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Efficacy of Hyperthermia and Polyunsaturated Fatty Acids on Experimental Carcinoma

Satoshi Kokura, Toshikazu Yoshikawa,[1] Toshiro Kaneko, Shoji Iinuma, Shunichiro Nishimura, Kiichi Matsuyama, Yuji Naito, Norimasa Yoshida, and Motoharu Kondo

First Department of Internal Medicine, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602, Japan

ABSTRACT

We investigated the efficacy of hyperthermia and γ-linolenic acid on experimental carcinoma. This study focused on polyunsaturated fatty acids that are substrates for free radical reactions. Oleic acid, linolenic acid, α-linolenic acid, or γ-linolenic acid was injected into the arteries feeding AH109A carcinoma implanted into rat hind limbs. Among these, γ-linolenic acid had the greatest effect on tumor tissue lipid peroxidation and demonstrated an antitumor effect. Consequently, γ-linolenic acid injection into the feeding artery of a tumor was performed immediately prior to hyperthermia. This combination therapy induced a high level of lipid peroxidation in tumor tissue and a significant antitumor effect. Hyperthermia combined with γ-linolenic acid produces free radical reactions by increasing the radical reaction substrate and may be an effective anticancer modality.

INTRODUCTION

We have demonstrated previously that free radical reactions and lipid peroxidation are important antitumor mechanisms of hyperthermic therapy (1). We then began aggressive efforts to develop new methods of cancer treatment using free radical reactions. In certain tissues, ischemia followed by reperfusion results in the formation of oxygen radicals (ischemia-reperfusion injury; Ref. 2). We demonstrated experimentally that this type of injury may occur in cancer tissue (3). In addition, when hypoxanthine and xanthine oxidase interact *in vitro,* a superoxide forms (4). When the same process occurs in cancer-bearing animals, the superoxide reduces the extent of the cancer (5), presumably through cancer cell destruction or apoptosis (6). However, because the free radical reaction is nonspecific, adverse effects may pose a problem for clinical application of reperfusion injury and superoxide production from hypoxanthine by xanthine oxidase. This study examined the unsaturated fatty acids that are the most important substrates for free radical reactions; we investigated the efficacy of cancer treatment using hyperthermia to trigger free radical reactions after these unsaturated fatty acids were introduced into cancer tissue.

MATERIALS AND METHODS

Experimental Animals and Tumors. We used 6-week-old male Donryu rats. Rat AH109A carcinoma was first transplanted into the peritoneum of the Donryu rats. Ascites containing 1×10^7 carcinoma cells were then s.c. grafted into the left hind limb of the rat. Rats were treated 7 days after implantation.

Administration of Unsaturated Fatty Acids. Four mg/kg oleic acid (18:1, n-9), linoleic acid (18:2, n-6), α-linolenic acid (18:3, n-3), or γ-linolenic acid (18:3, n-6) emulsified in 1 ml of physiological saline were administered intraarterially to the tumor via the left femoral artery. Polyunsaturated fatty acids were purchased from Sigma Chemical Co. (St. Louis, MO). As a control, 1 ml of physiological saline was administered in place of unsaturated fatty acids.

Hyperthermia. A RF[2] dielectric heater, the Thermotron RF I.V. (Yamamoto Vinyter Co., Osaka, Japan), which uses an 8-MHz dielectric system, was used. AH109A carcinoma implanted into the hind limb was locally heated using the RF I.V. During hyperthermia, rats were anesthetized by i.p. injection of 35 mg/kg of sodium pentobarbital. The tumor tissue temperature was monitored with a thermosensor (Yamamoto Vinyter Co.). The RF output was initially 20 W. After reaching 43°C, the output was adjusted to maintain a constant temperature. Heating was continued for 15 min.

Hyperthermia Combined with γ-Linolenic Acid. γ-Linolenic acid (4 mg/kg) emulsified in 1 ml of physiological saline was administered intraarterially to the tumor via the left femoral artery immediately prior to hyperthermia.

Evaluation of Tumor Growth. Seven days after hyperthermia, the major and minor axes of the tumors were measured, and volume was calculated with the following formula (7):

$$V = (\text{Longest diameter}) \times (\text{Shortest diameter})^2 \times 1/2$$

TBARS. The concentration of TBARS, an index of lipid peroxidation, was measured in tumor tissue using the method of Ohkawa *et al.* (8). In brief, animals from each treatment group were killed 60 min after treatment. Animals were killed by exsanguination via the abdominal aorta under i.v. sodium pentobarbiturate anesthesia (35 mg/kg). Tumor tissues were removed and homogenized with 1.5 ml of 10 mM potassium phosphate buffer (pH 7.8) containing 30 mM KCl in a Teflon Potter-Elvehjem homogenizer. The level of TBARS in the homogenates was expressed as nanomoles of malondialdehyde per mg protein using 1,1,3,3-tetramethoxypropane as the standard. Total protein in the homogenates was measured by the method of Lowry *et al.* (9).

