The Enhancement of Immunological Activity by Mild Hypothermia

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INTRODUCTION

In general, the term hypothermia is applied for the therapeutic method for the treatment of cancer using micro wave, RF wave thermal system or intra-tissue thermal device. It was found to be a tumor necrosis factor (TNF), which is one of cytokines secreted by macrophages"P"j. With remarkable progress in the instruments and technique in recent years, fundamental and clinical research showed extensive development"Q"j. At present, hypothermia is clinically very important as inter- disciplinary therapeutic method, and studies arebeing performed on combined effects with surgical treatment, radiotherapy, chemotherapy and gene therapy for the treatment of malignant tumor"R"j. Also, hypothermia is characterized by its selective thermal effect on tumor"S"j. In this sense, it is called mild hypothermia. There have been not many reports, which described mild hypothermia for the purpose of treating the cases with cancer. This suggests the possibility of immunological response by heating relatively mild temperature (39 - 42). In this respect, by experiments using mouse as model, we evaluated the effects of hypothermia under temperature of 42.5 and lower and demonstrated that the activation of immunological response is increased and anti-tumor effect
can be obtained.

MATERIALS AND METHODS

1) Transplantation of tumor

For the experiments, 15 C3H/HeJ mice were used for each treatment group. To each animal, SCC-VII tumor was transplanted subcutaneously in femoral region. After transplantation, when the tumor has grown to 4 5 mm in diameter, the experiment was started.

2) Thermal treatment at 40.0 degree and 41.0 degree

Each of the C3H/HeJ mice with SCC-VII tumor transplanted subcutaneously in femoral region was fixed on an acrylic fixture. With 10 mice in each group, only femoral region of each mouse was immersed in two different types of warm water tanks for 60 minutes respectively. After the treatment, the animals were divided into 4 groups. Group 1 was used for blood cell measurement, and blood was collected periodically from vein in tail. Group 2 was used for the preparation of tumor growth curve. Group 3 was classified for the measurement of NK cell activity. Group 4 was used as control without treating with

hypothermia therapy.

3)Thermal treatment before and after tumor transplantation

Mice were divided in the following groups with 15 mice in each group: a group treated with thermal treatment 12 hours after SCC-VII tumor was transplanted subcutaneously in femoral region 12 hours after the thermal treatment (40degree; 60 minutes), a control group transplanted with SCC-VII tumor and without thermal treatment. Then, NK cell activity and relative tumor volume were determined.

4)Thermal treatment every day and thermal treatment every other day

Each of the C3H/HeJ mice were transplanted with SCC-VII tumor subcutaneously in femoral region was fixed on acrylic fixture using adhesive tape. Then, the animals were divided to 10 mice in each group, and only femoral region was immersed in warm water tank, and thermal treatment was carried out. Thermal treatment was performed at 41.0 degree for 30 minutes, and on every other day for two groups, and five sessions of treatment were performed. After the first hypothermia, relative tumor volume was determined.

5)Blood cell counts

Each mouse was fixed on an acrylic fixture. Form each mouse in the 40.0degree group, the 41.0degree group, and no treatment group, 10 l of blood was collected from tail vein using infection needle and fine glass tube. To avoid accumulation of WBC due to wound, blood was collected from tip of tail toward root of tail. Sampling was performed before treatment, and 5, 10, 15, 25 and 30 hours after the treatment respectively. On the blood samples, WBC count, RBC count, hematocrit, platelet count, lymphocyte count, neutrophil count, and monocyte count were determined using hemocytometer (Nihon Kohden Corporation; MEK-6318).

6)Determination of anti-tumor effect from the measurement of tumor volume

The animals were divided into two groups: Trans-Heat group (T-H), for which SCC-VII tumor cells were transplanted atfirst and thermal treatment at 40.0degree or 41.0degree was performed for 60 minutes 12 hours after the transplantation, and Heat-Trans group (H-T), for which thermal treatment at 40.0degree or 41.0degree was performed at first, and 12 hours after, SCC-VII tumor cells were transplanted. Tumor volume was obtained from the formula of ab2/6 (a is longer diameter of tumor, and b is shorter diameter of tumor). The tumor volume after the first treatment was defined as 1, and relative tumor volume over time was obtained.

