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ORIGINAL ARTICLE

Nobuhiro Takiguchi · Norio Saito
Masao Nunomura · Keiji Kouda · Kenji Oda
Nobuaki Furuyama · Nobuyuki Nakajima

Use of 5-FU plus hyperbaric oxygen for treating malignant tumors: evaluation of antitumor effect and measurement of 5-FU in individual organs

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Abstract *Purpose*: Hyperbaric oxygen (HBO) has been shown to increase tumor radiosensitivity. Several reports indicate that it also increases sensitivity to alkylating agents, but other reports suggest that it may speed angiogenesis and tumor growth. To throw light on these questions, we investigated the effects of HBO and 5-fluorouracil (5-FU), individually and in combination, on Sarcoma 180 implants in mice. *Methods*: We administered 5-FU at a dose of 0.75 mg/mouse six times per week and HBO at 2 atm absolute pressure for 90 min six times per week, both 17 times in total. In combination treatment, HBO was administered immediately after 5-FU injection. *Results*: Over the treatment period, tumor diameter increased 277.8% in the untreated control group, 244.1% in the group receiving HBO monotherapy, 182.7% in the group receiving 5-FU monotherapy, and 138.5% in the group receiving combination therapy. Concomitant HBO increased accumulation of 5-FU in the tumors, liver, and kidneys, but not in the brain, of recipient animals. *Conclusions*: Based on the above results, we conclude that concomitant HBO enhances the effects of 5-FU.

Key words 5-FU · Hyperbaric oxygen (HBO) · Antitumor effect · Sarcoma 180

Introduction

When treating malignant tumors with radiation therapy, both basic and clinical research [4, 9] has shown that raising the oxygen partial pressure within the tumor can render it more susceptible to radiation. This makes combined therapy more effective than radiation therapy alone. Hyperbaric oxygenation (HBO) has therefore been the focus of considerable attention as a radiosensitizer since the 1950s [8, 11, 12, 14, 17, 20, 23]. Conversely, it is known that hypoxemia inhibits cell division and thus gives rise to radiation resistance [1, 2, 10, 22]. In the same way, the reduced cell division seen in areas of low oxygenation in solid tumors results in resistance to chemotherapy [21]. By supplying oxygen to hypoxic tissue, HBO accelerates tissue angiogenesis. Researchers hope that this characteristic of HBO can be used in conjunction with chemotherapy to overcome resistance by increasing both uptake of anticancer drug by the tumor and the susceptibility of tumor cells to the drug [18, 19].

However, a number of points remain unclear with regard to HBO-induced chemosensitization and the use of HBO in the treatment of malignant tumors: (1) Does HBO further promote tumor proliferation? (2) Does HBO affect the uptake of antineoplastic agents by the tumor? (3) Does HBO have negative effects on healthy tissue? [5, 7, 15]. Some reports indicate that oxygenation improves the uptake of antineoplastic agents and enhances antitumor effectiveness. However, there have also been reports that HBO promotes angiogenesis and tumor growth. In the present study, we looked at the effects on mouse tumors of separate and combined treatment with HBO and 5-FU in order to resolve some of these questions.

N. Takiguchi (✉) · N. Saito · M. Nunomura
K. Kouda · K. Oda · N. Nakajima
First Department of Surgery,
Chiba University School of Medicine,
1-8-1, Inohana, Chuo-ku, Japan
Tel.: +81-43-2602103; Fax: +81-43-2225241

N. Furuyama
Surgery Department,
Chiba University School of Medicine, Chiba, Japan

Subjects and methods

We implanted 5×10^5 cells of Sarcoma 180 subcutaneously into the lower right abdomen of 6-week-old male DDY mice. The mice were given unrestricted access to food (solid mouse feed CE-2; CLEA Japan Company) and were kept under identical experimental conditions unless noted otherwise. Mice were randomly divided into four groups, and treatment was begun 1 week after the subcutaneous implantation of cancer cells. Mice of group C (untreated,