α-Tocopherol. α-Tocopherol in the tumor tissue was measured by the method of Abe *et al.* (10).

Statistical Analysis. Results are presented as means ± SE for five to eight rats/group. Kruskal-Wallis analysis was used to determine variances. The two-tailed nonparametric Dunnett's test was used for comparison of group means. A level of $P < 0.05$ was accepted as statistically significant.

RESULTS

Tumor Tissue TBARS after Intraarterial Injection of Polyunsaturated Fatty Acids. The concentration of TBARS was measured in the tumor tissue 1 h after intraarterial injection of each polyunsaturated fatty acid. No significant difference in TBARS was seen compared with control (Fig. 1).

α-Tocopherol after Intraarterial Injection of Polyunsaturated Fatty Acids. Levels of α-tocopherol were similarly measured in tumor tissue 1 h after intraarterial injection of each polyunsaturated fatty acid. Compared to the control group (15.5 ± 1.34 μg/mg protein), a significant decrease was observed in the group treated with γ-linolenic acid (8.34 ± 1.47 μg/mg protein; Fig. 2).

Antitumor Effect of Polyunsaturated Fatty Acids. Seven days after treatment, the tumor volume of the control group was 5.89 ± 0.61 cm^3. The groups treated with oleic acid, linoleic acid, α-linolenic acid, and γ-linolenic acid had tumor volumes of 4.62 ± 0.67 cm^3, 4.97 ± 0.38 cm^3, 2.74 ± 0.59 cm^3, and 2.99 ± 0.41 cm^3, respectively. Tumor growth was significantly inhibited only by α-linolenic acid and γ-linolenic acid compared with control (Fig. 3).

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[1] To whom requests for reprints should be addressed. Phone: 81-75-251-5505; Fax: 81-75-252-3721.

[2] The abbreviations used are: RF, radiofrequency; TBARS, thiobarbituric acid-reactive substances.

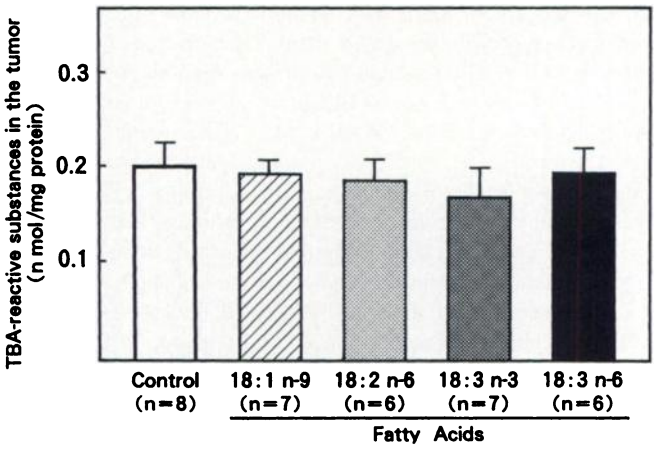

Fig. 1. TBARS in tumor tissue 1 h after intraarterial injection of polyunsaturated fatty acids. Results are expressed as means; *bars*, SE.

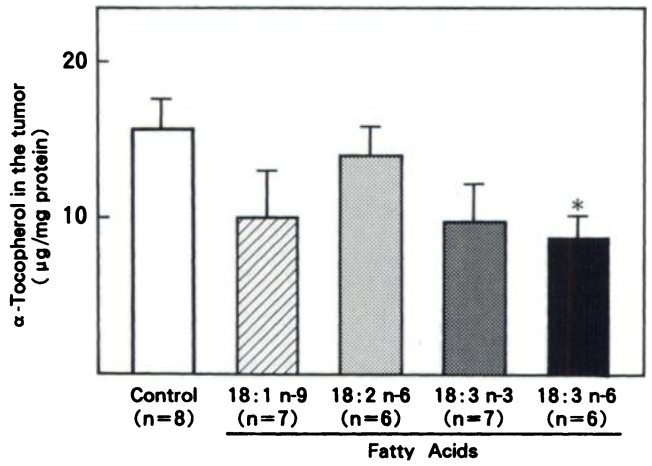

Fig. 2. α-Tocopherol in tumor tissue 1 h after intraarterial injection of polyunsaturated fatty acids. Results are expressed as means; *bars*, SE. *, $P < 0.02$ compared with control.

