The Use of Autologous Peripheral Blood Stem Cells as a Hemopoietic Support during Polychemotherapy of Children with Soft Tissue Sarcomas

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Intensification of cycle polychemotherapy in disseminated tumors considerably improves the efficiency of complex treatment. Reinfusion of peripheral blood stem cells as a factor of replacement treatment during hemopoietic suppression or disorders is now becoming more and more promising.

Key Words: *polychemotherapy; stem cells; soft tissue sarcomas*

Rabdomyosarcoma is one of the most incident tumors of soft tissues in children and adolescents: 50 to 65% soft tissue sarcomas (STS) are rabdomyosarcomas [12] and 13 to 19% are synovial sarcomas [2,8].

Complex treatment considerably improves the prognosis for patients with localized process, whereas survival of patients with disseminated stages of rabdomyosarcoma and synovial sarcomas did not significantly change over the last 30 years [6]. Five-year relapse-free survival after standard treatment regimens is <30%, therefore these patients can be assigned to prognostically unfavorable groups [3-5, 9-11].

Intensification of cyclic polychemotherapy (PCT) in disseminated tumor process considerably increases the efficiency of complex treatment, which gives hope for increasing the duration of remission and improving the prognosis for patients with disseminated process. However, survival improvement is associated with pronounced toxicity and high mortality. Toxicity of intensive chemotherapy (CT) leading to sustained hypoplasia of the bone marrow (BM) became the obstacle in further dose escalation and treatment intensification. The possibility of restoring the population of stem and polypotent cells via reinfusion of hemopoietic cells helped to overcome this obstacle and to intensify CT by several times.

Previous studies demonstrated the possibility of collection of peripheral blood stem cells (PBSC) after the initial course consisting of single injection of cyclophosphamide in a dose of 4 g/m^2 and stimulation with granulocytic-macrophage CSF and use of these cells after each of 4 courses of high-dose CT [13]. The possibility of many intensive CT courses with PBSC support was also shown. After 3 and more PCT courses, reinfusion of subtransplantation doses of peripheral stem cells mobilized with CSF after the first and second PCT courses can shorten the duration of neutropenic fever and, hence, the intervals between CT courses, which gives hope for improving the results of treatment of children with high-risk STS [1]. The possibility of using this method for the treatment of patients with stage IV rabdomyosarcoma is demonstrated for multicyclic intensive CT with PBSC support. Eight CT course (VACIME protocol) was performed. PBSC were collected 2 times: after the second and fourth PCT courses. Reinfusion of PBSC was performed after 3rd, 4th, 5th, and 6th courses. Surgery was performed after the 6th course and radiotherapy after the 8th course. Complete effect for the primary focus was obtained in 75% cases and very good partial effect was observed in 25% cases. The interval between the courses did not exceed 28 days. The severity of mucositis against the

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background of PCT was considerably lower during the same PCT with PBSC support. Despite PBSC support, the development of cumulative III-IV degree thrombocytopenic toxicity progressively aggravating from the first to the eighth course was noted. No cumulative granulopoietic toxicity was noted, but III-IV degree neutropenia was observed [5,7].

The aim of this study was to improve treatment efficiency and survival of children with high-risk STS.

MATERIALS AND METHODS

At the Institute of Children Oncology and Hematology, children with disseminated STS are treated according to an original protocol. Twenty-two patients with disseminated STS of different localization (7 boys and 15 girls aging 2-15 years, mean age 10.15±4.05 years) examined and treated at Institute of Children Oncology and Hematology, N. N. Blokhin Cancer Research Center, Russian Academy of Sciences were enrolled into the study protocol developed at the Musculoskeletal Tumor Surgical Department in cooperation with BM Transplantation Department in 1998-2008. Synovial sarcoma was morphologically confirmed in 8 patients (36.4%). Synovial sarcoma more often involved the lower extremities (5 cases, 62.5%), while upper extremities were involved in 2 cases (25%) and one patient (12.5%) had tumor of the body. In patients with rabdomyosarcoma, the tumor was located on the body in 7 cases (50.0%) and on the lower and upper extremities in 5 (35.7%) and 2 (14.3%) cases, respectively. At first examination, metastases in the lungs were found in 2 patients (9.1%), in the bones in 1 patient (4.56%), combined metastases were detected in 7 patients (31.8%), and metastases in lymph nodes were present in 12 patients (54.5%).

The control group for comparative assessment of the efficiency of therapy included 35 patients (21 boys and 14 girls aging from 4 months to 15 years, mean age 8.86±4.21) treated in 1990-1999. Synovial sarcoma was diagnosed in 11 patients (31.4%); it more often involved the lower extremities (7 cases, 63.6%), while upper extremities were involved in 3 cases (27.3%) and one patient (9.1%) had tumor of the body. In patients with rabdomyosarcoma, the tumor was located on the body in 8 cases (33.3%) and on the lower and upper extremities in 9 (37.5%) and 7 (29.2%) cases, respectively. At first examination, metastases in the lungs were found in 13 patients (38.2%), combined metastases in 7 patients (20.6%), metastases in soft tissues in 2 patients (5.9%), and metastases in lymph nodes in 12 patients (35.3%).

