

THE EFFECT OF CYTOSTATICS AND HYPERTHERMIA ON RAJI HUMAN LYMPHOMA CELLS

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Aim: To evaluate the effect of hyperthermia on cytostatic activity of chemotherapeutic drugs carboplatin, cisplatin, oxaliplatin, carmustine, gemcitabine and etoposide in human lymphoma cell culture. **Methods:** RAJI human lymphoma cells were incubated with cytostatics at 37 °C or 42 °C and evaluated for cell culture growth. **Results:** The number of viable cells after incubation with the drugs (except for gemcitabine) at 42 °C for 30 min was significantly lower than at 37 °C. There were synergism of cytostatic effects of platinum drugs (carboplatin, cisplatin and oxaliplatin) with cytostatic effect of 42 °C and the summation of cytostatic effect of carmustine or etoposide with the action of hyperthermia. The thermal enhancement ratio was 3.0 for oxaliplatin, 2.0 for cisplatin, 1.8–2.4 for carboplatin. The combination of platinum drugs with gemcitabine resulted in a significant enhancement of cytostatic activity. **Conclusion:** Our findings suggest that a gain in sensitivity of RAJI human lymphoma cells to platinum drugs occurs at 42 °C. **Key Words:** RAJI human lymphoma cells, hyperthermia, thermochemotherapy.

Intrinsic drug resistance of tumor cells or that acquired during the chemotherapy is considered to be the main cause of failures in the treatment of malignant tumors. Overcoming of the tumor drug resistance may open new perspectives in the management of patients for whom standard therapeutic approaches are ineffective.

To overcome the chemoresistance, different physicochemical modalities are currently used that can selectively enhance the sensitivity of tumor cells to anticancer therapy. Hyperthermia is regarded as the most promising modifying factor. It should also take into account the important fact of direct damaging effect of heat on the tumor.

The clinical experience gained until now confirms these theoretical prerequisites. Encouraging results of whole-body hyperthermia are achieved in chemotherapy for disseminated melanoma [1], metastatic sarcoma [2], recurrent ovarian cancer [3,4], pleural mesothelioma [5], malignant tumors in children [6] and other types of tumors [7–10]. In a number of cases hyperthermia makes it possible to reduce the doses of administered cytostatics by up to 50% with concurrent enhancement of antitumor effect and decrease of nephrotoxic, cardiotoxic and hepatotoxic effects of the chemotherapeutic agents.

In experimental models of various tumors, amplification of cytostatic antitumor effect with increased temperature was demonstrated. Numerous studies of tumor cell cultures reported increased cytotoxicity of cyclophosphamide, melphalan, ifosfamide, nitrosourea, bleomycin, doxorubicin, platinum preparations, etoposide, vincristine, taxanes, gemcitabine [11–25]. The death of tumor cells induced by cytostatics was found to rise with elevated temperature and prolonged duration of heating, and the highest-

cytotoxicity was recorded with concurrent exposure to a chemotherapeutic agent and hyperthermia.

The mechanism of enhancing antitumor activity of chemotherapeutic agents at elevated temperature has not been definitively determined. The available findings suggest that permeability of cell membranes and penetration of drugs into tumor cells are improved, and that the mechanisms of repair of primary and secondary damage of tumor cells are inhibited [26].

Notably, the results of experimental studies investigating the interaction between hyperthermia and cytostatic drugs are quite contradictory. The use of various cell lines and experimental tumors, treatment regimens, temperature levels and drug concentrations makes it difficult to interpret the results of the studies.

Investigation of possible thermal enhancement of human lymphoma cell sensitivity to cytostatic drugs used in the management of the disease is of interest for the development of thermochemotherapy schemes for malignant lymphomas. The objective of this study was investigation of the effect of hyperthermia on the cytostatic activity of chemotherapeutic agents used in the treatment of malignant lymphoma; the investigation was conducted on RAJI human lymphoma cell line.

MATERIALS AND METHODS

Chemotherapeutic drugs. The effect of the following chemotherapeutic agents was evaluated: carboplatin (chemocarb, Dabur Pharma Ltd., India), oxaliplatin (eloxatin, Sanofi-Winthrop Industria, France), cisplatin (cytoplatin, CIPLA Ltd., India), carmustine (BiCNU, Bristol-Meiers Squibb, Italy), etoposide (etoposide-Ebewe, Austria), gemcitabine (citogem, Dr. Reddy's Laboratories Ltd., India). The drugs were diluted with saline or distilled water for injections according to the package insert instructions, just before the experiment.

