PHARMACOLOGY AND TOXICOLOGY

Content of Testosterone and Activity of 5α-Steroid-NAD(P)Δ4-Oxidoreductase in Normal Bone Tissue and Malignant Bone Tumors

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Optimal conditions for measuring 5α-steroid-NAD(P)Δ4-oxidoreductase in primary bone tumors and in normal bone tissues are experimentally chosen *in vitro*. It is shown that the bone belongs to tissues with low activity of this enzyme. A considerable activation of 5α-steroid-NAD(P)Δ4-oxidoreductase is found in primary bone tumors, the content of testosterone being unchanged.

Key Words: *bone tissue; bone tumors; testosterone; 5α-steroid-NAD(P)Δ4-oxidoreductase*

According to current views, androgens are the factors of endocrine regulation of the growth and development of the bone tissue [4,5]. They produce direct effects, which is confirmed by the presence of specific receptors in osteoblasts, and act indirectly [7,8]. It is known that the development of primary bone tumors is accompanied by shifts in the endocrine status of the organism, in particular, increased plasma level of free testosterone (TS). A concept has been proposed that hyperandrogyny is a risk factor of bone sarcoma [2,3]. Biochemical mechanisms of androgenic activity in organs and tissues have been extensively studied [1]. In this context, the regulation of bone tissue in its carcinogenic transformation is of particular interest.

The aim of the present study was to compare the content of TS and activity of 5α-steroid-NAD(P)Δ4-oxidoreductase (5α-OR), the key enzyme in TS biosynthesis in normal bone tissue and in bone neoplasm.

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MATERIALS AND METHODS

The study was performed on experimental and clinical material. Optimal conditions for assaying 5α-OR activity were experimentally chosen in albino random-bred male rats weighing 180-200 g. In the clinical study, these parameters were determined in primary bone tumor bioptates obtained from patients of the Oncology Research Center, Russian Academy of Medical Sciences. Bioptates of metaphyseal parts of long tubular bones obtained at the Traumatology Department of the First Municipal Hospital (Moscow) during surgery for acute trauma served as the control.

The content of TS in homogenates of normal bone tissue and bone neoplasms was determined by radioimmunoassay using standard Amersham kits. Activity of 5α-OR was measured radiometrically with thin-layer separation as described elsewhere [6]. The data were processed using standard statistical tests.

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TABLE 1. Content of TS and Activity of 5α-OR in Normal Bone Tissue and Bone Tumors in Male and Female Patients

Parameter		Control	Malignant bone tumors
Content of TS, nmol/mg protein:			
	total	58.6±17.7 (8)	51.1±11.9 (13)
	males	82.3±32.3 (4)	56.5±16.2 (7)
	females	34.9±7.0 (4)	37.0±12.3 (6)
Activity of 5α-OR, nmol/min×mg protein:			
	total	0.025±0.002 (10)	0.071±0.010 (13)*
	males	0.025±0.004 (5)	0.082±0.017 (7)*
	females	0.024±0.004 (5)	0.059±0.006 (6)*

Note. *$p<0.05$ compared with the control. The number of patients is shown in parentheses.

Significance of the differences was evaluated using the nonparametric *U* Wilcoxon—Mann—Whitney test.

RESULTS

The content of TS was measured by a standard analytical method, and 5α-OR activity was measured using a method, which is usually applied in other androgen-dependent tissues. It has been found that the rate of 5α-dihydrotestosterone (DHT) formation from TS is proportional to sample weight and incubation time, while optimum pH lies around 6.5.

The mean 5α-OR activity in various tissues of albino random-bred male rats decreases in the following order: seminal vesicles → skeletal muscles → bone; the rate of TS—DHT conversion in the bone tissue approximates that in skeletal muscles, but is considerably lower than in seminal vesicles. These findings suggest that under physiological conditions the growth and development of bone tissue is regulated by TS, but not by its metabolites as in other androgen-dependent organs and tissues [4,5].

The aim of clinical study was to evaluate the relationship between the content of TS and 5α-OR activity in the bone tissue under conditions of tumor transformation in comparison with normal bone tissue.

The experimental group consisted of 13 nontreated patients with primary bone- and cartilage-forming tumors affecting various parts of the skeleton: osteogenic sarcoma (6), chondrosarcoma (4), and Ewing's tumor (3). Control group comprised 8 patients with bone fractures, treated at the Traumatology Department of the First Municipal Hospital (Moscow).

TABLE 2. Individual Values of TS Content and 5α-OR Activity in Malignant Bone Tumors

Sex		Age, years	Content of TS, nmol/mg protein	Activity of 5α-OR, nmol/min×mg protein
Osteogenic sarcoma				
	F	14	34.9	0.084
	F	12	25.3	0.042
	F	16	19.0	0.066
	M	21	117.1	0.069
	M	13	116.1	0.182
	M	16	32.0	0.049
Chondrosarcoma				
	F	33	12.5	0.063
	M	22	24.3	0.083
	M	59	24.3	0.063
	M	25	23.4	0.076
Ewing's tumor				
	F	19	95.6	0.050
	F	18	34.9	0.047
	M	13	58.6	0.054

We have found that the concentration of TS in bone samples varies from 13.7 to 144.1 nmol/mg protein, while 5α-OR activity lies within 0.021-0.028 nmol/min×mg protein (Table 1).

Clinical studies showed that the content of TS greatly varies in control and experimental groups. It was somewhat higher in males and lower in females; however, these differences were statistically insignificant (Table 1). The hormone concentration was practically the same in tumor tissues and normal bone samples (control group) in mixed groups and in males and females separately.

Primary tumor samples of practically all examined patients exhibited considerably elevated 5α-OR activity (2.8-fold in comparison with the control, Table 2). This parameter did not depend on sex, age, and histological structure of the tumor (Table 2) and weakly correlated with tissue level of TS (r=0.47, n=13).

Our findings suggest that elevated activity of 5α-OR, a key enzyme in the metabolism of androgens, rather than the tissue level of TS is a peculiarity of androgen regulation of the bone tissue. This presumably results in accumulation of DHT, a more potent androgen than TS, in bone tumors. These data provide a new insight into pathogenesis of primary bone tumors and are important for seeking new approaches to their treatment.

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