Synthesis of 5α-Dihydrotestosterone in Human Bone Tumors of Various Histological Structure

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In human primary bone tumors of various histological origins, metabolism of testosterone led to the formation of 5α-reduced metabolites (mainly 5α-androstane-3α,17β-diol, and 5α-androstane-3β-17β-diol), but not 5α-dihydrotestosterone. Transformation of 5α-dihydrotestosterone to other metabolites in human primary osteosarcomas is assumed to be associated with its inactivation or involvement of metabolites of this androgen in unknown regulatory mechanisms in bone tumors. Therefore, *in vitro* evaluation of 5α-reductase activity in human primary osteosarcomas and other tumors by 5α-dihydrotestosterone formation could yield erroneous results.

Key Words: *bone tumors; androgens; metabolism*

Pathogenesis of bone tumors is obviously associated with enhanced androgen biosynthesis. On the other hand, these tumors can produce some androgens [1, 2,4]. This implies the involvement of androgens in the regulation of some functions in bone tumors and the presence of androgen-metabolizing enzymes in the bone tissues.

Metabolism of androgens in various target tissues has long been studied, but the data on bone tissues are contradictory. These tissues were shown to express some enzymes of androgen metabolism [11,12].

Our recent experiments showed that androgens are metabolized in human primary osteosarcomas containing the main enzymes of androgen metabolism. Activities of 3α-hydroxysteroid oxidoreductase (3α-HSOR) and 3β-hydroxysteroid oxidoreductase (3β-HSOR) far surpassed those of other enzymes. In spite of relatively high activity of Δ^4steroid:NAD(P)-oxidoreductase (5α-R) in osteosarcomas, biotransformation of testosterone did not lead to the formation of 5α-dihydrotestosterone (5α-DHT) in the majority of tumors. However, other 5α-reduced metabolites, 5α-androstane-3α, 17β-diol (3α-D) and 5α-androstane-3β,17β-diol (3β-D) were found in these tumors.

Here we compared the formation of 5α-DHT from testosterone in osteosarcoma, chondrosarcoma, and giant cell tumor and benign bone neoplasms.

MATERIALS AND METHODS

Patients with osteosarcoma (n=19), chondrosarcoma (n=10), giant cell bone tumor (n=7), and benign bone tumor (n=5) were examined.

Testosterone androgens 5α-DHT, 3α-D, 3β-D, 5α-androstane-3,17-dione, 4-androstene-3,17-dione (A4) were form Sigma, and other reagents from Merck and Beckman. [1,2,6,7-^{3}H]-Testosterone (^{3}H-testosterone, specific activity 80-110 Ci/mmol) and 5α-dihydro-[1,2,6,7-^{3}H]-testosterone (^{3}H-DHT, specific activity 80-105 Ci/mmol, Amersham) were used.

Silufol plates (15×15 cm) coated with Silica gel (100 μ) were used for separation of metabolites and purification of ^{3}H-testosterone and ^{3}H-DHT. Chromatography was performed 3 times in benzene:acetone: ethanol system (9:1:0.5 volume ratio). This procedure allowed us to separate not only the main androgen metabolites, but also 3α-D and 3β-D stereoisomers.

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Androgen metabolites in bone tumor samples were assayed as described elsewhere with minor modification [6]. Bone tumor samples were powdered in liquid nitrogen using a porcelain mortar, and 0.05 M Na-phosphate buffer (pH 7.4) in a volume of 700 µl/200 mg tissue was added. These samples were then defrosted, and the homogenate was centrifuged at 5000*g* for 10 min under cooling in an Optima TM TLC centrifuge (Beckman). The supernatant was used for measuring activities of androgen metabolism enzymes. ^{3}H-Testosterone or ^{3}H-DHT (98% purity, 8-9× 10^4 cpm/sample) and ethanol solution of testosterone or 5α-DHT, respectively, were put in 12×70-mm glass tubes to a final steroid concentration in the incubation solution of 10^{-7} M. The solvent was evaporated in a water bath at a temperature below 60°C. Into cooled tubes (in ice), 50 µl tumor homogenate (or 50 µl Na-phosphate buffer in the control) and 50 µl Na-phosphate buffer containing 100 µg D-glucose, 100 µg D-glucose-6-phosphate, 25 µg $NADH_2$, 25 µg $NADPH_2$, and 50 mU glucose-6-phosphate dehydrogenase were added. The samples (3 experimental and 3 control for each substrate) were incubated for 1 h at 37°C, and 10 µl 1 N HCl and 10 µl ethanol solution of unlabeled androgens containing the analyzed metabolites in concentrations of 1 mg/ml were added. Lyophilized steroids were dissolved in 100 µl absolute ethanol:benzene mixture (9:1). The samples (80-90 µl) were placed on a plate and chromatographed. The plate was then dried at 80°C for 10 min, and androgen spots were developed in iodine vapors, cut out, and transferred into vials with a scintillator. Radioactivity was measured in an LS 6500 spectrometer (Beckman).