Cell culture. The investigations were conducted on RAJI human lymphoma cell line (Russian Collection of Cell Cultures, Cytology Institute of Russian Academy of Sciences, St. Petersburg). The cell culture was

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Abbreviations used: IC50 – drug concentration resulting in growth inhibition by 50% vs. the control; TER – thermal enhancement ratio.

grown in RPMI-1640 culture medium supplemented with 10% fetal calf serum.

Cell treatment. To perform the experiments, the cell culture was plated in culture vials, 200,000 cells in 2 ml of the culture medium in each. Twenty-four hours later 50–200 μ l of cytostatic solution were added into the vials for different final concentrations. The vials with the cell culture were incubated in heating bath circulator EXATERM U3 (Julabo, Germany) at 37 °C or 42 °C for 15 or 30 min and for 48 h in the thermostat at 37 °C. After adding 0.1% trypan blue to the suspension, live (unstained) cells were counted in hemocytometer. Three vials with cells were used for each drug concentration.

Regression analysis of the data was employed to calculate the rate of growth inhibition of the tumor cell culture (IC_{50} — drug concentration resulting in growth inhibition by 50% versus the control). Thermal enhancement ratios (TER) for inhibition of cell proliferation for each chemotherapeutic drug were calculated as IC_{50} for drug alone divided by IC_{50} for drug combined with hyperthermia [27].

The values obtained were processed using standard statistical methods of Origin 7.0.

RESULTS AND DISCUSSION

The study of RAJI human lymphoma cell culture growth demonstrated that the effect of 42 °C temperature was more prominent with increased duration of heating. The heating of the cells for 15 min did not actually affect the number of viable cells, whereas the increase in thermal treatment duration up to 30 and 60 min resulted in the reduction of viable cell numbers to 64% and 42%, respectively.

Fig. 1 presents the results of RAJI human lymphoma cells incubation with chemotherapeutic agents at different concentrations in temperature settings of 37 °C or 42 °C for 15 and 30 min. The findings suggest that examined drugs induce significant inhibition of human lymphoma cell culture growth at 37 °C, with growing cytostatic effect upon increasing the final concentration of the drug in the culture medium. The calculation of cytostatic activity rate (IC_{50}) demonstrated that RAJI human lymphoma cell culture at 37 °C is most sensitive to etoposide (IC_{50} = 0.1 μ g/ml), oxaliplatin (IC_{50} = 0.3 μ g/ml) and cisplatin (IC_{50} = 0.8 μ g/ml). IC_{50} was 8.5 μ g/ml for carboplatin, 9.3 μ g/ml for carmustine and 6.5 μ g/ml for gemcitabine (Table 1).

Table 1. Cytostatic activity of chemotherapeutic agents at 37 °C and hyperthermia setting

Agent	IC_{50} , μ g/ml		
	37 °C	42 °C, 15 min	42 °C, 30 min
Cisplatin	0.8	0.4 (TER = 2.0)	0.4 (TER = 2.0)
Carboplatin	8.5	4.8 (TER = 1.8)	3.6 (TER = 2.4)
Oxaliplatin	0.3	0.1 (TER = 3.0)	0.1 (TER = 3.0)
Carmustine	9.3	8.9 (TER = 1.0)	9.2 (TER = 1.0)
Gemcitabine	6.5	4.7 (TER = 1.4)	8.4 (TER = 0.8)
Etoposide	0.1	0.1 (TER = 1.0)	0.1 (TER = 1.0)

Incubation of RAJI human lymphoma cells with oxaliplatin at 42 °C for 15 min resulted in significant reduction of cell number compared to 37 °C. Effectiveness of gemcitabine and etoposide was not changed, and the enhancement of the effect of carboplatin,

cisplatin and carmustine at 42 °C for 15 min was observed only at certain concentrations of the drug. Cell incubation at 42 °C for 30 min significantly increased the efficiency of all investigated drugs, except for gemcitabine (Fig. 1).

