ONCOLOGY

Neoadjuvant Chemotherapy and the Cyclooxygenase Pathway of Arachidonic Acid Metabolism in Osteogenic Sarcoma

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Arachidonic acid metabolites are measured in primary osteogenic sarcomas, benign neoplasms, and tumor-like bone lesions. In typical osteogenic sarcomas, the contents of E and $F_{2\alpha}$ prostaglandins are significantly higher than in benign bone tumors or tumor-like bone lesions. Neoadjuvant chemotherapy has different effects on the levels of prostaglandins E and $F_{2\alpha}$, thromboxane B_2, and prostaglandin $F_{1\alpha}$ in osteogenic sarcomas, its effectiveness depending on the mechanism of drug action and individual sensitivity of tumor cells to this drug. A negative correlation is established between the content of prostaglandins E in osteogenic sarcoma and the degree of therapeutic pathomorphosis of the tumor in patients treated with adriamycin during the preoperative period. A single transfusion of a suspension of nonactivated allogenic bone marrow cells markedly reduces the content of prostaglandins E in osteogenic sarcoma.

Key Words: *osteogenic sarcoma; prostaglandins; neoadjuvant chemotherapy*

Osteogenic sarcoma (OS) is a tumor that often develops during puberty. It has a number of morphological variants and is characterized by an extremely aggressive course, poor response to therapy, and early hematogenous dissemination [7,12]. The investigation into prostaglandins, potent endogenous bioregulators, may be helpful for better understanding of the mechanisms underlying the metastasizing of osteosarcoma and for the development of biochemical criteria to evaluate the susceptibility of osteosarcoma to chemotherapy.

An enhanced prostaglandin synthesis in malignant cells has been demonstrated previously [1,2,10]. The ability of the cyclooxygenase blockers to control tumor growth may be associated with production of prostanoids by neoplastic cells [2], since cyclooxygenase is a key enzyme of the arachidonic acid metabolism. Considerable attention has been focused on prostaglandins E (PGE) because they are involved in division and proliferation of tumor cells [10].

Previously, we showed that the PGE content is higher in poorly differentiated OS (osteoblastic, anaplastic, and telangiectasic) than in moderately and highly differentiated OS [5].

In this study we compared the contents of the arachidonic acid metabolites other than PGE in OS in order to find out how they are influenced by neoadjuvant therapy, taking into account therapeutic pathomorphosis of primary tumor. This enabled us

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to establish additional criteria for evaluating the metastasizing activity of OS.

MATERIALS AND METHODS

A total of 166 patients with primary osteosarcoma and 28 patients with benign bone tumors or tumor-like bone lesions were examined using clinical, roentgenological, and morphological methods. Most of the patients (72%) were in their twenties.

In all patients, clinical and roentgenological diagnosis was confirmed by histological examination of the tumor or tumor-like lesion. Morphological variants of OS were identified according to the classification [8]. Patients with the following morphological variants of OS were included in the study: osteoblastic (40% of patients with OS), anaplastic (21.7%), telangiectasic (17.5%), periosteal (9.2%), chondroblastic (9.2%), and highly differentiated (2.4%). In the majority of patients (74%), the tumor was located in the epi- and metaphyses of the bones forming the knee joint. Benign bone neoplasms or tumor-like bone lesions (n=28) included chondroblastoma (6 cases), enchondroma (2), osteoblastoma (3), osteoid osteoma (1), osteocartilaginous exostosis (13), benign fibrous histiocytoma (1), aneurysmal osseous cyst (1), and ossifying hematoma (1).

Some patients were examined by angiography, radioisotope scanning of the skeleton, computerized tomography, ultrasonography, and biochemical tests for hepatic, renal, and pancreatic functions.

Generalized OS (metastases in the lungs) was diagnosed in 7% of the patients.

Therapeutic pathomorphosis of the tumor [6] was evaluated in all patients receiving neoadjuvant therapy during the preoperative period.

