ONCOLOGY

New Therapeutic Approach to the Prevention of Metastases of Spontaneous Osteogenic Sarcoma to the Lungs

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Transfusion of allogenic bone marrow to prevent metastasizing of osteogenic sarcoma was studied as an alternative to aggressive chemotherapy in cases when it is impossible to preserve the limb. The study was carried out on 62 dogs with spontaneous osteogenic sarcoma. A single preoperative transfusion of allogenic bone marrow was carried out in 42 dogs one day before the limb amputation and 20 animals were treated by surgery alone (amputation or exarticulation). All the animals were observed for the rest of their lives. In comparison with the control, in the group treated by bone marrow transfusion the median of metastases-free period was notably longer: 20 dogs survived for a long time, some of them died from natural causes in old age. Relative indications for bone marrow transfusion in osteogenic sarcoma are defined with consideration for age and tumor volume.

Key Words: *osteogenic sarcoma; bone marrow transfusion; metastases*

Despite the progress in the treatment of osteogenic sarcoma (OS), this therapy requires further improvement [4,7,8]. The potential of the bone marrow (BM) transfusion, an alternative of intensive chemotherapy, has been studied [1].

Hematogenic metastases to the lungs of highly malignant sarcoma in Syrian hamsters were suppressed by a single transfusion of intact BM cells from normal allogenic donors. The BM cells effectively suppress metastases to the lungs represented by individual or small groups of cells, but the treatment is ineffective after formation of micrometastases [1]. A three-year survival improved in 24 patients treated by transfusions of allogenic BM in comparison with patients treated by surgery [2]. Further course of disease after treatment with BM was not reported. We studied the remote results of the treatment with BM transfusions. Dogs with spontaneous OS, a classical analog of human tumors with hematogenic metastases, but with a 4-6 times more rapid progress [6] were observed.

Our purpose was to evaluate the remote results, the outcome of treatment, and the prospects for the application of this method. We evaluated the effectiveness of preoperative transfusion of whole (BM_W) and separated (BM_S) BM, the relationship between the treatment effectiveness and animal age and the tumor size, and defined the indications for BM transfusions in the treatment of osteosarcoma in humans.

MATERIALS AND METHODS

The study was carried out in 62 dogs with spontaneous OS. The animals were treated at the Experimental Therapy Clinic of the Oncology Research

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Center in 1986-1995. The majority of dogs were of large and giant breeds, weighing 9-89 kg, aged 1-12 years (mean age 6.5±0.7 years). Experimental group (BM transfusions) consisted of 42 dogs, control (surgery) of 20 dogs. All the animals were examined by general clinical methods and special laboratory tests. Sex, age, breed, body weight, status of organs, constitution, family cancer history, disease duration, tumor size and site, a history of bone diseases, traumas, and provocative incidents were taken into consideration. The dates of the disease onset, beginning of the follow-up, operation, development of metastases, death or euthanasia were recorded. The disease was diagnosed by clinical, x-ray, cytological, histological methods, and by autopsy.

The primary tumor node was examined by x-rays in two projections, and the lungs were x-rayed. In the studies of the primary tumor node, the stage of the process was roentgenologically estimated by multiplying three maximum diameters of the tumor [5]. We think that a tumor of 125 cm^3 corresponds to stage I, up to 700 cm^3 to stage II, more than 700 cm^3 without remote metastases to stage III, and tumor of any size with remote metastases to stage IV [5].

Cytological studies were carried out routinely using Romanowsky-Leischmann's staining. Simultaneously with cytological studies, material for histological studies was collected by transbiopsy. Postoperative material was analyzed by histological methods (at least 10 blocks per specimen).

The majority of dogs were autopsied; parenchymatous organs and tumor metastases were collected for histological examination.