Time Course Changes in TBARS after Hyperthermia and Polyunsaturated Fatty Acid. Hyperthermia was combined with injection of γ-linolenic acid, which was the only polyunsaturated fatty acid to show both an antitumor effect and reduced α-tocopherol. TBARS were measured prior to treatment and at 1 and 48 h after treatment. There was 0.076 ± 0.010 nmol/mg protein of TBARS in the tumor tissue prior to treatment. After 48 h, the treatment groups of hyperthermia alone, γ-linolenic acid alone, and hyperthermia combined with γ-linolenic acid had 0.106 ± 0.004 nmol/mg protein, 0.126 ± 0.026 nmol/mg protein, and 0.252 ± 0.034 nmol/mg protein of TBARS, respectively. Posttreatment sequential measurements of tumor tissue TBARS demonstrated that only the combined treatment group had significantly increased TBARS for 48 h (Fig. 4).

α-Tocopherol with Combined Hyperthermia and Polyunsaturated Fatty Acid. Levels of α-tocopherol were measured in tumor tissue 1 h after treatment. Compared to the control group (24.7 ± 3.86 μg/mg protein), a significant decrease was observed in the group treated with hyperthermia and γ-linolenic acid (11.5 ± 2.60 μg/mg protein; Fig. 5).

Antitumor Effect with Combined Hyperthermia and Polyunsaturated Fatty Acid. Tumor volume was measured on day 7 after combined γ-linolenic acid and hyperthermia. A significant antitumor effect compared with control (10.5 ± 1.49 cm^3) was seen with hyperthermia alone (5.88 ± 0.73 cm^3), γ-linolenic acid alone (5.53 ± 0.68 cm^3), and γ-linolenic acid combined with hyperthermia (2.91 ± 0.65 cm^3). Pretreatment with γ-linolenic acid followed by hyperthermia showed the greatest inhibition of tumor growth compared with either treatment alone (Fig. 6).

DISCUSSION

Cancer treatment is broadly classified into categories such as surgery, chemotherapy, radiation therapy, immunotherapy, and hyper-

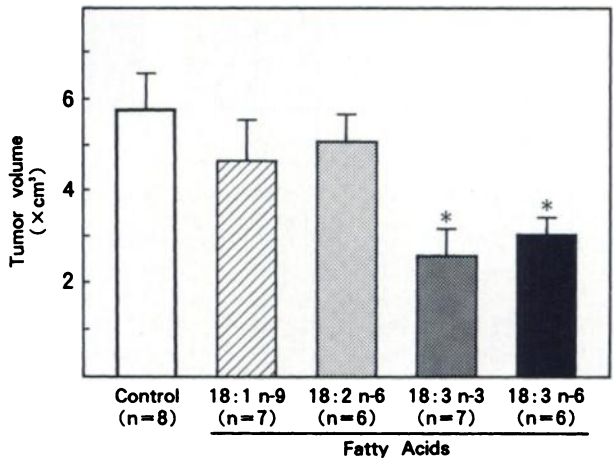

Fig. 3. Antitumor effect with polyunsaturated fatty acid injection. Results are expressed as means; *bars*, SE. *, $P < 0.01$ compared with control.

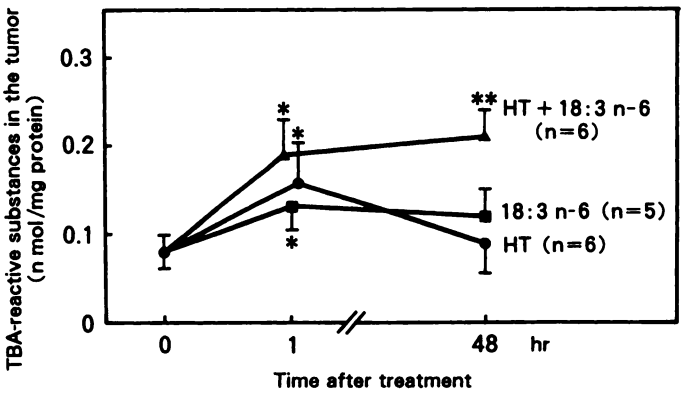

Fig. 4. Time course changes in TBARS in tumor tissue after hyperthermia (*HT*) alone, γ-linolenic acid (18:3, n-6) alone, and HT plus γ-linolenic acid. Each point indicates the mean; *bars*, SE. The significance between the value at time zero and each value after treatment is shown. *, $P < 0.05$; **, $P < 0.01$.

