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Effects of Tumor Necrosis Factor and Hyperthermia on Meth-A Tumors

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The combined effects of purified human natural tumor necrosis factor (TNF) and hyperthermia were investigated in a transplanted TNF-sensitive Meth-A tumor model. We assessed the sequence and interval for the two treatments, the temperature that caused maximal heat sensitization, and the effects of pH modification on this combination therapy. Tumor response was evaluated by means of a tumor growth delay assay. TNF at a dose of 50 JRU/g caused significant tumor growth delay. A synergistic effect of TNF and hyperthermia was observed when TNF was administered 10 min before heating. This thermal enhancement of the action of TNF became more prominent with an increase in the heating temperature. Tumor growth delay was maximal when TNF was given immediately before or after hyperthermia. However, after an increase in the time interval to more than 2 h, there was no enhancement of growth delay. Injection of glucose (5 g/kg) caused a significant fall in pH at 10 and 30 min after administration. Further enhancement in tumor growth delay was seen with the trimodality of glucose, TNF, and heat compared to combined treatment with heat and either TNF or glucose at a hyperthermia of 42°C. This effect was not obtained with heating to 40°C. TNF appears to be a potent heat sensitizer when an appropriate temperature and time interval between hyperthermia and TNF administration are used. Trimodality treatment with hyperthermia, TNF, and glucose may be a new method of anticancer therapy.

Key words: Tumor necrosis factor — Hyperthermia — Meth-A tumor — Glucose

Numerous experimental studies[1,2] have demonstrated synergism between hyperthermia and radiation or various chemotherapeutic agents in their cytotoxic effect. Recent reports[3–5] indicate that this synergism also exists between hyperthermia and tumor necrosis factor (TNF) both *in vitro* and *in vivo*, which raises the possibility of the clinical use of this combined modality of treatment. The important issues that need to be clarified before clinical application include the sequence and interval for applying heat and TNF, the heating temperatures causing maximal heat sensitization, and the effects of pH modification on the synergism between heat and TNF, none of which has yet been reported. We accordingly investigated these effects in a murine transplanted tumor model.

MATERIALS AND METHODS

The right thighs of Balb/c mice were subcutaneously inoculated with 2×10^5 viable Meth-A tumor cells and the tumors were treated when the mean diameter had reached 8–10 mm. Hyperthermia was applied for 30 min by immersion of the tumor-bearing leg in a water bath (Toyo Seisakusho Co. Ltd., model ET-45P) which was maintained at the desired temperature. The accuracy of the water bath temperature was within 0.05°C. Intratumor temperatures were 0.3°C lower than the water bath temperature within 2 min after the mice were immersed. All temperatures mentioned in this paper refer to the intratumor temperatures.

TNF was produced from a B cell acute lymphatic leukemia line (BALL-1 cells) sensitized with hemagglutinating virus of Japan (HVJ), and was kindly supplied by Mochida Pharmaceutical Co. Ltd. (Tokyo). TNF at doses of 10–50 JRU/g was administered via the tail vein.

Glucose was given by the intraperitoneal injection of 0.01 ml/g of dextrose solution (Otsuka Pharmaceutical Co. Ltd., Tokyo). The tumor response was evaluated by means of a tumor growth delay assay. Three diameters (*a, b, c*) of each tumor were measured every two days after treatment, by using a caliper, and the tumor volume was calculated as an ellipsoid using the formula $\pi abc/6$. The time required for treated tumors to grow to 3 times their initial volume (growth time) was determined.

The pH of the Meth-A tumors was measured using miniature capillary glass electrodes. The pH electrode was manually inserted into the tumor center and the reference electrode was placed in contact with the mouse tail using electrocardiogram electrode cream. Animals were anesthetized intraperitoneally with sodium pentobarbital, 67.5 mg/kg, and subjected to treatment. An additional small dose of anesthetic was given if needed. Each treatment group consisted of at least 12 animals unless otherwise stated. The data points shown in this paper represent the mean values, and the standard errors are given.

RESULTS

Figure 1 shows the growth delay times for tumors treated with TNF alone or with TNF plus hyperthermia at 40, 42 and 44°C. TNF was administered 10 min before the start of heating. The delay time was significantly prolonged with TNF alone at a dose of 50 JRU/g compared to that with no treatment. When TNF was administered before heating, tumor growth was delayed more than with heating alone, indicating thermal sensitization. This thermal enhancement by TNF was more prominent with an increase in heating temperature. Tumor eradication occurred in 25 and 17% of the tumors treated by hyperthermia at 44°C in combination with 30 and 40 JRU/g of TNF, respectively, while no such response was obtained to 44°C hearting alone.