According to the type of neoadjuvant therapy, the patients were divided into 7 groups:

Group 1 included 65 patients who received intra-arterial infusion of adriamycin (30 mg/m^2) for 3 days followed by a 12-14-days radiation therapy (total dose 20 Gy) involving the entire affected area. Surgical removal of the tumor was performed on day 17-19.

Group 2 consisted of 19 patients who received 1 to 3 intra-arterial infusions of platinum preparations (platin, platidiam, or cisplatin) in a total dose of 150 to 750 mg depending on individual tolerance.

In group 3 (n=18), a suspension of nonactivated allogenic bone marrow cells was infused 16-18 h before surgical removal of OS to prevent hematogenous metastases in the lungs. A second infusion of the bone marrow suspension was performed one day after surgery. This method of prevention metastasizing in the lungs was developed at the Laboratory of Antitumor Immunity (Institute of Carcinogenesis, Oncology Research Center) [9].

Group 4 patients (n=5) were treated with high doses of methotrexate (total dose 10-12 g).

Group 5 (n=9) received 1-9 courses of mono- or polychemotherapy with adriamycin, methotrexate, vincristine, and/or cyclophosphane. In addition, some patients received radiation therapy (total dose 40 Gy). All patients of group 5 were treated at the Clinic for Tumors of the Musculoskeletal System (Oncology Research Center). Six of them had no distant metastases or tumor reoccurrence; in 3 patients, radiation therapy was ineffective. Surgical removal of the tumor was performed in all patients, after which they received adjuvant chemotherapy.

Groups 6 and 7 served as the controls.

Group 6 included 50 patients with typical OS. They received no specific therapy during the preoperative period.

Group 7 consisted of 28 patients with benign bone tumors or tumor-like bone lesions. Prior to analysis of the arachidonic acid metabolites, these patients received no specific treatment.

The arachidonic acid metabolites were extracted with ethyl acetate [12]. Their content was measured using radioimmunoassay kits for the determination of PGE and $PGF_{2\alpha}$ (Clinical Assays), thromboxane B_2 (TxB_2), and prostacyclin ($PGF_{1\alpha}$) (Amersham). The results were expressed in ng/g tissue and analyzed using the software for statistical processing of medical data.

RESULTS

Table 1 shows the contents of eicosanoids in OS. The PGE content in typical OS ranged from 0.7 to 56.1 ng/g tissue. The PGE content of benign bone tumors and tumor-like bone lesions varied from 0.5 to 3.2 ng/g tissue. In OS of patients who received no neoadjuvant therapy, the PGE content was higher than in benign bone tumors or tumor-like lesions.

The $PGF_{2\alpha}$ levels were higher in OS of these patients (8.5±1.4 ng/g tissue) than in benign bone neoplasms and tumor-like bone lesions (3.2±0.8 ng/g).

Osteosarcomas had higher contents of TxB_2 than benign bone tumors and tumor-like bone lesions (Table 1).

The levels of prostanoids in OS depended on the type of therapy. In some patients, the PGE content correlated with the intensity of therapeutic pathomorphosis of the tumor (Table 1).

After surgical removal and histological examination, the OS from patients of groups 1, 2, and 5 were classified into grades 1-2 and 3-4 according to the intensity of tumor pathomorphosis. All tumors from

TABLE 1. Neoadjuvant Chemotherapy and Arachidonic Acid Metabolism in Osteogenic Sarcomas (*M±m*)