BM donors were young mongrel dogs. Before collecting BM, the dogs were examined, vaccinated, helminths were eliminated, biochemical and clinical analyses of the blood were performed, immunological studies were carried out, and the donor-recipient blood compatibility was tested. The interval between BM collections in one donor was at least 6 months. When selecting the donors, special attention was paid to ruling out cancer. Immunological studies of donor blood and BM were aimed at evaluating the cytotoxic activity of natural killer cells and their capacity to activation.

BM was obtained by exfusion from a spongy bone. The operation was performed under total anesthesia under sterile conditions. BM was collected from the tubercle of the humerus, the upper flaring portion of the ilium, and the great trochanter by Kassirsky's needles: 150 ml from one side and 150 from the other into a sterile bag with anticoagulant. The total count of nuclear cells in the suspension and myelogram were determined immediately. If BM_S suspension was planned for transfusion, BM was centrifuged at 2000 rpm for 10 min at 18°C and separated in a special device, isolating the BM cells. The resultant BM or the centrifugate volume was adjusted to 500 ml with 5% glucose, polygluquine, or medium 199.

The treatment of the dogs consisted in a single transfusion of BM one day before the limb amputation. BM dose was at least 100×10^6 nuclear cells/kg. BM was drip-infused in the involved limb vein with constant clinical monitoring. The suspension of BM cells was injected immediately after myelograms and total counts of nuclear cells in the suspension for transfusion were evaluated.

The compatibility was tested by cross tests and biological compatibility only before the transfusion of BM_W. If there was no incompatibility reaction for 5-10 min, infusion was carried out at a rate of 60 drops/min.

Surgical operation (amputation or exarticulation of the limb) was performed under routine multicomponent anesthesia. The amputation was always beyond the involved bone. After the dog had recovered after the operation, it was returned to its master and checked up (including x-ray examination of the lungs) every month and after 3 months, every 3 months.

The median of the metastases-free period (in days) was the criterion for assessing the results. The duration of the metastases-free period was counted starting from the day of operation till the moment when metastases in the lungs were detected.

Our objective was the prevention of hematogenic metastases to the lungs. Therefore, only detection of metastases in the lungs was considered as the end of metastases-free period. Lymphogenic metastases and hematogenic metastases to other organs are not typical of OS and were neglected.

Results were statistically processed by Kaplan-Mayer's method.

RESULTS

In control dogs, the median of metastases-free period was 55 days and there were no long survivors, while in experimental group this value was 122 days and 20% dogs survived for 365-1901 days, $p=0.0002$, $t=13.3$).

In studies of the effect of BM on metastases of OS we had to refuse from BM_W injections because of numerous complications resulting in pulmonary artery thromboembolism and to use BM_S free from erythrocytes. In dogs transfused BM_S, the median of metastases-free period was 119 days and in those transfused BM_W it was 129 days ($p<0.1$, $t=2.5$, Figs. 1 and 2).

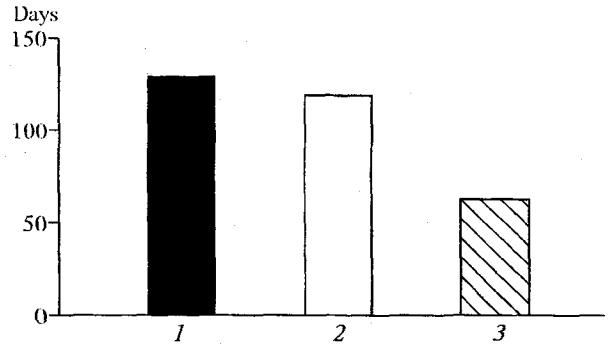

Fig. 1. Median of metastases-free period in groups treated by transfusions of whole (*1*) and separated (*2*) bone marrow suspension and in the control (*3*).