TNF (30 JRU/g) was given at various time intervals (0, 0.5, 1, 2, 6, 12, 24, 48 and 72 h) before and after hyperthermia (42°C for 30 min) to determine the optimal sequence and time interval for the two treatments as shown in Fig. 2. Tumor growth delay time was maximal when TNF was administered immediately before or after hearting. With an increase in the time interval to more than 2 h, no enhancement of growth delay was observed for the combination of TNF and hyperthermia.

The pH of control tumors was 6.91±0.04. Injection of TNF at a dose of 30 JRU/g into mice resulted in a pH drop in the Meth-A tumors. The mean pH declined by

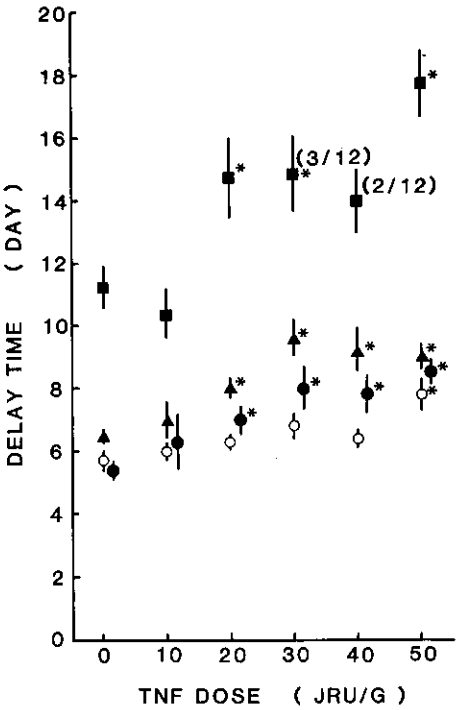

Fig. 1. Tumor growth delay time as a function of TNF dose for tumors treated with hyperthermia at 37 (○), 40 (●), 42 (▲) and 44°C (■). Bars shown indicate the mean±SD. An asterisk indicates a statistically significantly different ($P<0.05$) delay time from that of similar treatment without TNF.

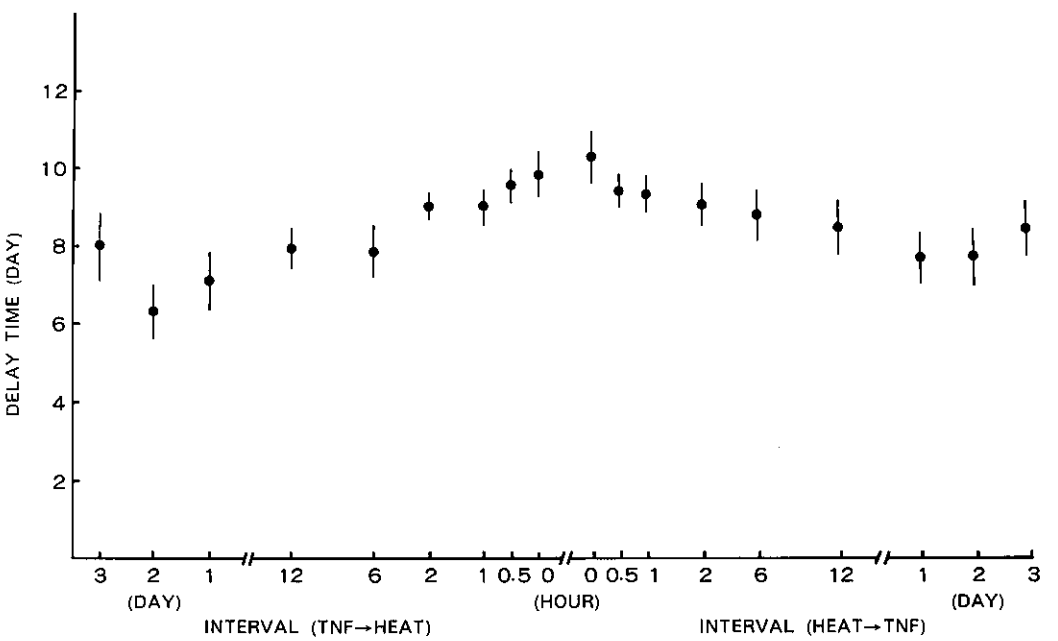

Fig. 2. Tumor growth delay time as a function of the time interval between TNF administration (30 JRU/g) and hyperthermia (42°C, 30 min). Bars shown indicate the mean±SD.