Grade of therapeutic pathomorphosis of tumor	No. of patients	Arachidonic acid metabolites, ng/g tissue			
		PGE	$PGF_{2\alpha}$	TxB_2	$PGF_{1\alpha}$
Adriamycin+radiotherapy					
1-2	31	13.0±1.6[1]	8.3±1.6	2.0±0.3	2.1±0.3
3-4	34	4.7±0.6[2]	5.8±1.4	2.7±0.4	2.4±0.4
Platinum preparations					
1-2	11	6.4±1.6	4.2±0.6	3.3±0.6	2.1±0.4
3-4	8	4.2±0.8	3.1±0.2	3.9±0.7	2.7±0.4
Transfusion of bone marrow cell suspension					
1-2	18	4.6±0.6	4.3±2.1	5.6±1.5	3.7±1.3
High-dose methotrexate					
1-2	5	11.7±2.5	10.3±2.5	1.5±1.0	1.4±0.9
Polychemotherapy+radiotherapy					
1-2	6	24.6±5.4[3]	5.8±1.9	2.3±0.4	2.0±0.4
3-4	3	4.3±1.2[4]	3.8±1.2	2.9±0.4	3.0±0.4
Control specimens					
Osteosarcomas untreated before surgery	50	10.1±1.2[5]	8.5±1.3[1]	3.6±0.4[1]	1.5±0.2
Benign bone tumors or tumor-like bone lesions	28	2.4±0.46[6]	3.2±0.8[2]	0.9±0.1[2]	1.1±0.2
p	1 *vs.* 2<0.05	1 *vs.* 2<0.05 3 *vs.* 4<0.05 5 *vs.* 6<0.05	1 *vs.* 2<0.05		

group 4 patients (treatment with high doses of methotrexate) and group 3 patients (a single infusion of bone marrow cell suspension) were of grade 1-2. The intensity of therapeutic pathomorphosis, which can be evaluated as the percent of destroyed tumor cells, may be indicative of tumor susceptibility to the prescribed therapy.

We have hypothesized a close relationship between the effectiveness of therapy assessed by morphological characteristics of primary focus of OS, the levels of prostanoids in the tumor, and the degree of therapeutic pathomorphosis of the tumor. However, the intensity of therapeutic pathomorphosis was associated only with the PGE level.

Thus, among the patients given intra-arterial infusion of adriamycin and a course of radiation therapy as well as among the patients after chemotherapy and/or radiotherapy, the decrease in the PGE content was greater in OS with grade 3-4 therapeutic pathomorphosis than in those with grade 1-2. The PGE level in OS with grade 1-2 therapeutic pathomorphosis after adriamycin therapy did not differ significantly from that in the OS of patients who had not received neoadjuvant therapy.

By contrast, no association between the PGE level and the intensity of therapeutic pathomorphosis of the tumor was found in the patients treated with intra-arterial infusions of platinum preparations (group 2): PGE levels were considerably and to a similar extent reduced in osteosarcomas of grade 1-2 and grade 3-4.

Interestingly, PGE levels were markedly decreased in all OS from patients given a single infusion of nonactivated allogenic bone marrow cells (group 3). It should be noted that the PGE level in this group differed slightly from the mean PGE content in OS with grade 3-4 therapeutic pathomorphosis.

Although after neoadjuvant therapy the $PGF_{2\alpha}$ content in osteosarcomas was lower than in the control (untreated patients), it did not differ significantly in osteosarcomas with grade 1-2 and 3-4 therapeutic pathomorphosis. The lowest $PGF_{2\alpha}$ content was recorded in patients treated with platinum preparations.

Neoadjuvant therapy induced no significant decrease in the TxB_2 and $PGF_{1\alpha}$ contents of OS. A tendency toward an increase in these parameters was observed in tumors with grade 3-4 therapeutic pathomorphosis compared with those of grade 1-2 and OS from patients given an infusion of bone marrow cell suspension.

A tendency toward an enhanced synthesis of prostanoids displayed by malignant neoplasms may be associated with the stimulatory effect of PGE on cell proliferation and, consequently, on the formation of metastases [1,10].

Immunocompetent cells are involved in the processes of tumor metastasizing [3,9]. PGE is known to inhibit the cytostatic activity of effector cells (natural killer cells, macrophages, and neutrophils), thus promoting metastasizing [4].

Our results suggest that OS cells more actively synthesize PGE and $PGF_{2\alpha}$ than cells of benign bone tumors and tumor-like bone lesions, which agrees with the observation that the ability to synthesize eicosanoids is increased in transformed animal and human cells [10]. Our morphometric studies suggest that sarcomatous cells contain higher levels of PGE compared with the stromal components of OS [12].

