# ONCOLOGY

# Adjuvant Effect of High-Frequency Hyperthermia of the Abdominal Cavity on the Course of Transplanted Leukemia in AKR Mice

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The adjuvant effect of high-frequency hyperthermia of the abdominal cavity was studied in 2-month-old mice of highly leukemic AKR strain treated with cyclophosphamide. Weights of organs, parameters of the peripheral blood, and cytological imprints of organs were compared in experimental and control animals. A more pronounced antitumor effect was observed in animals exposed to hyperthermia. These data provide the basis for clinical use of hyperthermia of the abdominal cavity.

**Key Words:** *leukemia; hyperthermia; abdominal cavity; cyclophosphamide*

Controlled hyperthermia used in the treatment of cancer produces a direct cytodestructive effect on tumor cells and an adjuvant effect consisting in sensitization of the tumor to drugs and radiation exposure [1,3-5,10]. The development of modern hyperthermic trend in oncology consists in the creation of selective hyperthermic methods: perfusion, local, and intracavitary [6-9].

A method for local hyperthermia of the abdominal cavity was developed in Kirov Institute of Hematology and Blood Transfusion. The method is based on the exposure of the current-conducting fluid pre-infused into the abdominal cavity to high-frequency electric current. Transplanted leukemia in syngeneic AKR mice was selected as an experimental model for investigation of the antitumor mechanisms of this method. Original mice obtained in 1981 from Laboratory of Experimental Biological Models, Academy of Medical Sciences of USSR, were inbred in Laboratory of Experimental and Clinical Studies, Kirov Institute of Hematology and Blood Transfusion. This strain is characterized by high (95%) incidence of spontaneous lymphocytic leukemia at the age of 9-12 months [2].

We studied the adjuvant effect of hyperthermia in mice with transplanted leukemia treated with cyclophosphamide.

## MATERIALS AND METHODS

The study was carried out on a Vulkan-1 device for HF-hyperthermia (Polet Firm). Fifty AKR/J mice of both sexes were used in the experiments. The animals were narcotized with calypsol (0.1 mg) intramuscularly (in the hind paw) and fixed (by the limbs) in the supine position to the manipulation table. The anterior abdominal wall was treated with an antiseptic solution. Normal saline (current-conducting fluid) heated to 37°C in a water bath was injected into the abdominal cavity in a volume of 5-7 ml sufficient for hyperthermia and causing no compression of the diaphragm. Needle electrodes were implanted through the ileac areas parallel to the inner surface of the anterior abdominal wall and to each other. The electrodes were sub-

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**TABLE 1.** Weights of Organs (g) in AKR Mice with Transplanted Leukemia ($n$=8; $M\pm m$)

| Group | Spleen | Thymus | Heart | Kidneys | Liver |
|---|---|---|---|---|---|
| 1st | 0.0970±0.0082 | 0.1540±0.0117 | 0.1600±0.0046 | 0.4290±0.0188 | 1.350±0.049 |
| 2nd | 0.0990±0.0219 | 0.1710±0.0236 | 0.1300±0.0035* | 0.2720±0.0073* | 1.080±0.067 |
| 3rd | 0.0560±0.0016* | 0.0730±0.0059*++ | 0.1340±0.0054** | 0.3980±0.0159+ | 1.230±0.059 |

**Note.** *$p$<0.001, **$p$<0.01 compared to group 1; +$p$<0.001, ++$p$<0.01 compared to group 2.

merged into the current-conducting fluid and only insulated sections of the needles contacted with the anterior abdominal wall to prevent burns to soft tissue. A transducer was inserted along the midline into the abdominal cavity; it was positioned at equal distances from the working zones of the needle electrodes and was also submerged in the current-conducting fluid. Communicating wires leading through a regulator to a generator were connected to the needle electrode plugs. A block for temperature measurements was attached to the plug of the temperature transducer. The maximum temperature in the abdominal cavity induced by high-frequency electromagnetic field and heated current-conducting fluid did not exceed 43°C. The duration of hyperthermia was 15-20 min. Hyperthermia with these parameters was well tolerated by the animals.

Transplantation of leukemia to AKR mice was performed as follows. A mouse with signs of spontaneous leukemia was sacrificed, the spleen was removed and cut into fragments with scissors, and the fragments were passed through a capron net with a glass stick. The resultant splenocyte suspension was washed and diluted with normal saline to a final concentration of $2.0\times10^9$/liter. The cells were transplanted to 2-month-old AKR mice weighing 26.2±3.1 g. Each mouse intraperitoneally received 0.5 ml suspension. The animals were then divided into 3 groups (8 per group) and inoculated (intraperitoneally) with tumor cells.

Group 1 mice with transplanted leukemia were controls. One day after transplantation of tumor cells the animals were subjected to sham intervention (injection of normal saline, introduction of needle electrodes and thermal transducer without hyperthermia). These animals received no cyclophosphamide.