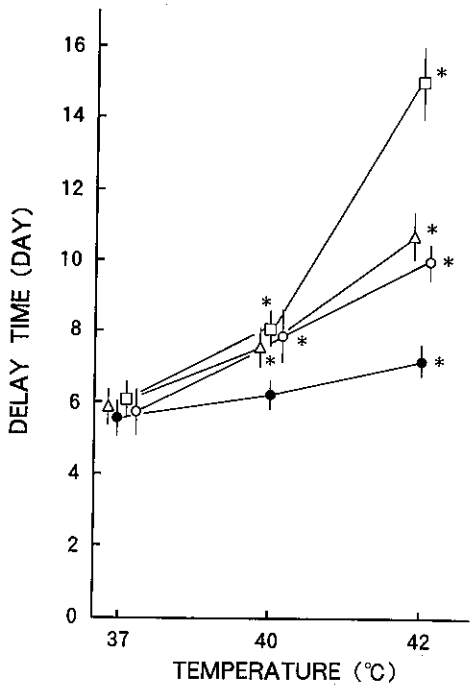

Fig. 3. Tumor growth delay time as a function of heating temperature for tumors treated with hyperthermia alone (●), hyperthermia plus glucose (○), hyperthermia plus TNF (△) and the trimodality of hyperthermia, glucose and TNF (□). An asterisk indicates a statistically significantly different ($P<0.05$) delay time from that of the treatment at 37°C.

0.07 ± 0.03 and 0.12 ± 0.05, respectively, at 10 and 30 min after TNF injection. The value 30 min after injection was statistically significantly different ($P<0.05$) from that of the control. Figure 3 shows the combined effects of TNF, heat, and glucose assessed by the tumor growth delay assay. Glucose (5 mg/g) and TNF (30 JRU/g) were given 30 and 10 min before the start of hyperthermia, respectively. Tumor growth was significantly delayed by combined treatment with glucose or TNF plus hyperthermia at 42°C when compared to hyperthermia alone. This effect was remarkably increased by the trimodality of heat, glucose and TNF. No significant increase in growth delay was demonstrated when hyperthermia was performed at 40°C.

DISCUSSION

A synergistic effect of hyperthermia and TNF has been previously shown both *in vitro* and *in vivo*. The present study demonstrated that this combined effect depends greatly on the heating temperature achieved and the time interval between heat and TNF administration. The effect was increased by an increase in heating temperature, and hyperthermia at 44°C with 30 or 40 JRU/g of TNF caused a substantial rate of tumor eradication in addition to a remarkable increase in the growth delay time. On the other hand, the combined effect was minimal with heating at 40°C, which is not in agreement with a previous report.[4] This discrepancy may be due to differences in the tumor size, the dose of TNF and the origin of the TNF used. It was also shown that the synergistic effects were maximal when TNF was given immediately before or after heating, and synergism was not apparent after an increase in the time interval between hyperthermia and TNF administration to over 2 h. These findings should be useful in designing clinical regimens for combined treatment with hyperthermia and TNF.

Several mechanisms explaining the synergistic effects of TNF and heat have been proposed, including increased turnover of the TNF-receptor complex[3] and TNF-induced accumulation of cells in the S-phase.[5] The present study showed a substantial decrease in intratumor pH following TNF administration. Since the sensitivity of cells to heat is increased under low pH conditions, intratumoral pH reduction might be involved in the thermal enhancement achieved by TNF.

Glucose is well-known as a potent modifier of tumor pH.[6,7] The fact that the combined effect of heat and TNF was enhanced further by the addition of glucose also suggests that intratumoral pH reduction may have a role in the enhanced cellular response to heat following TNF administration. It is noteworthy that the combination effect of glucose was apparent with treatment at 42°C for 30 min, which is clinically applicable. Glucose has been clinically applied as an adjuvant to hyperthermia,[8] and trials have demonstrated a substantial decrease in pH in human tumors without any serious complications of hyperglycemia. The problem is that the dose of glucose used in this study is appreciably higher than that of those clinical trials. Nonetheless, trimodal therapy with hyperthermia, TNF, and glucose administration could possibly achieve eventual clinical application.

In conclusion, TNF seems to be a potent heat sensitizer provided that the temperature, the time interval between heating and TNF injection, and the dose of TNF are appropriately designed. The synergism of heat and TNF was enhanced further by glucose administration, which suggests that the clinical use of trimodal therapy might be worthwhile.

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