PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 169, 413–415 (1982)

Antitumor Effect of Combined Interferon and Hyperthermia in Mice (41367)

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Abstract. Combined therapy consisting of either exogenous interferon or interferon inducers (polyinosinic–polycytidylic acid or Newcastle disease virus) together with local hyperthermia exerted a greater antitumor effect in C57Bl/6 mice bearing the transplantable Lewis lung carcinoma than either treatment alone.

Interferon and interferon inducers exhibit an antitumor activity in a number of experimental tumor systems in mice (reviewed in (1, 2)). It has been observed that a combination of different antitumor agents often provides a greater antitumor effect than either agent alone, i.e., a combination of interferon with chemotherapy (3, 4), or chemotherapy or X irradiation with hyperthermia (5–9). There is also one report suggesting that combined therapy of interferon or polyriboinosinic–polyribocytidylic acid (poly(I)·poly(C)) with microwaves was more effective than microwave therapy alone (10). It was therefore of interest to determine the antitumor effect of exogenous interferon and inducers of endogenous interferon in combination with local hyperthermia. The results of experiments reported herein show that such combined therapy exerted a greater antitumor effect than either treatment alone in C57Bl/6 mice bearing the transplantable Lewis lung carcinoma (3LL).

Materials and Methods. *Mice.* Male and female C57Bl/6J mice were obtained from a colony at the Weizmann Institute and were 3–6 months old at time of experimentation.

Tumor cells. The Lewis lung carcinoma (11) was maintained by serial transplantation of tumor cells in C57Bl/6J mice. Tumor cells (2×10^5) in 0.2 ml were injected intramuscularly in the left hind leg.

Interferon and control preparations. Mouse interferon was prepared from C-243 cells induced with Newcastle disease virus. The methods of production, concentration, and assay of the interferon preparations have been described previously (12). The control preparation was prepared in a manner identical to that for interferon with the exception that the viral inducer was omitted.

Interferon inducers. Newcastle disease virus (NDV), Herts strain, was propagated in 10-day-old embryonated chicken eggs. Multiplicity of infection was calculated on the basis of NDV infectivity in $TCID_{50}$/ml in primary chick embryo fibroblast cultures. Poly(I)·poly(C) was prepared by mixing equimolar concentrations of poly(I) and poly(C) (purchased from Boehringer GmbH, Mannheim, West Germany) at room temperature in phosphate-buffered saline. Polymerization was checked by the development of hypochromicity using a Beckman spectrophotometer.

Heating system and temperature measurements. The heating system consisted of (a) a hot air blower; (b) a heat-insulated cylinder (diameter 10.5 cm, length 24 cm); and (c) a temperature control unit as previously described (5). For local heating the tumor-carrying limbs of four mice were inserted into the four holes drilled in the upper part of the cylinder. Hot air was blown in through an insulated pipe connecting the blower with the cylinder (5). The hot air atmosphere was achieved by passing the hot air through an attenuator to reduce turbulence. Homogeneity was achieved and confirmed by a system of valves and thermocouples installed for temperature stability and control. Simultaneous temperature measurements were made in the cylinder, in the tumor (to specify intratumor temperature), and in the rectum of the mouse with a PT-6 ther-

0037-9727/82/030413-03$01.00/0

TABLE I. EFFECT OF POLY(I)·POLY(C) OR NDV WITH LOCAL HYPERTHERMIA (LH) ON SURVIVAL OF C57Bl/6 MICE BEARING THE 3LL TUMOR

Expt. No.	Treatment: Poly(I)·poly(C)	Treatment: NDV	Treatment: LH	No. of mice	Mean survival ± SE (days)	
1[a]	−	−	−	9	13.9 ± 0.3	NS
	−	−	+	10	13.5 ± 0.3	*
	−	+	−	11	15.1 ± 0.3	NS
	−	+	+	11	16.2 ± 0.6	
	+	−	−	11	15.2 ± 0.4	*
	+	−	+	11	17.4 ± 0.5	
2[b]	−	−	−	11	14.2 ± 0.3	NS
	−	−	+	10	13.3 ± 0.3	
	−	+	−	11	14.0 ± 0.6	**
	−	+	+	11	17.3 ± 0.6	

[a] In experiment 1, 0.2 ml of poly(I)·poly(C), 500 μg/ml, and NDV (10^8 $TCID_{50}$/ml) was injected ip on Days 7 and 14 after implantation of tumor. Six hours after each treatment the tumor-bearing leg was heated to 42.7° (intratumor temperature) for 1 hr.

[b] In experiment 2, 0.2 ml of NDV (10^8 $TCID_{50}$/ml) was injected 7 days after tumor inoculation, and 6 hr thereafter the tumor-bearing leg was heated to 42.7° (intratumor temperature) for 1 hr.

* $P \leq 0.01$.

** $P \leq 0.001$; NS, not significant.

mocouple (Bailey). Intratumor temperature was determined with fine-gauge Teflon-coated copper–constantan thermocouples (Type IT-1, Bailey). The leads from the installed thermocouples were connected to an electronic control unit which included a continuous digital temperature readout and an XY recorder.

Results. Local hyperthermia of the 3LL tumor-bearing limb of C57Bl/6 mice applied after tumor implantation did not modify survival time (Tables I and II). Treatment of tumor-bearing mice with the interferon inducers poly(I)·(C) and NDV afforded a slight but significant increase in survival (Expt. 1, Table I). This effect was increased by combined local hyperthermia for poly(I)·(C) in experiment 1 (Table I) and for NDV in experiment 2 (Table I).

