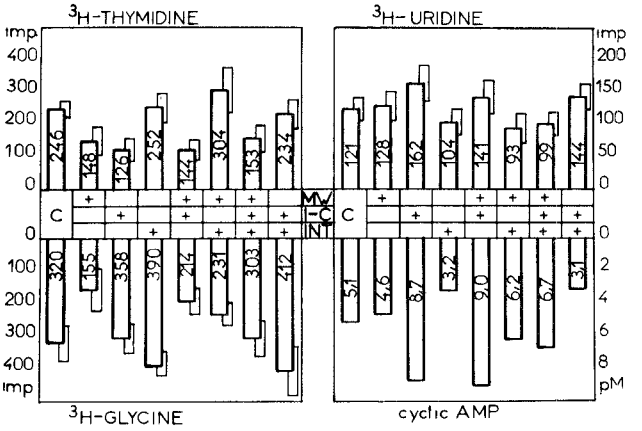

Fig. 2. Incorporation of ^{3}H-thymidine, ^{3}H-uridine, and ^{3}H-glycine and intracellular levels of cyclic AMP in tumor tissues harvested from mice 16 days after transplantation of sarcoma 180 tumor cells. All values are expressed in cpm impulses/(imp) 1 mg of freshly isolated tumor tissue. C: control; MW: microwave hyperthermia; I-C: injected with poly I-poly C; INT: injected with interferon. The results represent mean values (± SD) for 6 mice.

IV. Discussion

Whole-body microwave irradiation for 2 h daily over a period of 2 weeks caused a regression of sarcoma 180 tumors in 50 percent of the tumor-bearing mice. Concomitantly, survival of the animals was markedly prolonged. Similar observations have been reported by Dietzel [4] and Overgaard and Overgaard [17] for a variety of solid tumors.

The inhibitory effect of microwave hyperthermia may be enhanced by simultaneous treatment with interferon and interferon inducers such as poly I-poly C. Application of all three factors resulted in a regression of tumors in 66 percent of the mice. Further experiments are required to establish the mechanism of tumor regression caused by microwave hyperthermia, interferon, and poly I-poly C. Whether interferon (and interferon inducers) and microwave hyperthermia display any synergism in their modes of action also needs further elaboration.

The inhibitory effect of poly I-poly C on the development of experimental neoplasms is well-documented [1], [2], [8], [15], [18], [19], [29], [32]. Occasionally, enhancement of tumor growth by interferon inducers has been

reported [6]. The antitumor activity of poly I-poly C most probably depends on mechanisms other than interferon induction (see, e.g., [2] and [30]). Depending on the host-tumor system employed, poly I-poly C may owe its antitumor activity to a direct or macrophage-mediated cytotoxic action on the tumor cells [30]. In some systems, a potentiation of cell-mediated immunity should also be considered. It is noteworthy that immunostimulating agents such as streptolysin S or *Corynebacterium parvum* were found to enhance the tumor-inhibiting effects of general and local microwave hyperthermia [27], [28]. Streptolysin S and corynebacteria are known to stimulate the specific and nonspecific activity of macrophages and T-lymphocytes.

The antitumor effect of interferon is also well-documented [8]–[12]. Interferon preparations have been reported to inhibit the development of both primary solid malignant tumors and pulmonary metastases in mice [10]. However, the mechanism of action of interferon in tumor-bearing animals is poorly understood [12].

The sensitivity *in vitro* of tumor cells, e.g., mouse L-929 cells, to the cytotoxic action of double-stranded RNA's poly I-poly C is markedly enhanced if the cells have been treated with interferon before their exposure to the double-stranded RNA [22]. Such a mechanism may also operate *in vivo*. In addition, microwave hyperthermia might, through mechanisms yet to be resolved render tumor cells more sensitive to the direct cytotoxic action of interferon and poly I-poly C, thereby providing a plausible basis for synergism in the antineoplastic activities of hyperthermia and interferon or its inducers.

References

[1] R. S. Bart and A. W. Kopf, "Inhibition of the growth of murine malignant melanoma with synthetic double-stranded ribonucleic acid, *Nature*, vol. 224, pp. 372–373, 1969.

[2] E. De Clercq, "Effect of mouse interferon and polyriboinosinic acid-polyribocytidylic acid on L-cell tumor growth in nude mice. *Cancer Res.*, vol. 37, pp. 1502–1506, 1977.

[3] J. A. Dickson and H. D. Shah, "The effect of hyperthermia (42°C) on the biochemistry and growth of a malignant cell line," *Europ. J. Cancer*, vol. 8, pp. 561–571, 1972.

[4] F. Dietzel, *Tumor und Temperatur*. München: Urban u. Scharzenberg, 1975.

[5] F. Dietzel, W. Kern, and G. Klobe, "Hyperthermiebehandlung der maligner Tumoren," *Med. Klin.*, vol. 68, 182–186, 1973.

[6] A. F. Gazdar, "Enhancement of tumor growth rate by interferon inducers," *J. Nat. Cancer Inst.*, vol. 49, pp. 1435–1438, 1972.

[7] A. G. Gilman, "A protein binding assay for adenosine-3′-5′-monophosphate. *Proc. Nat. Acad. Sci. USA*, vol. 67, pp. 305–312, 1970.

[8] I. Gresser and C. Bourali, "Exogenous interferon and inducers of interferon in the treatment of BALB/c mice inoculated with RC 19 tumour cells," *Nature*, vol. 223, pp. 844–845, 1969.

[9] ——, "Antimtumor effects of interferon preparation in mice," *J. Natl. Cancer Inst.*, vol. 45, pp. 365–376, 1970.