7)Measurement of NK cell activity

For the T-H group for which SCC-VII tumor cells were transplanted and thermal treatment at 40.0degree for 60 minutes was performed 12 hours after transplantation and the H-T group (for which thermal treatment at 40.0 degree was performed for 60 hours, and SCC-VII tumor cells were transplanted 12 hours after the thermal treatment, NK cell activity was determined using Cr51-labeled YAC-1 cells. Cr51 was added together with culture solution and this was incubated for 2 hours, and YAC-1 cells were labeled with Cr51. Blood cells separated from spleen were added to a 96-well plate together with

culture solution. Then, YAC-1 cells labeled with Cr51 at various concentrations were added together with culture solution, and this was incubated at 37degree for 3 hours. Supernatant solution of the 96-well plate was placed into a 100-l test tube, and Cr51 was determined using autowell scintillation counter. When NK activity is high, NK cells attack and destroy YAC-1 cells. From the graph of YAC-1 cell concentration and count of the supernatant, the gradient was obtained. The ratio of activity to the control group was calculated, and NK cell activity was evaluated.

RESULTS

1) Changes of blood cell count after thermal treatment at 40.0degree or 41.0degree for 60 minutes respectively, WBC count, RBC count, hematocrit, platelet count, neutrophil count, monocyte count, and lympocyte count were determined over time before treatment and 5, 10,15, 25, and 30 hours after treatment using hemocyteter (Nihon Koden Corporation; MEK-6318). As a result, transient increase was observed in WBC count, monocyte count, and lymphcyte count after thermal treatment. The change of monocyte count showed the highest peak 15 hours after the treatment in both groups treated at 40.0degree and 41.0degree. The increase of WBC count, monocyte count and lymphocyte count after mild hypothermia were observed, and the increase of to induce inflammatory sever, which triggers the increase of immunological activity.

2) NK activity caused by hypothermia before and after tumor transplantation.

From animals transplanted with tumor after thermal treatment (H-T group; Heat-12hr-Transplant), and from the group treated with thermal treatment after tumor transplantation (T-H group; Transplant-12hr-Heat), spleen was extracted 12 hours after the final treatment, and activity of NK cells contained in WBC determined by Cr51 labeling method. When the control group was regarded as 1.00, it was 1.50 in H-T group, and 1.75 in T-H group. The increase of NK cell activity due to mild hypothermia suggests the activation of the entire immunological response, and we can expect much on anti-tumor effect of this therapy.

3) Anti-tumor effect by hypothermia before and after tumor transplantation

When the number of days required until the time when the tumor grew 4 times in size was evaluated from tumor growth curve in T-H group (treated with hypothermia after tumor transplantation), H-T group (transplanted with tumor after hypothermia), and the control group without treatment. The results were 3.6 days in H-T group. When the control was regarded as 1.00, it was 1.22 in T-H group, and 1.58 in H-T group, and this confirms that tumor growth is delayed when tumor is transplanted after hypothermia treatment. The findings of growth inhibition in the tumor transplanted after hypothermia suggest that it was induced by the increase of WBC count, monocyte count, and lymphocyte count and by the activation of NK cells.

4) Anti-tumor effect by thermal treatment every day and every other day

The number of days required until the time when the tumor grew 4 times in size was evaluated from tumor growth curve in three groups: ED-H group (heating every day) (the

group treated with mild hypothermia at 42degree for 30 minutes every day after transplantation of SCC-VII tumor cells (5105 cells/0.05 ml), EOD-H group (heating every other day) (the group treated with thermal treatment every other day), and in the control group without treatment. The results were 3.5 days in the control group, 4.3 days in EOD-H group, and 5.6 days in ED-H group. When the control was regarded as 1.00, it was 1.22 in EOD-H group, and 1.60 in ED-H group. In general, hypothermia is conducted 2-3 times per week because of the resistance to heat, but the results of the present study suggest that there is no needs to take special care about the resistance to heat.