11 mice) received food and water and were kept under normal atmospheric pressure. Mice of group O (HBO monotherapy, 10 mice) were kept under the same conditions as the group C mice, except for treatment with HBO. HBO treatment was given six times per week for 90 min each time, 17 times in total, in a hyperbaric chamber with 100% oxygen at 2 atm absolute pressure. Mice of group F (5-FU monotherapy, 9 mice) were treated six times per week, 17 times in total, by intraperitoneal injection of 5-FU dissolved in physiological saline solution at a dose of 0.75 mg/day (approximately 25 mg/kg body weight). Mice of group OF (5-FU plus HBO, 11 mice) were treated with both 5-FU and HBO. Mice were treated with 5-FU in the same way as those of group F, followed by hyperbaric oxygenation treatment in an identical manner to mice of group O. On the 27th day after Sarcoma 180 implantation, all surviving mice were killed. All experimental procedures were approved by the Institutional Review Board of Chiba University.

For the duration of the experiment, tumor diameter was measured twice weekly by calipers. Changes in body weight and food consumption during the experiment were also calculated. Mean food consumption was calculated as the amount of food consumed by one mouse during the 20-day period of the experiment. Also, 2 h after the final 5-FU dosing in groups F and OF, 5-FU concentrations in the various organs were measured by HPLC [19]. Peripheral blood cell counts were determined at the completion of treatment. After the mice were killed, hematoxylin-eosin stained specimens were prepared from each organ for histologic evaluation. The Tukey-Kramer multiple comparisons procedure and the Wilcoxon test were used for statistical analysis.

Results

On the last day of treatment, tumor-related death (peritoneal hemorrhage) occurred in two mice in group C. All mice in the other groups survived and were killed at the conclusion of the study. The following results were obtained.

Mean food consumption

Mean food consumption per animal in each group during the treatment period was calculated as 82.7 g in group C, 92.8 g in group O, 79.0 g in group F, and 76.4 g in group OF. Food consumption was highest in group O, and tended to decrease in the groups treated with 5-FU.

Changes in body weight

Body weight at the start and completion of treatment is shown in Fig. 1. There was no significant difference in body weight among the groups at the start of treatment. Over the course of treatment, weight gain was significantly lower in the groups treated with 5-FU (groups F and OF) than in groups C and O ($P < 0.05$, $P < 0.01$).

Tissue concentrations of 5-FU

Concentrations of 5-FU in different organs (tumor, liver, kidneys, and brain) of animals treated with this drug are shown in Table 1. In both groups F and OF, drug concentrations were higher in the tumor than in other tissues ($P < 0.01$). Furthermore, drug concentrations in tumor, liver, and kidneys were consistently higher in group OF than in group F ($P < 0.05$). In both groups, the concentration of 5-FU in the brain was approximately 40 ng/g, the lowest concentration found in any of the organs measured.

Changes in tumor diameter

Changes in tumor diameter over the course of treatment are shown in Fig. 2. There were no significant differences among the groups in tumor diameter at the start of treatment. However, the difference in tumor diameter between group C and group OF at the conclusion of

Table 1 Tissue concentrations of 5-FU (ng/g)

Tissue	Group F ($n=9$)	Group OF ($n=11$)
Tumor	$1350 \pm 1345^{**}$	$1709 \pm 1310^{*,**}$
Liver	75 ± 69	$91 \pm 45^{*}$
Kidney	475 ± 294	$919 \pm 935^{*}$
Brain	38 ± 25	39 ± 9