The present study showed that different preoperative therapies have different effects on PGE levels in OS. These differences may depend on the mechanisms of action of chemotherapeutic agents and on individual sensitivity of tumor cells to drugs and ionizing radiation. For example, the PGE content is markedly reduced in OS with grade 3-4 therapeutic pathomorphosis, in which >75% of cells are irreversibly changed after adriamycin therapy (group 1) or chemotherapy and/or radiotherapy (group 5).

On the other hand, OS from some adriamycin-treated patients had grade 1-2 therapeutic pathomorphosis and contained virtually the same amount of PGE as OS of untreated patients. This is consistent with the observation that PGE are synthesized predominantly by tumor cells but not by stromal components [10].

It was reported that the mechanism of action of platinum drugs on the metabolism of arachidonic acid, a precursor of PGE, has certain specific features, and that these drugs modify the activity of cyclooxygenase, a key enzyme of the PGE synthesis [1]. This may account for a decrease in the PGE content in all OS (regardless of the degree of their therapeutic pathomorphosis) from patients treated with platinum preparations.

The PGE levels in osteosarcomas are important for the prognosis of hematogenous dissemination of the disease. Previously, we proposed a method for evaluating the degree of OS malignancy and, consequently, for prognosis of hematogenous metastases [12]. The prognosis was unfavorable for more than 70% of patients receiving preoperative adjuvant therapy with the PGE content in primary tumor ≥9.4 ng/g tissue, regardless of the type of therapy. However, it should be remembered that a decrease in the PGE content of OS treated with platinum preparations may be associated with the specific mechanism of action of these preparations and that the effectiveness of therapy must be confirmed only by morphological evaluation of therapeutic pathomorphosis of the tumor. The above-mentioned method of predicting the development of metastases cannot be applied to these patients.

The patients given a single infusion of allogenic bone marrow cells are of special interest. On the one hand, experiments with cultured animal tissues showed that the contact of effector cells with tumor cells stimulates the release of PGE from the surface of tumor cells and lowers the cytotoxic activity of immunocompetent cells [3,4]. This indicates that PGE plays an important role in the mechanisms whereby tumor is protected against the cytotoxic effect of immunocompetent cells [9]. On the other hand, administration of nonactivated allogenic bone marrow cells prevents the development of hematogenous metastases and reduces their occurrence in experimental animals [9]. A considerable decrease in the PGE content of OS after infusion of bone marrow cells is probably associated with the release of PGE from the surface of sarcomatous cells. However, this should be confirmed by further investigations, at least by the determinations of plasma PGE concentrations before and after transfusion of bone marrow cells. Prevention of metastasizing by this method will permit a retrospective analysis in patients with OS.

Our findings [1] and those of other researchers indicate that PGE are involved in tumor growth. An enhanced synthesis of these prostaglandins was observed in tumor cell clones with a high metastatic potential [4]. Consequently, the drugs modifying the arachidonic acid turnover in the membranes of tumor cells may change proliferative activity (metastasizing potential) of the tumor. At the current stage of clinical investigations, none of existing approaches to this problem can be preferred. Nevertheless, we believe that the blockers of PGE synthesis in combination with current therapies provide a new strategy in the treatment of patients with OS.

From our results it can be concluded that:

1. The contents of prostaglandins E and $F_{2\alpha}$ are significantly higher in OS than in benign bone tumors or tumor-like bone lesions.

2. Neoadjuvant therapy has different effects on the contents of the arachidonic acid metabolites in OS, depending on the mechanisms of action of the drugs and individual sensitivity of tumor cells to them.

3. There is a negative correlation between the PGE content in OS and the intensity of therapeutic pathomorphosis of the tumor in patients receiving prolonged adriamycin therapy during the preoperative period.

4. A single transfusion of nonactivated allogenic bone marrow cells results in a marked reduction in the PGE and $PGF_{2\alpha}$ contents of osteosarcoma.

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