THE SENSITIVITY OF CHEMIORESISTANT HUMAN TUMOR EXPLANTS TO LYSIS BY ACTIVATED AND NONACTIVATED AUTOLOGOUS LYMPHOCYTES: A PILOT STUDY

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**Aim:** The comparative study of antitumor action of peripheral blood lymphocytes (PBL) and lymphokin-activated killer cells (LAK) on autologous cell of chemoresistant and chemosensitive human soft tissue sarcomas and tumors of epithelial origin. **Materials and Methods:** Tumor explants (15 samples of soft tissue sarcomas, 10 samples of cervical and ovarian carcinomas) were cultivated with autologous lymphocytes in double diffusion chambers. Evaluation of the results was done on the basis of morphological criteria of explants growth. **Results:** The results have shown that in the patients with the resistant soft tissue sarcomas and carcinomas LAK possess more pronounced antitumor action, than non-activated lymphocytes, whilst PBL possess higher antitumor action on sensitive epithelial tumors than that on soft tissue sarcoma. **Conclusion:** LAK possess antitumor action toward chemoresistant tumors of different origin and localization. **Key Words:** chemoresistance, soft tissue sarcoma, epithelial tumors, lymphocytes, LAK, Il-2.

The development of chemoresistance of tumors limits the efficacy of therapy of cancer patients; that's why the search for ways to overcome chemoresistance is extremely important. It is well recognized now that immunotherapy may be perspective for treatment of chemoresistant tumors. Few studies evidencing that resistant tumor cells reveal pronounced sensitivity to the action of killer cells (cytotoxic T-lymphocytes — CTL, natural killer (NK) cells) support such hypothesis [1—5]. Antitumor activity of non-activated effector cells, lymphokin-activated killer cells (LAK) and co-cultivation with cytokines (most often — interleukin-2 (Il-2), rarely — interferons (IFNγ)) has been studied.

Sometimes Il-12 and Il-18 were used; their action in the presence of INFγ elevated the activity of NK cells toward chemoresistant sarcomas [6]. Interestingly, immunotherapy has been shown to be more effective toward tumors resistant to hormonal therapy (in particular, prostate carcinoma) [7]. However, these studies were performed mostly in vitro, whilst in vivo studies are scarce, as well as the research carried on freshly isolated tumor cells.

In our earlier publication [8], we have demonstrated that tumors resistant to doxorubicin (melanoma B16 and MC-rhabdomyosarcoma) revealed increased sensitivity to the action of LAK, and different fractions of lymphocytes possess different activity. By in vivo transplantation of MC-rhabdomyosarcoma cells resistant to doxorubicin, it has been shown that immunotherapy and its variants in some cases may suppress tumor growth, and result in elevation of life span of experimental animals [9].

In the present study we aimed to compare antitumor action of peripheral blood lymphocytes (PBL) and LAK toward autologous human tumors of soft tissues and epithelial ones that were chemoresistant or chemosensitive. **MATERIALS AND METHODS**

Samples of tumor tissue were obtained from the biopsy specimens or surgically removed tumors of 25 patients (21 to 60 years old) with soft tissue sarcoma (rhabdomyosarcoma, malignant mesenchymoma, undifferentiated sarcoma, synovial sarcoma, fibrosarcoma, dermatofibrosarcoma, neurosarcoma (15 patients)) and epithelial tumors (cervical, ovarian or endometrial carcinoma (10 patients)), cured in the Institute of Oncology, AMS of Ukraine (Kyiv, Ukraine). 18 patients were at stages I, II, and 7 patients — at stages III, IV of the disease. None of the patients received radiotherapy prior to biopsy/surgery.

Lymphocytes were isolated from total heparanized blood by centrifugation in Ficoll-Verografin density gradient.

Sensitivity to chemotherapy was determined by the method developed by us [10]: 0.02 mg/ml doxorubicin (Ebeve, Austria), 0.06 mg/ml cyclophosphane (Latvia), 0.02 mg/ml vincristine (Teva Pharmaceutical Industries LTD, Israel), 0.005 mg/ml methotrexate (Teva Pharmaceutical Industries LTD, Israel), 0.045 mg/ml carboplatin (Sicor Inc, USA) were added in the medium for cultivation of tumor explants in diffusion chambers; the drugs were selected according the rate of their application on therapy of soft tissue and epithelial tumors.

To receive LAK, the cells were incubated with roncoleukin-2 (1000 MU/ml) (BIOTEX, Russia) for 2 h at 37 °C, then twice washed and added in diffusion chambers.

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Abbreviations used: Il — interleukin; LAK — lymphokin-activated killer cells; PBL — peripheral blood lymphocytes.
Tumor cells and lymphocytes were co-cultivated for 5 days in complete RPMI-1640 medium (Sigma, USA) at 37 °C in atmosphere of 5% CO₂. Then the filters of diffusion chambers were fixed, stained by Karacchi hematoxylin, treated by spirits (50 °, 70 °, 96 °, 100 °) and xylene, and preparations for microscopic examination were prepared using canadian balsam.