The calculation of cell culture growth inhibition using IC_{50} criterion and taking into account the reduced number of cells due to the effect of increased temperature alone demonstrated that synergetic amplification of the cytostatic effect by hyperthermia occurred with oxaliplatin, carboplatin and cisplatin: drug IC_{50} decreased from 0.3 at 37 °C to 0.1 μ g/ml at 42 °C for oxaliplatin, from 8.5 to 4.8–3.6 μ g/ml for carboplatin, and from 0.8 to 0.4 μ g/ml for cisplatin. Summation of drug and hyperthermia effects was observed with carmustine and etoposide, as drug IC_{50} did not change. Hyperthermic exposure with gemcitabine produced a small amplification of cell growth inhibition with 15 min duration of heating (IC_{50} decreased from 6.5 to 4.7 μ g/ml compared to that at 37 °C) or reduction of the effect with 30 min duration of heating (IC_{50} increased from 6.5 to 8.4 μ g/ml compared to that at 37 °C). The thermal enhancement ratio (TER) of the cytostatic effect (the ratio of drug IC_{50} at 37 °C to IC_{50} at 42 °C) was 3.0 for oxaliplatin, 2.0 for cisplatin, 1.8–2.4 for carboplatin, 1.0 for carmustine, 1.0 for etoposide. The TER for gemcitabine was 1.4 with 15 min duration of heating. The increase in heating duration up to 30 min resulted in gemcitabine effect reduction (see Table 1). The differences between the effects at the temperatures of 37 °C and 42 °C for all platinum preparations were statistically significant.

The data obtained suggest that lymphoma cells growth inhibition by alkylating chemotherapeutic agents carboplatin, cisplatin, oxaliplatin, carmustine and plant-derived drug etoposide increases under hyperthermia. For platinum drugs synergetic activity with hyperthermia was observed, for carmustine or etoposide there was the summation of cytostatic effects of drug and hyperthermia.

It should be noted that there are contradictory reports about the interaction of hyperthermia and gemcitabine. Simultaneous application of gemcitabine and heating led to the decreased cytotoxicity [16, 22], had no influence on cytotoxicity [19] or augmented cytotoxic effect of the drug [23, 28, 29]. Our data did not allow to consider gemcitabine as a promising drug for thermochemotherapy of lymphomas. But as gemcitabine in combination with platinum drugs is used for the treatment of refractory lymphoma, we investigated the effect of hyperthermia on the cytostatic effect of this combination.

The data presented on Fig. 2 indicate that the combination of platinum drugs with gemcitabine (5 μ g/ml) at the temperature of 37 °C results in significant inhibition of RAJI human lymphoma cell culture growth, the effect of cytostatics increasing with the rise of the final concentration of platinum agents. A convincing corroboration of antitumor effect enhancement is the reduction of drug IC_{50} . While IC_{50} for cisplatin alone was 0.8 μ g/ml, for carboplatin alone

8.5 µg/ml, and for oxaliplatin alone 0.3 µg/ml (see Table 1), it decreased to 0.06 µg/ml for cisplatin with gemcitabine (5 µg/ml), to 0.5 µg/ml for carboplatin with gemcitabine, and to 0.03 µg/ml for oxaliplatin with gemcitabine (Table 2). Thus, cytostatic activity of platinum preparations in combination with gemcitabine increased 13-fold for cisplatin, 17-fold for carboplatin, and 10-fold for oxaliplatin. Gemcitabine alone (5 µg/ml) at 37 °C reduced the number of RAJ1 human lymphoma cells only 2-folds. Thus, we can conclude about the synergistic action of gemcitabine with the platinum drugs at 37 °C.

Incubation of the cells with platinum drugs in combination with gemcitabine at 42 °C for 15 min virtually did not affect the cytostatics activity compared to that at 37 °C. The increased duration of cell incubation

at 42 °C up to 30 min led to the reduction in the number of viable cells compared to the numbers observed at 37 °C (Fig. 2). The calculation of cytostatic effect rates for platinum drugs, taking into account the reduced number of cells due to the effect of increased temperature alone, demonstrated that it was the case of summation of cytostatic effect of platinum drugs with gemcitabine and cytostatic effect of hyperthermia as drug's IC₅₀ did not actually change after exposure to hyperthermia (see Table 2).