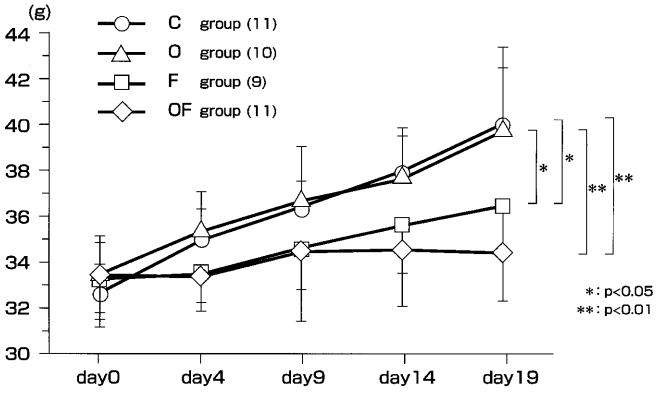

Fig. 1 Changes in body weight

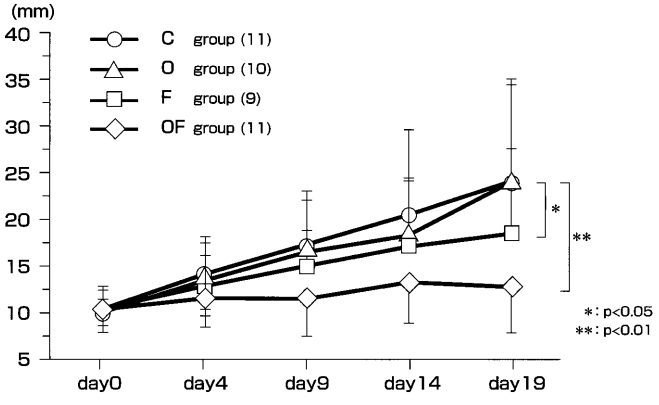

Fig. 2 Changes in tumor diameter

treatment was statistically significant ($P < 0.01$). Differences in the rate of tumor growth between groups F and OF on the one hand and groups C and O on the other first appeared 4 days after the start of treatment.

Peripheral blood cell counts

Peripheral blood cell counts at the conclusion of treatment are shown in Table 2. These values were significantly lower for groups F and OF than for groups C and O ($P < 0.01$), findings which we attribute to 5-FU-induced lymphopenia. No differences in hemoglobin values were noted among the groups.

Histologic changes

Histologic examination of hematoxylin-stained specimens showed no abnormalities in the brain, liver, bone marrow, kidneys, heart, or spleen, and no differences among groups. Histologic examination of tumors showed evidence of centralized necrosis in all tumors, with the larger tumors showing particularly extensive centralized necrosis. This made it necessary to study the leading edge of the tumor margin in order to provide a histologic evaluation of the effects of treatment. Tumor invasion, as determined by evaluation of tumor margins, was noted in ten mice in group C, seven mice in group O, five mice in group F, and two mice in group OF. Thus, from a histologic standpoint, tumor invasiveness was most strongly inhibited in group OF, followed by group F and group O in that order.

Discussion

Although there have been dramatic advances in the effectiveness of antineoplastic agents, it would be an overstatement to say that current treatments are adequately effective against tumors. Attempts to potentiate the antitumor affects of chemotherapy have led to a number of adaptations in the method of administration, along with application of biochemical modulation theories, use of multidrug therapy, and concomitant use of auxiliary treatments.

HBO was described in 1953 by Gray et al. [9], who reported experimental evidence that the use of HBO concomitantly with radiation therapy has a direct effect on the susceptibility of tumors to irradiation, and that this concomitant treatment increases the effectiveness of radiation therapy. Since that time, there have been a number of reports, based on both basic and clinical research, confirming that HBO increases tumor oxygenation and reduces tumor radiation resistance [4, 8, 11, 12]. Because of the unique characteristics of HBO, there have been hopes that this procedure might increase the uptake of anticancer drugs into the tumor and also increase the susceptibility of tumor cells to the drug, thus helping to overcome tumor resistance to radiation therapy and/or chemotherapy [21].

Butler et al. [3] showed that the mechanism of cell damage is identical for alkylating agents and for radiation, and in 1961 Krementz et al. [16] reported the efficacy of concomitant treatment with nitrogen mustard and HBO. There was considerable research on concomitant use of HBO and anticancer therapy in the 1960s and 1970s, but HBO therapy is not very widely employed at present. This is due not only to difficulties connected with the facilities required for HBO treatment, but also to reports that HBO may be associated with an increase in the frequency of cancer recurrence and metastasis [6, 13].