As can be seen in Table II, daily injection of mouse interferon (but not a control preparation) resulted in a clearcut increase in

TABLE II. EFFECT OF INTERFERON AND LOCAL HYPERTHERMIA (LH) ON SURVIVAL OF C57Bl/6J MICE BEARING THE 3LL TUMOR

Treatment[a]: Interferon	Treatment[a]: Control preparation	Treatment[a]: LH	No. of mice	Mean survival ± SE (days)	
−	−	−	10	19.4 ± 0.5	NS
−	−	+	9	18.7 ± 0.5	
−	+	−	10	17.7 ± 1.3	***
−	+	+	10	18.3 ± 0.9	
+	−	−	9	25.2 ± 0.5	*
+	−	+	10	27.0 ± 0.5	

[a] Partially purified interferon (titer 3.2×10^{-6} /ml) and control preparation (titer 2×10^{-1}) injected daily (0.25 ml ip) from −1 day to 8 days after tumor inoculation. Local hyperthermia (42.7° for 1 hr) was applied to tumor-bearing leg 2, 5, and 7 days after tumor implantation.

* $P < 0.05$.

*** $P < 10^{-9}$; NS, not significant.

survival time (in accord with previous results (13)), and this effect was increased by local hyperthermia treatment.

Discussion. Local hyperthermia alone did not exert any beneficial effect on mice bearing the 3LL tumor in these or in our previous experiments (8). However, when this treatment was associated with either exogenous or endogenous interferon therapy an increase in mouse survival time was observed, relative to either treatment alone. Although the mechanism of this effect is unknown, some experimental data suggest that the inhibitory effect of interferon on viral multiplication and cell division (determined *in vitro*) is enhanced at elevated temperatures (14–16).

Although the differences between the different groups in our experiments were small, we believe that these results are of interest since they may provide an insight into the mechanism of action of antitumor drugs or biologic substances such as interferon as well as suggesting possible approaches to antitumor therapy in man.

This work was supported in part by grants from the Gulton Foundation, Englewood, New Jersey, DRET (Contracts 78-34-210 and 80-34-522), and the Fondation pour la Recherche Médicale Française.

1. Gresser I. Antitumor effects of interferon. In: Becker F, ed. Cancer—A Comprehensive Treatise. New York, Plenum, Vol 5:p521, 1977.
2. Gresser I, Tovey MG. Antitumor effects of interferon. Biochim Biophys Acta **516**:231–247, 1978.
3. Chirigos MA, Pearson JW. Cure of murine leukemia with drug and interferon treatment. J Nat Cancer Inst **51**:1367–1368, 1973.
4. Gresser I, Maury C, Tovey MG. Efficacy of combined interferon cyclophosphamide therapy after diagnosis of lymphoma in AKR mice. Eur J Cancer **14**:97–99, 1978.
5. Yerushalmi A. Treatment of a solid tumor by local simultaneous hyperthermia and ionizing radiation: Dependence on temperature and dose. Eur. J. Cancer **12**:807–813, 1976.
6. Yerushalmi A. Cure of a solid tumor by simultaneous administration of microwaves and X-ray irradiation. Rad Res **64**:602–610, 1975.
7. Yerushalmi A. Combined treatment of a solid tumor by local hyperthermia and actinomycin D. Brit J Cancer **37**:827–832, 1978.
8. Yerushalmi A, Hazan G. Control of Lewis lung carcinoma by combined treatment with local hyperthermia and cyclophosphamide: Preliminary results. Israel J Med Sci **15**:462–463, 1979.
9. Yatvin MB, Clifton KH, Dennis WH. Hyperthermia and local anesthetics: Potentiation of survival of tumor-bearing mice. Science **205**: 195–196, 1979.
10. Szmigielski S, Luczak M, Bielec M, Janiak M, Kobus M, Stewart II WE, De Clercq E. Effect of microwaves combined with interferon and or interferon inducers (Poly I-poly C) on development of sarcoma 180 in mice. J Microwave Power **11**:174–175, 1976.
11. Sugiura K, Stock CC. Studies in a tumor spectrum. III. The effect of phosphoramides on the growth of a variety of mouse and rat tumors. Cancer Res **15**:31–38, 1955.
12. Tovey MG, Begon-Lours J, Gresser I. A method for the large scale production of potent interferon preparations. Proc Soc Exp Biol Med **146**:809–815, 1974.
13. Gresser I, Bourali-Maury C. Inhibition by interferon preparations of a solid malignant tumour and pulmonary metastases in mice. Nature New Biol **236**:78–79, 1972.
14. Gresser I, Brouty-Boye D, Thomas M.-T, Macieira-Coelho A. Interferon and cell division. II. Influence of various experimental conditions on the inhibition of L1210 cell multiplication in vitro by interferon preparations. J Nat Cancer Inst **45**: 1145–1153, 1970.
15. Ito Y, Nishiyama Y, Shimokata K, Nagata I, Kunii A. Temperature sensitivity of interferon susceptibility in L cells persistently infected with hemagglutinating virus of Japan (HVJ). Virology **89**:342–345, 1978.
16. Heron I, Berg K. The actions of interferon are potentiated at elevated temperature. Nature (*London*) **274**:508–510, 1978.

Received June 16, 1981. P.S.E.B.M. 1982, Vol. 169.