DISCUSSION

When cancer cells proliferate, oxygen, nutrition and productive components must be supplied to the cells from the host just as in the case of normal cells, and it is believed that cancer secretes various types of inductive factors and growth factor to adjust the conditions for division and growth in human body. In order that cancer cells avoid the attack from the immunocompetent cells and continue to grow, cancer cells must behave just as normal cells and receive the supply of oxygen and the like from the host. For this purpose, cancer cells induce and form new capillary blood vessels from the neighboring blood vessels of the host and creates advantageous conditions in human body"T"j. Also, blood vessel with abundant blood flow grows in the growth of tumor blood vessel, while blood vessel retracts and disappears when blood flow is low or is stopped, and this is demonstrated from ecological observation"U"j. Radiotherapy andhypothermia have the same effects as the case of carcinostatic agent as described above so far as direct purpose of the therapy is necrosis of cancer cells and inhibition of growth. From the viewpoint of the risk of side effect, hypothermia is safer, and mild hypothermia is much safer. In the dynamics of tumor blood vessel in hypothermia, there are factors such as blood flow increase of normal cells, blood flow stagnation at central part of the tumor, poor supply of oxygen and nutrition from these reasons, and decrease of pH value"V"j. From the results of the present study, it is confirmed that neutrophil count, WBC count, monocyte count and lymphocyte count increase and NK cells are activated before the other cells when treatment is performed with mild hypothermia at mild temperature of 42.5degree or lower. Further, when tumor was transplanted after the treatment by hypothermia, the delay of tumor growth was observed. If we set up a hypothesis from these results, anti-tumor effect is not direct physical damage caused by mild hypothermia, but it efficiently induces a series of immunological responses such as the induces a series o immunological responses such as the increase of neutrophil count, monocyte count and lymhocyte count in the dynamics of tumor blood cells due to heating, and activation of NK cells. As described above, H-T group, while T-H group was higher in NK cell activity. This may be attributed to the fact that a series of immunocompetent cells responded after hypothermia treatment (a peak was reached 5-15 hours after the treatment by hypothermia in the present study). Also, the site treated with hypothermia was the site of the tumor transplanted subcutaneously in hemopoietic organs in bone marrow of femoral region. Further, this may have induced the increase of WBC,

neutrophil, moncyte and lymphocyte and activation of NK cells. Also, it was observed that the treatment everyday by mild hypothermia has higher anti-tumor effect than the treatment given every other day. This suggests that the effects with higher efficiency can be expected by the heating procedure (total physical quantity of heat and timing of treatment). Hasegawa et al. reported that thermal effects could be reinforced if heated quickly even when the temperature and the time for treatment were the same8"j. If would be possible to apply the treatment quantitatively, it would contribute to immunological activity, and the scope of the therapy may be expanded to the preventive treatment. Further, the activation of immunological potency as discussed in this article would contribute to the improvement of postoperative prognosis of cancer treatment such as postoperative prognosis of the patients. It would also be much helpful to the inhibition of metastatic tumor.

CONCLUSION

The results of the present study confirmed: (1) Anti-tumor effect can be given by thermal treatment at relatively mild temperature (mild hypothermia at 39degree-42degree); (2) The increase of neutrophils is dependent on the quantity of heat added; (3) immunological response of monocytes and lymphocytes is associated with it; (4) Activity of the immunological potency as a whole such as activation of NK cells was also confirmed.

REFERENCES

1)Ivan M.Roitt, et al:-Tomomi ed, Immunology CMI,121-132 JPN.(1986)

2)K.Sugimati et al: Effect of Hypothermia on Cancer. JPN J. Hyperthermic Oncology.Vol.4, 79-98 (1988)

3)Terashima H et al: Combined radiotherapy and local external hypothermiain advanced cancer. J UOEH Vol.9, No.2, 171-180 (1987)

4)Hasegawa T: The biological ground on hypothermia for malignant tumors. Reserch Reports of Suzuka Univ. of Med. Sci. No.3, 3-9, (1996)

5)Folman J et al: Angiogenic factors. Science, Vol.235, 422-447 (1987)

6)M.Abe et al: Vasculogenesis and angiogenesis. Experimental Medcine Vascular Biology. Vol.16,No.5, 570-576 (1998)

7)T.Hasegawa et al:Effects of HypothermiaInduced Changes in pH Value on Tumor Response and Thermotolerance. Thermotherapy for Neoplasia,Inflammantion and Pain. M.Ksakas Ed, 433-438 Springer(2001)

8)T.Hasegawa et al:Effects of HypothermiaEnhancement of Hyperthermic Effects Using Rapid Heating. Thermotherapy for Neoplasia,Inflammantion and Pain. M.Ksakas Ed, 439-444 Springer(2001)