5-FU is often used as a primary agent for chemotherapy of solid tumors. In our present research, we used Sarcoma 180 tumors in mice to investigate the effects of HBO on tumor proliferation and the outcome of 5-FU chemotherapy. Parameters included in our evaluations included changes in body weight, tissue concentrations of 5-FU, tumor diameter, and histologic changes. Treatment began 1 week after implantation, when tumor diameters were approximately 10 mm. We observed no instances of spontaneous regression from this tumor diameter.

The increase in tumor diameter was inhibited from the 4th day after the start of treatment in both group F and group OF, being particularly notable in the latter. The increase in tumor diameter was slightly inhibited in group O. These results indicate that HBO does not promote tumor growth, and that it does play a role in chemosensitization.

We found a correlation between increasing tumor diameter and body weight changes in the mice studied, suggesting that body weight is related to tumor proliferation and to the amount of ascites present. After the tumor was removed, we found almost no differences between the groups in the weight of the remaining carcasses.

Since anorexia is one of the side effects of chemotherapy, food consumption was measured in this study as an indicator of appetite. Mean food consumption per mouse during the treatment period was highest in group O, followed by group C, group F, and group OF in that order. However, food consumption tended to be lower in the animals treated with 5-FU, and was not improved appreciably by concomitant treatment with HBO. It appears from these findings that, although tumor

Table 2 Peripheral blood laboratory values (conclusion of treatment)

Group	White blood cells (/μl)	Hemoglobin (g/dl)	Hematocrit (%)
C ($n = 11$)	9772 ± 6735	14.7 ± 1.4	44.6 ± 3.6
O ($n = 10$)	10965 ± 15080	14.0 ± 1.4	41.4 ± 4.4
F ($n = 9$)	$2754 \pm 1120^{**}$	13.3 ± 1.7	$38.3 \pm 4.3^{*}$
OF ($n = 11$)	$3236 \pm 1598^{**}$	13.4 ± 1.3	$38.2 \pm 4.5^{*}$

proliferation was more strongly inhibited by the concomitant use of 5-FU and HBO than by HBO monotherapy, the addition of HBO to the treatment regimen did not affect appetite as measured by food consumption. To our knowledge, this is the first time that such findings have been reported.

Measurement of tissue 5-FU concentrations showed that tissue uptake of 5-FU following intraperitoneal administration was enhanced by concomitant treatment with HBO, and that concomitant 5-FU/HBO therapy resulted in an elevated tumor concentration of 5-FU. These findings indicate that combined therapy enhanced the antitumor effects of treatment in comparison to 5-FU monotherapy. Our research also showed that concomitant use of HBO did not increase 5-FU transport into the brain. These results are important because, in conjunction with the absence of histologic abnormalities in the brain specimens studied, they suggest that concomitant use of HBO will not aggravate brain damage.

Leukocyte counts were lower in the groups treated with 5-FU than in the untreated group ($P < 0.01$). Hemoglobin levels tended to be lower in the combination treatment group than in the untreated group. No effects on hemoglobin level or hematocrit were noted in the group treated with HBO alone. Similar effects of HBO have previously been reported by Farmer et al. [6] and by Hitchcock et al. [13].

Because histologic studies frequently show the presence of spontaneous centralized tumor necrosis, particularly in large-diameter tumors, antitumor efficacy should be investigated at the tumor margins. Tumor invasiveness was commonly seen in the nonchemotherapy groups, but in the chemotherapy groups, particularly the group receiving concomitant treatment with HBO, noninvasive carcinoma was frequently found.

The results of this research indicate that there are no serious adverse reactions to 5-FU treatment in combination with HBO, and that concomitant HBO enhances the antitumor effects of 5-FU. We believe that 5-FU plus HBO combination therapy could be an appropriate therapeutic regimen for the treatment of cancer, and look forward to the development of clinical applications.

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