For characterization of tumor growth morphologic criteria were used: destruction of tumor cells, the absence of tumor cell migration from explant, migration of single tumor cells from explant, formation of monolayer of different density; formation of cell conglomerates; formation of spheroids.

Expression of IL-2 receptors was studied by the method of indirect fluorescence using CD25-MoAbs (MedBioSpectr, Russia).

RESULTS AND DISCUSSION

Upon the study of clinical material we have faced some problems due to appearance of different variants of chemoresistance of the studied tumors.

In the patients with epithelial tumors (n = 10), 3 tumors were sensitive to anticancer drugs, and 7 — resistant to one or more drugs (in 1 case, tumor cells were resistant to all used drugs); the resistance to carboplatin was registered the most frequently, whilst to doxorubicin — rarely (Table 1).

PBL of the patients from the group with chemosensitive tumors possess antitumor activity in 2 cases from 3 studied, and their activation with IL-2 results in elevation of antitumor activity in all cases (see Table 1). At the same time, in the group with chemoresistant tumors, antitumor action of PBL wasn’t pronounced, and activation with IL-2 has no effect in 2 cases, moderate — in 2 cases, and resulted in marked elevation of antitumor activity of LAK — in 3 cases (manifesting itself in the absence of tumor cell migration, or migration of single cells, and their destruction) (Fig. 2, 3).

The studied tumors of soft tissues were divided in 2 groups: sensitive to chemotherapy (8 patients, 53.3%), and resistant to one or more drugs (7 patients, 47.7%). The most frequently, the resistance to doxorubicin has been registered, however, in any of studied cases the resistance to methotrexate has been revealed. In a part of tumor samples from the patients with soft tissue sarcomas (n = 8) the sensitivity to doxorubicin only has been evaluated; that’s why these data are not included in Table 2.

Table 1. Sensitivity of explants of epithelial tumors to different anticancer drugs and action of PBL and LAC

<table>
<thead>
<tr>
<th>№</th>
<th>Diagnosis</th>
<th>Sensitivity to the action of chemoterapies</th>
<th>Antitumor action of lymphocytes on the growth of tumor explants</th>
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<td></td>
<td></td>
<td>Doxorubicin</td>
<td>Carboplatin</td>
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<td>-</td>
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Notes: "+" — sensitive; "-" — insensitive.
The study of antitumor action of PBL from the patients with chemosensitive soft tissue tumors has been registered only in 3 cases, but activation of LAK with Il-2 significantly elevates that index (75%). Analogously, the study of antitumor action of PBL from the patients with soft tissue tumors resistant to one or more chemopreparations has shown that PBL of 3 patients from 7 examined possess antitumor action, but there was a marked elevation of antitumor activity of LAK in all patients from that group compared to the sensitive tumors. Peculiarities of growth of tumor explants evaluated by morphological criteria has shown after activation with Il-2, antitumor action of LAK toward sensitive tumors manifests itself in migration of single cells from explants, initial stage of monolayer formation and absence of destruction, whilst in the chemoresistant group — in the destruction of tumor cells in the majority of cases (see Table 2, Fig. 3, 4).

Fig. 3. The growth of rhabdomyosarcoma cells (control), х 200

Fig. 4. Inhibition of the growth of rhabdomyosarcoma cells upon co-cultivation with LAK, х 200

Table 2. Sensitivity of explants of soft tissue tumors to different anticancer drugs and action of PBL and LAK

<table>
<thead>
<tr>
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<th>Antitumor action of lymphocytes on the growth of tumor explants</th>
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Notes: “+” — sensitive; “-” — insensitive.
The increased sensitivity to action of LAK of chemoresistant tumor cells may be caused by altered expression of some membrane proteins in tumor cells — Pgp [2, 22] or p170 [5, 23]. Also, the ability of tumor cells influenced by mediators secreted by LAK, to produce active compounds (in particular, ATP) that may affect cytotoxic activity of lymphocytes, should be considered as well [24]. Other publications reported on the role of II-1β-receptor and NF-kappaB expression in chemoresistant pancreatic tumor cells [25–29].

At last, in the experiments on MC-sarcoma model it has been shown that in ascitic fluid of mice the content of adenosine is increasing, that causes the inhibitory effect on LAK cell cytotoxicity; such inhibition occurs via subtype of adenosine receptor (AdoR2A) upon increased level of cAMP [30]. These data may explain why in some cases LAK demonstrate no cytotoxicity toward tumor cells.

The multiplicity of factors influencing antitumor activity of LAK toward chemoresistant tumors and the sensitivity of the latest to action of LAK may in part explain our data on association of elevation of activity of LAK and chemoresistance of tumors. Activation of lymphocytes with II-2 could level the intensity of anti-tumor action of PBL of the patients with soft tissue and epithelial tumors.

REFERENCES