Table 2. Cytostatic activity of platinum drugs in combination with gemcitabine

Drugs	IC ₅₀ , µg/ml		
	37 °C	42 °C, 15 min	42 °C, 30 min
Gemcitabine + cisplatin	0.06	0.06	0.05
Gemcitabine + carboplatin	0.5	0.5	0.4
Gemcitabine + oxaliplatin	0.03	0.03	0.03

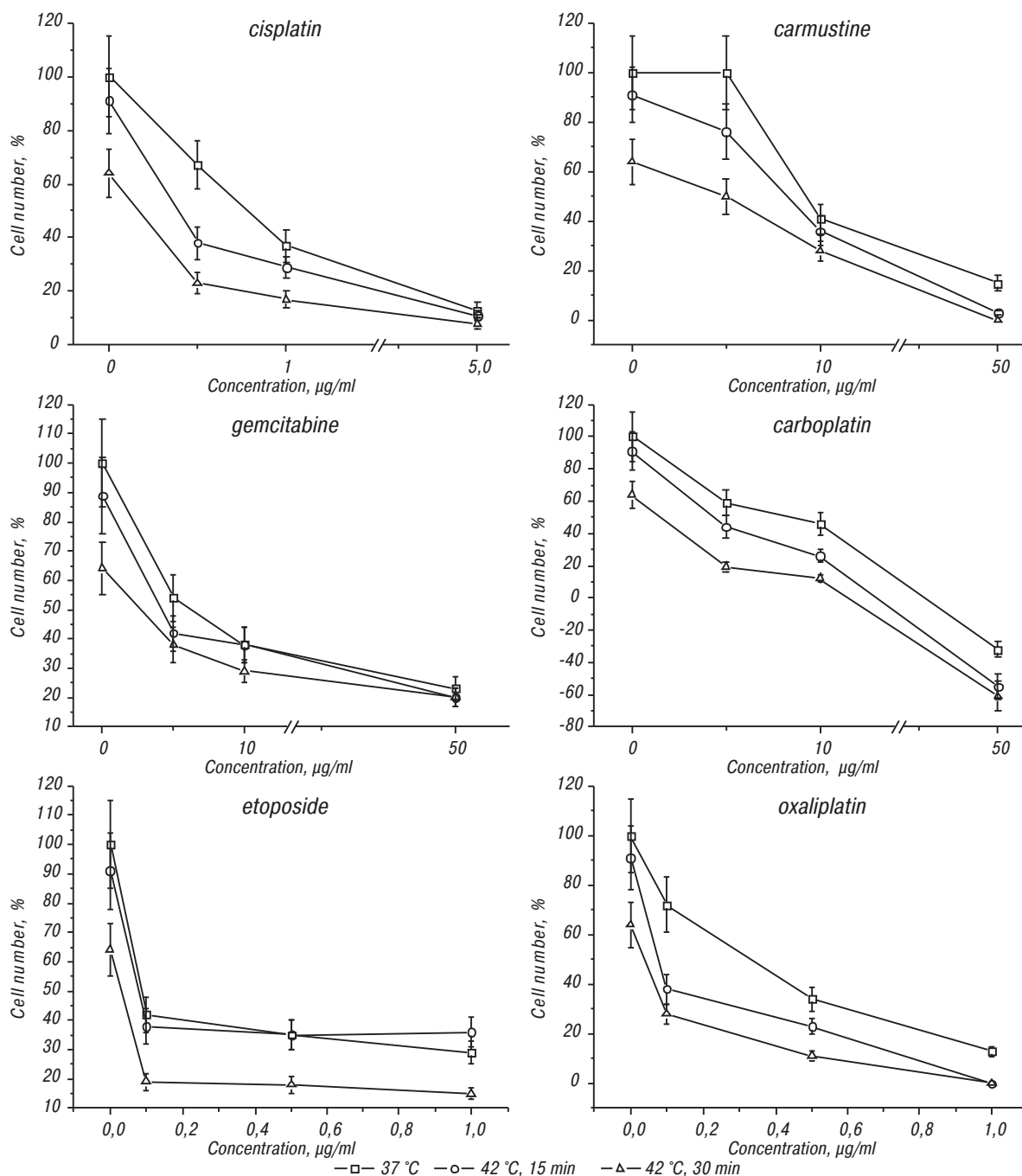


Fig. 1. Cytostatic effect related to drug concentration and cell incubation temperature