In group 2 hyperthermia was also simulated (injection of normal saline and insertion of instruments into the abdominal cavity without subsequent hyperthermia) after tumor cell inoculation. One day after the sham intervention the animals were injected (intraperitoneally) with a single dose of cyclophosphamide (30 mg/kg).

Group 3 mice were subjected to local hyperthermia 1 day after inoculation of tumor cells and after the next 24 h (2 days after tumor transplantation) were intraperitoneally injected with cyclophosphamide in a dose of 30 mg/kg. On day 10 after the start of the experiment the animals were sacrificed by cervical dislocation. Peripheral blood parameters, ALT, AST, weights and cytological parameters of the spleen, thymus, heart, kidneys, and liver were studied.

## RESULTS

The weights of the spleen, thymus, and heart in group 3 mice were significantly lower than in group 1 animals, while the weights of the spleen and thymus were virtually the same in groups 1 and 2. The weights of the heart and kidneys were significantly lower in group 2 animals. The weights of the thymus and kidneys in group 3 were significantly lower than in group 2 (Table 1). Comparison of the peripheral blood parameters showed that hemoglobin content and erythrocyte count in group 3 were higher than in group 2, leukocytosis in groups 2 and 3 was significantly lower than in group 1. The counts of granulocytes, lymphocytes, and blasts were significantly lowered in group 3 compared to the control, while in group 2 only granulocyte and lymphocyte counts decreased significantly. Analysis of liver enzymes showed significantly lower levels of

**TABLE 2.** Peripheral Blood Parameters in AKR Mice with Transplanted Leukemia ($n$=8; $M\pm m$)

| Group | Hemoglobin, g/liter | Erythrocytes, $10^{12}$/liter | Leukocytes, $10^9$/liter | Granulocytes, $10^9$/liter | Lymphocytes, $10^9$/liter | Blasts, $10^9$/liter | ALT, μcat/liter | AST, μcat/liter |
|---|---|---|---|---|---|---|---|---|
| 1st | 130.20±0.94 | 5.380±0.218 | 5.510±0.358 | 1.570±0.212 | 4.000±0.198 | 0.10±0.04 | 0.250±0.019 | 0.850±0.124 |
| 2nd | 147.30±1.98* | 4.45±0.02*** | 4.04±0.85*** | 0.650±0.122** | 2.41±0.259* | 0.07±0.03 | 1.01±0.00* | 1.240±0.057*** |
| 3rd | 133.60±1.89+ | 5.500±0.115+ | 2.320±0.232* | 0.660±0.101* | 1.630±0.185+++ | 0.00±0.00+++ | 0.16±0.01+ | 0.73±0.71+ |

**Note.** *$p$<0.001, **$p$<0.01, ***$p$<0.05 compared to group 1; +$p$<0.001, ++$p$<0.01, +++$p$<0.05 compared to group 2.

**TABLE 3.** Cytological Analysis of the Spleen in AKR Mice with Transplanted Leukemia (*M*±*m*)

| Group | Splenic cells, in % | | | |
|---|---|---|---|---|
| | blasts | prolym-phocytes | lympho-cytes | myeloid |
| 1st | 22.5±4.2 | 37.0±5.1 | 35.8±5.8 | 4.3±0.7 |
| 2nd | 18.6±5.7 | 32.4±4.3 | 43.4±8.7 | 8.1±2.6 |
| 3rd | 0*+ | 1.6±0.5**++ | 94.8±0.9 | 4.0±0.7 |

**Note.** *$p<0.001$, **$p<0.01$ compared to group 1; +$p<0.001$, ++$p<0.01$ compared to group 2.

ALT and AST in group 3 mice in comparison with group 2. A significant difference was observed between groups 1 and 2 (Table 2). Comparison of organ imprints showed the absence of tumor cell infiltration in the spleen in group 3. The content of prolymphocytes in the spleen was significantly lower in group 3 compared to groups 1 and 2. The counts of other cells in organ were virtually the same in animals of all groups (Table 3).

The study of the isolated effect of local hyperthermia of the abdominal cavity on the course of transplanted leukemia in AKR mice receiving no drug therapy showed no inhibition of the leukemic process.

Hence, local hyperthermia of the abdominal cavity had an adjuvant effect and in combination with cyclophosphamide inhibited the tumor process in animals. The cytostatic effect of combined treatment appreciably surpassed that of drug therapy alone. The absence of tumor cells in the peripheral blood and cytological imprints of the spleen and absence of tumor infiltration in the liver confirm higher efficiency of cyclophosphamide in combination with hyperthermia. On the other hand, the increase of tumor cell sensitivity to the drug was not paralleled by higher myelotoxicity and hepatotoxicity, which was confirmed by unchanged levels of hemoglobin, erythrocytes, and liver enzymes.

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