Local Microwave Hyperthermia (43°C) and Stimulation of the Macrophage and T-Lymphocyte Systems in Treatment of Guerin Epithelioma in Rats*

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Summary. Rats bearing Guerin epithelioma were exposed to 3 or 6 sessions of local microwave hyperthermia (LMwH) (2450 MHz, tumor temperature 43°C, time of exposition 45 min) on the 3rd or 5th week after implantation of tumor cells. In some of the animals LMwH was combined with the administration of immunopotentiating (IP) substances—purified Streptolysin S (SLS) (1 mg/kg b.w.) or Corynebacterium parvum (CP) (1 mg/kg b.w., intratumorally)—injected 3 times weekly on the 4th and 6th weeks after implantation of tumor cells. All the animals were immunized with bovine serum albumine (BSA) 2 and 3 weeks after tumor implantation. Size of tumors was measured each 4 days and the growth curves were analysed. Eight weeks after implantation all the animals were killed and the following tests were performed: weight of tumors, BSA antibodies titre (passive hemagglutination test using BSA-glutaraldehyde coupled sheep red blood cells), 3H-leucine and 3H-uridine incorporation to phytohemagglutinin (PHA)-stimulated spleen lymphocytes and to mixed tumor cell-lymphocyte cultures, cytotoxicity of isolated spleen lymphocytes and peritoneal macrophages to 51Cr-labeled primary cultures of Guerin tumor cells, lysozyme (muramidase) activity in blood serum.

LMwH inhibited growth of Guerin tumors and the effect was enhanced by combined treatment with IP substances. Combined application of LMwH and CP resulted in complete disappearance of 9 of 12 tumors, while LMwH and IP applied separately led to eradication of 2–5 tumors of 12.

LMwH stimulated reactivity of the T-lymphocyte and macrophage systems enhancing both non-specific (increased production of BSA antibodies, high reactivity of spleen lymphocytes to PHA, increased serum lysozyme levels) and specific (increased cytotoxicity of lymphocytes and macrophages to cultured tumor cells and higher reactivity of lymphocytes to mitomycin-inhibited tumor cells) immune responses.

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The role of immunostimulation by LMwH is stressed as an important factor in tumor-inhibiting effect of hyperthermia in addition to the well known selective heat killing of neoplastic cells.

**Key words:** Microwaves, hyperthermia, immunopotentiation, cancer therapy, immunotherapy

Many reports have been recently published on high sensitivity of neoplastic cells to elevated temperatures (40–44 °C) and on the use of general and local hyperthermia in cancer treatment both in experimental animals and in human patients with the hope for selective heat killing of tumor cells (for the most recent reviews, see Bronk, 1976; Dietzel, 1975; Har-Kedar and Bleezen, 1976; Leith et al., 1977; Szmigielski and Bielec, 1976; Thrall et al., 1976, Wizenberg and Robinson, 1976). Inhibition of RNA and protein synthesis, injury of lysosomes and cell membrane damage were found in neoplastic cells exposed to intensive hyperthermia (42–44 °C), nevertheless the mechanisms of action and the sequence of metabolic and functional changes in the heated cells are not known in detail. Still less is known about the reaction of tumor bearing host to hyperthermia (Dickson, 1976; Dickson and Calderwood, 1976; Dickson and Muckle, 1976). Physiologic tolerance of mammalian organisms limits the use of intensive hyperthermia only to local heating of solid tumors (Dietzel, 1975) and for these aims the best sources of heat energy seem to be microwave radiation (Guy, 1976; Guy et al., 1974) and ultrasound. Irradiation of solid tumors in mice with microwaves results in inhibition of tumor growth (Dietzel, 1975; Overgaard, 1976; Overgaard and Overgaard, 1976) and under certain conditions total cure may be observed (Mendecki et al., 1976). However, Marmor et al. (1977) on the base of well controlled studies of cell survival after localized heating of tumors with radiofrequency electromagnetic radiation demonstrated that cell killing by elevated temperatures, although being an important factor, cannot account for eradication of tumor mass and other mechanisms, presumably resulting from the host's response to tumor heating, must be involved.

Possible use of local microwave hyperthermia (LMwH) in the near future as tumor inhibiting factor in human patients (Kramer, 1976; Leith et al., 1977) stresses the urgent need for better understanding of the mechanisms of its action in vivo and for developing the most promising combinations of hyperthermia with other forms of cancer therapy.

The aim of this work was to study the effect of LMwH combined with immunopotentiating (IP) substances presently used in cancer therapy (Mathe, 1976; Scott, 1974; 1975) on growth of Guerin epithelioma and reactivity of the immunologic systems in rats, as virtually nothing is known about the reaction of these systems to intensive hyperthermia in vivo (Dickson, 1976; Kramer, 1976; Leith et al., 1977; Marmor et al., 1977; Suit, 1976).

**Material and Methods**

1. **Experimental**

Inbred, 3 months old male albino WA/F 66 Wistar rats were used for all experiments. Guerin epithelioma was implanted subcutaneously in the intrascapular region (10⁷ viable cells in 0.25 ml of