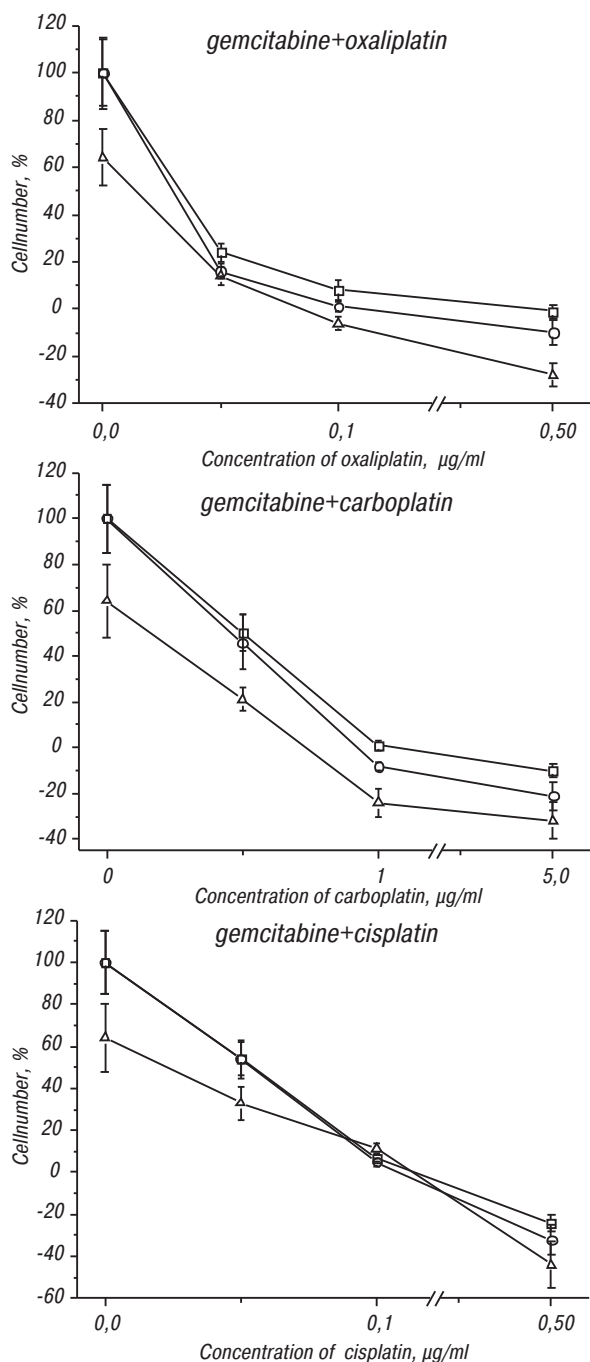


Fig. 2. The impact of hyperthermic exposure (42 °C, 15 or 30 min) and gemcitabine (5 µg/ml) on cytostatic effect of platinum drugs

The results of the study allowed to assert that growth inhibition of RAJI cells by carboplatin, cisplatin, oxaliplatin, carmustine and etoposide was increased by the exposure to 42 °C for 30 min. Summation of drug and hyperthermia cytostatic effects was observed for carmustine and etoposide, synergic effect — for all platinum drugs. The thermal enhancement ratio was 3.0 for oxaliplatin, 2.0 for cisplatin and 1.8–2.4 for carboplatin.

The combination of platinum agents with gemcitabine at 37 °C produces a synergic effect of the cytostatics. In such combination, IC₅₀ of platinum drugs was reduced 10-fold for oxaliplatin, 13-fold for cisplatin, 17-fold for carboplatin. Incubation of the cells with platinum preparations in combination with gemcitabine at 42 °C for 30 min causes a reduction in the

number of viable cells compared to cell incubated at 37 °C, which is a result of summation of cytostatic effects of drugs and hyperthermia.

The obtained results give grounds to continue the study on animal models with feasible prospects of clinical application.

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