[10] ——, "Inhibition by interferon preparations of a solid malignant tumour and pulmonary metastases in mice," *Nature New Biol.*, vol. 236, pp. 78–79, 1972.

[11] I. Gresser, C. Bourali, J. P. Lévy, D. Fontaine-Brouty-Boyé, and M. T. Thomas, "Increased survival in mice inoculated with tumor cells and treated with interferon preparations," *Proc. Nat. Acad. Sci. USA*, vol. 63, pp. 51–57, 1969.

[12] I. Gresser, C. Maury, and D. Brouty-Boyé, "Mechanism of the antitumor effect of interferon in mice," *Nature*, vol. 239, pp. 167–168, 1972.

[13] A. W. Guy, J. F. Lehmann, and J. B. Stonebridge, "Therapeutic application of electromagnetic power," *Proc. IEEE*, vol. 22, pp. 55–75, 1974.

[14] G. M. Hahn, J. Braun, and I. Har-Kedar, "Thermochemotherapy: Synergism between hyperthermia (42–43°C) and adriamycin (or bleomycin) in mammalian cell inactivation," *Proc. Nat. Acad. Sci USA*, vol. 72, pp. 937–940, 1975.

[15] H. B. Levy, L. W. Lax, and A. S. Rabson, "Inhibition of tumor growth by polyinosinic polycytidylic acid," *Proc. Nat. Acad. Sci. USA*, vol. 62, pp. 357–361, 1969.

[16] D. S. Muckle and J. A. Dickson, "The selective inhibitory effect of hyperthermia on the metabolism and growth of malignant cells," *Brit. J. Cancer*, vol. 25, pp. 771–778, 1971.

[17] K. Overgaard and J. Overgaard, "Investigations on the possibility of a thermic tumour therapy—Short wave treatment of a transplanted isologous mouse mammary carcinoma," *Europ. J. Cancer*, vol. 8, pp. 65–86, 1972.

[18] M. Potmesil and A. Goldfeder, "Inhibitory effect of polyinosinic: Polycytidylic acid on the growth of transplantable mouse mammary carcinoma," *Proc. Soc. Exp. Biol. Med.*, vol. 139, pp. 1392–1397, 1972.

[19] J. S. Rhim and R. J. Huebner, "Comparison of the antitumor effect of interferon and interferon inducers," *Proc. Soc. Exp. Biol. Med.*, vol. 136, pp. 524–529, 1971.

[20] J. E. Robinson, M. J. Wizenberg, and W. A. McCready, "Combined hyperthermia and radiation suggest an alternative to heavy particle therapy for reduced oxygen enhancement ratios," *Nature*, vol. 251, pp. 521–522, 1974.

[21] J. E. Robinson, M. J. Wizenberg, W. A. McCready, and E. Edelsack, "Tumour response to a three-fraction regimen combining hyperthermia and X-radiation," *Radiation Res.*, vol. 59, pp. 185–191, 1974.

[22] W. E. Stewart II, E. De Clercq, A. Billiau, J. Desmyter, and P. De Somer, "Increased susceptibility of cells treated with interferon to the toxicity of polyriboinosinic:polyribocytidylic acid," *Proc. Nat. Acad. Sci. USA*, vol. 69, pp. 1851–1854, 1972.

[23] W. E. Stewart II, E. De Clercq, and P. De Somer, "Antiviral and nonantiviral activity of highly purified interferon," *Nature New Biol.*, vol. 246, pp. 141–143, 1973.

[24] H. D. Suit and H. Shwayder, "Hyperthermia—Pontential as an antitumor agent," *Cancer*, vol. 34, 122–129, 1974.

[25] S. Szmigielski and M. Bielec, "Microwaves as a tool for intensive local hyperthermia in cancer treatment," Presented at *Proc. Int. Symp. Cancer Treatment by Hyperthermia*, Washington, Apr. 28–30 1975, American College of Radiology.

[26] S. Szmigielski, M. Luczak, and M. Wiranowska, "Effect of microwaves on cell function and virus replication in cell cultures irradiated in vitro," *Ann. N.Y. Acad. Sci.*, vol. 247, pp. 263–272, 1975.

[27] S. Szmigielski, M. Janiak, G. Pulverer, W. Hryniewicz, and J. Jesljaszewicz, "Local microwave hyperthermia (43°C) and stimulation of the macrophage and T-lymphocyte systems in treatment of Guérin epithelioma in rats," *Ztschr. Krebsforsch*, vol. 91, pp. 35–48, 1978.

[28] S. Szmigielski, G. Pulverer, W. Hryniewicz, and M. Janiak, "Inhibition of tumor growth in mice by microwave hyperthermia, streptolysin S and colcemide," *Special Suppl. Radio Sci*, vol. 12, pp. 185–189, 1977.

[29] D. Webb, W. Braun, and O. J. Plescia, "Antitumor effects of polynucleotides and theophylline," *Cancer Res.*, vol. 32, pp. 1814–1819, 1972.

[30] A. J. Weinstein, A. F. Gazdar, and H. L. Sims, "Lack of correlation between interferon induction and antitumor effect of poly I.poly C.," *Nature New Biol.*, vol. 231, pp. 53–54, 1971.

[31] A. Wüst, K. Norpoth, U. Witting, and W. Oberwittler, "Der in vitro Effekt der Hyperthermie auf den Einbau von Nukleinsaureverlaufern in Tumoren und normalen Gewebe," *Ztschr. Krebsforsch.*, vol. 79, pp. 193–201, 1973.

[32] L. D. Zeleznick and B. K. Bhuyan "Treatment of leukemic (L-1210) mice with double-stranded polyribonucleotides," *Proc. Soc. Exp. Biol. Med.*, vol. 130, pp. 126–128, 1969.