The main and control groups were comparable by the sex, age, and histological diagnosis. Treatment program in the control group implied induction PCT; here it consisted in PCT courses performed before local control including radiotherapy with surgery (in case of operable tumor) or without it. At the stage of induction, an IVA combination was used: vincristine (1.5 mg/m^2, stream intravenous infusion on day 1), actinomycin (1500 μg/m^2), holoxan (3 g/m^2, drip intravenous infusion on day 2).

The protocol of treatment for children of the main group included the following stages: induction intensive PCT with collection of PBSC after the 2nd or 3rd course on the assumption of complete BM sanation over 4 courses, broad-field radiotherapy of the lungs in case of their metastatic involvement after the 2nd PCT course, surgical removal of the tumor; radiotherapy of the primary focus (if surgical treatment is impossible) or the bed of the removed tumor, consolidating PCT courses similar to induction ones with hemopoietic support with subtransplantation doses of PBSC.

Induction PCT in disseminated STS (4 courses) included cyclophosphamide (400 mg/m^2, drip intravenous infusion on days 1-5), etoposide (100 mg/m^2/day, drip intravenous infusion on days 1-5), and carboplatin (500 mg/m^2, drip intravenous infusion on day 4).

PBSC were collected after 2 courses of induction CT in the absence of BM impairment (light microscopy data).

Leukopheresis was performed on Baxter CS-3000plus or CobeSpectra constant-flow separator. The result of PBSC separation, content of CD34$^+$ cells, was evaluated on a flow cytofluorometer using CD34 анти-HPCA-2 monoclonal antibodies (Becton Dickinson) in Laboratory of Radioimmunology (N. N. Blokhin Cancer Research Center). The collected material (PBSC) was frozen in liquid nitrogen using dimethylsulfoxide as a cryopreserving agent and stored in the BM Bank of Institute of Clinical Oncology, N. N. Blokhin Cancer Research Center).

Broad-filed irradiation of the lungs (in case of their metastatic involvement) in a total focal dose of 10.8-12 Gy was performed after the 2nd PCT course after collection of PBSC.

At the stage of local control, primarily organ-sparing surgery was performed; wide excision of soft tissue tumors was performed in 18 cases (81.8%).

Radiotherapy of the tumor bed or primary tumor focus was carried out in a total focal dose of 45-57 Gy, radiotherapy of metastases was performed in the total focal dose of 30 Gy after completion of induction CT.

The stage of consolidation in the treatment of children with STS consisted of 4 PCT courses. On day 7 after each PCT course, PBSC in small doses (0.9-1.5±0.1×10^6 CD34$^+$/kg body weight) were reinfused. The interval between the courses was 21-27 days (mean interval 26.00±0.54 days).

RESULTS

Immediate efficiency of induction CT in the main group was 80%. Second and third degree therapeutic pathomorphosis was found in 46.6 and 20% operated patients. Evaluation of the toxicity of consolidation therapy showed that IV degree leukopenia, thrombocytopenia, and anemia accompanied 74.6, 53.7, and 29.9% courses. The decrease in absolute leukocyte count (ALC) below 1000 cell/μl was observed on day 8.35±0.36 of CT. The minimum ALC decrease was 100 cell/μl. The maximum leukocyte drop was observed on day 11.35±0.34. The duration of leukopenia in cases with ALC<1000 cell/μl varied from 1 to 21 days (mean duration 7.47±0.49 days). The recovery of leukocyte count to ALC>1000 cell/μl was observed on day 16.00±0.45 of CT.

The decrease in absolute platelet count (APC) below 75,000 cell/μl was observed on day 9.64±0.5 of CT. The maximum APC decrease was 4000 cell/μl. The maximum platelet drop was observed on day 13.26±0.40. The duration of thrombocytopenia in cases with APC<75,000 cell/ml varied from 5 to 23 days (mean duration 11.45±0.73 days). The recovery of platelet count to ALC>75,000/μl was observed on day 21.58±0.74 of CT. The decrease in hemoglobin content to 79 g/liter was observed on day 8.39±0.56 of CT. The minimum decrease in hemoglobin content was 44 g/liter. The maximum drop in hemoglobin content was observed on day 12.79±0.62. The duration of anemia in cases with hemoglobin <79 g/liter varied from 1 to 20 days (mean duration 8.70±0.71 days). The recovery of hemoglobin content above 79 g/liter was observed on day 16.72±0.68 of CT. One patient died from complications developing against the background of consolidation PCT.

Five-year relapse-free survival was 21.2±9.9% for patients receiving protocol treatment and 3.7±3.6% in the group of retrospective control. The survival in the control and main groups differed significantly (p=0.0001).

The use of intensive regimen of CT by the cyclophosphamide–vepeside–carboplatin protocol with hemopoietic support with autologous PBSC improved 5-year survival of patients with prognostically unfavorable STS.

Collection of PBSC after induction CT in all children with high-risk STS is possible after the second or third course of induction CT. The use of subtransplantation doses of autologous PBSC reduces hematological toxicity, shortens the period of febrile neutropenia, and allowed us to perform intensive PCT program. Hemopoietic support with PBSC during CT courses preceded by radiotherapy can reduce the severity of thrombocytopenia (caused by CT courses performed after induction courses) and the incidence of grave organotoxic complications.

Thus, the use of subtransplantation doses of PBSC in children with unfavorable prognosis is an efficient way of reducing hematological toxicity after repeated CT courses.

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