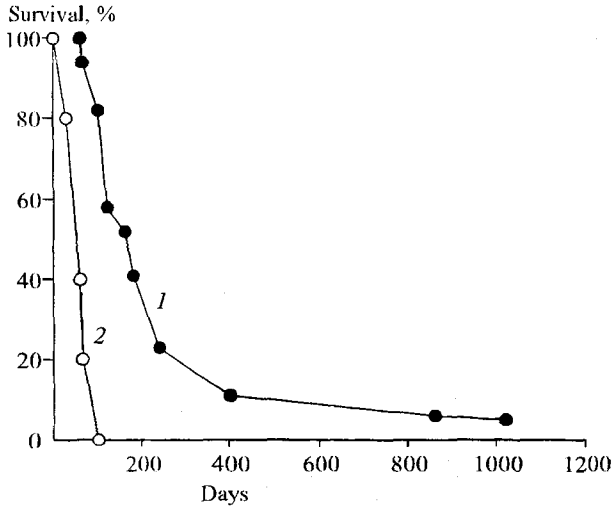

Fig. 2. Survival without metastases of animals transfused bone marrow (*1*) and subjected to amputation alone (*2*).

Since the material was heterogeneous, statistical analysis was carried out on a sampling. For more reliable estimation of the effectiveness of BM_W and BM_S, the animals with the most typical localization of OS (humerus and radius) of a most typical age (4-9 years) were selected in each group and a sampling totality was thus formed. In the group transfused BM_S, the median of metastases-free period was 183 days and in the group transfused BM_W it was 106 days. In this case, too, the difference between the groups was statistically insignificant (p=0.5, t=0.3). Since BM_W and BM_S infusions are equally effective, we united these two groups and compared them with the control group, which matched for age and localization of primary OS. In the BM transfusion group the median of metastases-free period was 152 days *vs.* 53 days in the control (p=0.000009, t=24).

The majority of dogs were presented with stages II (n=14) and III (n=21) of the process, judging from the size of the tumor. The differences between the two groups were analyzed; the median of metastases-free period was 67 and 148 days, respectively (p=0.01, t=5.8).

Genetic disorders in the mechanisms of antitumor defense have been paid much attention of late. The prognosis is initially unfavorable for young animals (aged up to 2-3 years) with OS (G. R. Rutteman, 1997). For evaluating the relationship between the effectiveness of the therapy and the animal age, we divided the animals into two unequal groups: aged under 2 years and aged 2-10 years. In young dogs the median of metastases-free period was 77 days vs. 259 days in older dogs (p=0.06, t=3.5).

Studies of cytotoxic activity of natural killer cells in blood donors yielded virtually the same data, as well as blood analysis of the recipients by the first examination. In the recipients with metastases the activity of natural killer cells and their capacity to activation were much lower than at the beginning of the disease, but these differences were statistically insignificant.

Transfusion of BM significantly prolonged the duration of the metastases-free period in dogs with spontaneous OS in comparison with the controls. There was no difference between preoperative infusion of BM_W and BM_S. Therefore, erythrocytes and part of neutrophils possess no significant antitumor activity, at least in these animals. The use of BM_S is preferable due to a lower incidence of complications (pulmonary artery thromboembolism) and lack of erythrocyte and protein loading and need in evaluating the blood group compatibility.

Our data indicate that the prognosis is initially unfavorable for young animals under 2 years in comparison with the rest dogs, which may be explained by apoptosis disorders. The method cannot be recommended for very young animals.

The effect of the tumor volume on the duration of metastases-free period looks paradoxical. Metastases developed later in animals with the third stage of the process than in those with the second stage. In our study the animals with small tumors were subjected to limb amputation for pathological bone fractures or rapid involvement of soft tissues and skin. It is obvious that aggressive OS rapidly develops metastases, and the prognosis is the worst for animals with these tumors. By contrast, slowly growing tumors reaching large size may run a more benign course.

This method cannot compete with chemotherapy at the early stages of the process, because it does not help preserve the limb, but in local disseminated processes BM transfusion can be the method of choice with effectiveness comparable to that of polychemotherapy.

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