The content of each metabolite (in fmol) was determined considering the substrate concentration and the ratio between radioactivity in the spot and total radioactivity in all spots. Enzyme activities (5α-R, 3α-HSOR, 3β-HSOR, and 17β-HSOR) were estimated by the content of the corresponding metabolite (or total content of metabolites) per 1 mg total protein of the homogenate per 1 h. During incubation with testosterone, 5α-R activity was determined by the formation of 5α-DHT, 3α-D, and 3β-D, while 17β-HSOR activity was evaluated by the formation of A4. During incubation with DHT, 3α-HSOR and 3β-HSOR activities were determined by the formation of 3α-D and 3β-D, respectively.

The concentration of total protein in the homogenate was measured by the method of Lowry [8] on a DU 650 spectrophotometer (Beckman).

RESULTS

Activity of the main enzyme of androgen metabolism (5α-R) was determined by the total content of 5α-reduced metabolites formed from testosterone (5α-DHT,

TABLE 1. Formation of 5α-DHT and Ratio of Androgen-Metabolizing Enzyme Activities in Primary Bone Tumors

Tumor	Patient, No.	5α-DHT, fmol/mg protein	5α-R/hydroxylases*
Osteosarcoma (*n*=19)	1	968	0.584
	2	252.9	0.080
	3	1891.8	0.616
	4	1648	0.330
	5	124.6	0.181
	6	62.25	0.018
	7	104.5	3.54
Chondrosarcoma (*n*=10)	1	106.25	0.110
	2	96.55	8.076
	3	405.7	0.378
	4	53.57	0.011
	5	356.5	0.097
Benign tumor (*n*=5)	1	238.7	0.064
	2	48.48	0.015
Giant cell tumor (*n*=7)	1	87.5	0.002
	2	41.02	0.031
	3	1087.69	0.085
	4	30	0.219

Note. *Total activity of steroid hydroxylases (17β-HSOR, 3α-HSOR, and 3β-HSOR).

3α-D, and 3β-D). In our opinion it is of fundamental importance for bone tumor, because synthesis of these metabolites (i.e. 5α-R activity) was found in all tumors, but 5α-DHT was found only in some neoplasms (Table 1).

Our findings showed that in all primary bone tumors of various histological types, 5α-DHT was not the final metabolite of testosterone, but was transformed to other 5α-reduced androgen metabolites (mainly 3α-D and 3β-D). The ratio between 5α-R activity (by 5α-DHT formation) and the total activity of 3 steroid hydroxylases (17β-HSOR, 3α-HSOR, and 3β-HSOR) was far below 1 in the majority of studied tumors (Table 1). Only in 2 patients (patient 7 with osteosarcoma and patient 2 with chondrosarcoma), this value surpassed 1. However, steroid hydroxylase activities in tumors of these patients were much lower than in other tumors. Thus, steroid hydroxylase activities in the majority of studied primary bone tumors of various histological types were considerably higher than 5α-R activity. This suggests that metabolism of the main androgen testosterone in bone tumors is directed to inactivation of 5α-DHT but not to its accumulation in tumor cells.

The regulatory effects of androgens in bone tumors are not necessarily mediated through the receptor mechanism [5,7]. Not only DHT, but also testosterone can act as the main androgen of the receptor-mediated regulatory mechanism under conditions of high 3α-HSOR and 3β-HSOR activities, since not only bone tumors express androgen receptors [3,10]. These results indicate that 5α-DHT-mediated regulatory mechanisms can change in primary bone tumors, and/or 3α-D and 3β-D formed from 5α-DHT are involved in regulatory processes in these neoplasms. It can not be excluded that 3α-D serves as a 5α-DHT reserve pool in bone tumors (similarly to other tissues) [9]. Studying androgen metabolism in bone tumors *in vitro*, 5α-R activity can not be estimated by the formation of 5α-DHT only, without considering other 5α-reduced metabolites (primarily 3α-D and 3β-D). Thus, 5α-R activity should be determined from the total content of 5α-reduced androgens. This is obviously true when testosterone metabolism in various tissues is studied. At the same time, the use of 5α-DHT as a substrate provides more precise information on 3α-HSOR and 3β-HSOR activities.

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