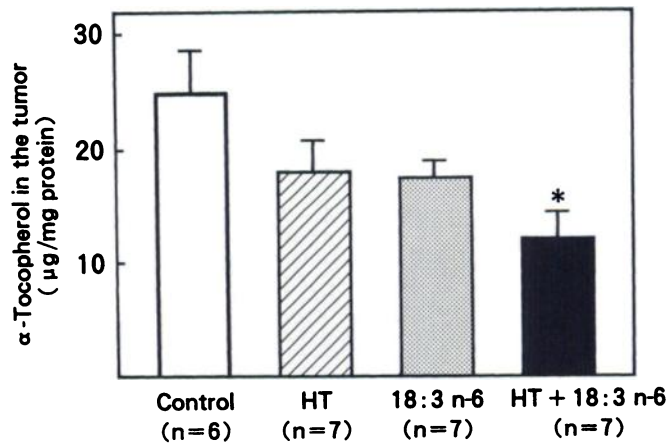

Fig. 5. α-Tocopherol in tumor tissue 1 h after hyperthermia (*HT*) alone, γ-linolenic acid (18:3, n-6) alone, and HT plus γ-linolenic acid. Results are expressed as means; *bars*, SE. *, $P < 0.02$ compared with control.

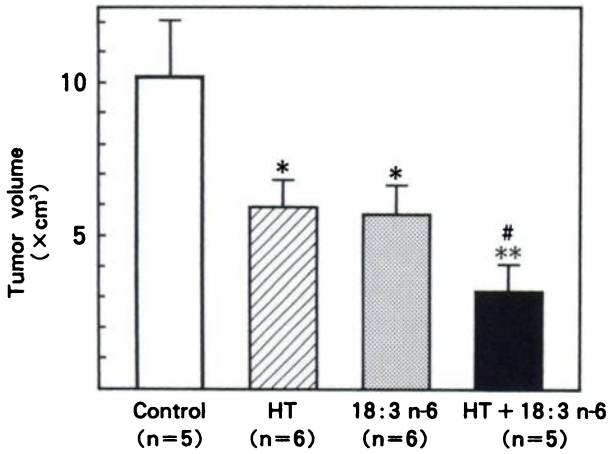

Fig. 6. Antitumor effect of hyperthermia (*HT*) plus γ-linolenic acid. Results are expressed as means; *bars*, SE. *, $P < 0.02$ compared with control. **, $P < 0.01$ compared with control. #, $P < 0.05$ compared with HT or γ-linolenic acid alone.

thermic therapy. Advances have been made in each of these areas, but resistance to treatment remains common. We have previously investigated cancer treatment using the free radical reaction (3, 5). Three approaches to this treatment may be considered: (*a*) strengthening radical production itself; (*b*) increasing the substrate for the radical reaction; and (*c*) decreasing the elimination of radicals. In the present study, we injected polyunsaturated fatty acids, which are radical reaction substrates, into the feeding arteries of tumors to reinforce the radical reactions in the cancer. Immediately after enriching the tumor tissue with polyunsaturated fatty acids, we performed hyperthermia to trigger the radical reaction.

We first examined the degree of tumor tissue lipid peroxidation and the antitumor effect of polyunsaturated fatty acids. γ-Linolenic acid was found to have the greatest effect on tumor tissue lipid peroxidation by virtue of reduction of α-tocopherol levels. γ-Linolenic acid also had an antitumor effect, although slight. On the basis of these results, we selected γ-linolenic acid as the radical reaction substrate. When hyperthermia was performed immediately after injection of γ-linolenic acid, lipid peroxidation in the cancer tissue was significantly higher than with hyperthermia or γ-linolenic acid alone. Combined treatment also had the strongest antitumor effect. The antitumor effects of polyunsaturated fatty acids have been extensively studied *in vitro*. Several reports have shown that polyunsaturated fatty acids exhibit cytotoxic activity against cancer cells at concentrations that have no effect on normal cells (11–13). However, there are little data regarding the mechanism of this effect. Some investigators have shown an association with lipid peroxidation (11, 12), whereas others have focused on suppression of arachidonic acid metabolism (13). Although the present study found a slight antitumor effect of intraarterial injection of γ-linolenic acid, extremely high doses would likely be required for significant antitumor effects. An antitumor effect has been demonstrated with polyunsaturated fatty acids *in vitro,* but they have failed to show efficacy *in vivo* (14–16). An antitumor effect in this study was obtained with polyunsaturated fatty acids administered to increase the levels of radical reaction substrates, followed by a trigger for radical reactions, hyperthermia. Although the prospect of obtaining an antitumor effect with polyunsaturated fatty acids alone is attractive, there are several problems with clinical application of this approach (such as hemolysis and decreased fatty acid availability because of binding to albumin.) These will be subjects of future work. In summary, this investigation found that hyperthermia combined with γ-linolenic acid significantly increases free radical reactions in cancer tissue and is effective in suppressing tumor growth.

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