Reductase Activity of 17β-Hydroxysteroid Oxidoreductase in Prostatic Tumors of Different Histological Structure

V. G. Degtyar', T. V. Babkina, I. A. Kazantseva*, A. P. Morozov*, M. F. Trapeznikova*, and N. E. Kushlinskii

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 139, No. 6, pp. 684-687, June, 2005
Original article submitted March 16, 2005

Reductase activity of 17β-hydroxysteroid oxidoreductase in biopsy specimens of prostatic cancer and benign hyperplasia, and prostatic intraepithelial neoplasia and serum concentrations of testosterone, 5α-dihydrotestosterone, 4-androstene-3,17-dione were compared in patients and healthy individuals. Reductase activities of 17β-hydroxysteroid oxidoreductase in soluble fraction of prostatic biopsy specimens decreased in the following order: prostatic cancer>prostatic intraepithelial neoplasia>>benign prostatic hyperplasia. No differences in serum concentrations of testosterone, 5α-dihydrotestosterone, 4-androstene-3,17-dione between these three groups of patients were found, while the mean serum concentration of these androgens in patients with prostatic tumors did not surpass the threshold normal values for men. Hence, high reductase activity of 17β-hydroxysteroid oxidoreductase can be associated with pathogenetic mechanisms of human malignant prostatic tumors.

Key Words: *prostatic cancer; benign prostatic hyperplasia; prostatic intraepithelial neoplasia; 17β-hydroxysteroid oxidoreductase*

Androgens play a key role in the regulation of normal and tumor-transformed growth of the prostatic gland [3,5]. Depending on the period of development, human gonads release different androgens into the blood, but qualitative and quantitative composition of androgens in prostatic cells depends on activity of androgenesis enzyme systems in the prostatic gland, rather than on their secretion by gonads and adrenals [2]. It is androgen metabolism that determines the content of each androgens in prostatic gland cells.

Now the role of only two important androgens in human prostatic gland is well studied: testosterone and 5α-dihydrotestosterone (DHT), while physiological roles of other androgen metabolites are only hypothesized. Presumably, various pathologies of the prostatic gland are caused by changes in activities of androgenesis enzymes. In prostatic cancer these changes can shift the ratio between androgen metabolites formed in the organ, *e. g.*, increase DHT content in hyperplastic prostate and decrease DHT and increase testosterone and 4-androstene-3,17-dione (A4) contents in prostatic cancer [8,9].

Testosterone transformation into DHT catalyzed by 5α-reductase is the most important reaction of androgen biotransformation in the prostate [4,10]. Activity of 17β-hydroxysteroid oxidoreductase (17β-HSOR) catalyzing mutual conversion of the main androgens (testosterone and A4) is also essential for biological effects of androgens. Six 17β-HSOR isoenzymes differing by activity and substrate specificity were detected in humans, but the prostate contains mainly type 5 17β-HSOR isoenzyme. Reductase and dehydrogenase activities of this enzyme in human prostate are normally low, though reductase activity (testosterone formation) predominates. The ratio of 17β-HSOR reductase/dehydrogenase activities can appreciably change in prostatic cells in some prostatic diseases [2,5,8,9], but the data of different authors are contradictory.

N. N. Blokhin National Center for Cancer Research, Russian Academy of Medical Sciences, Moscow; *M. F. Vladimirskii Moscow Regional Research and Clinical Institute

0007-4888/05/1396-0715

We studied 17β-HSOR reductase activity in biopsy specimens of prostatic tumors of different histological structure and measured serum concentrations of the main androgens (testosterone, DHT, A4) in these patients.

MATERIALS AND METHODS

The study was carried out in patients with prostatic cancer (11 patients), benign prostatic hyperplasia (32 patients), and prostatic intraepithelial neoplasia (8 patients).

The following chemicals were used: inorganic salts, D-glucose (E. Merck), NADH and NADPH, A4 (Sigma), testosterone and progesterone (Koch-Light Lab.), hydrochloric acid, organic solvents (benzene, acetone, ethanol), crystal iodine, scintillation fluid ZhS-8 (Beckman scintillation fluid in some cases), ether for narcosis (stabilized), [1,2,6,7-^{3}H]4-androstene-3,17-dione (specific radioactivity 100 Ci/mol) from Amersham Pharmacia Biotec UK Ltd.

The [^{3}H]androgen substrate was purified and androgen metabolites were separated by chromatography on plates (15×15 cm) with a thin (100 μ) silicagel (Silufol) layer (Kavalier) in a benzene—acetone—ethanol system (9:1:0.5 v/v/v, 3 times).

Fragments of prostatic tumor biopsy specimens were crushed in a porcelain mortar in liquid nitrogen, Na-phosphate buffer (0.01 mol/liter, pH 7.4) was added (1 ml buffer/200 mg tissue), and the mixture was defrosted on ice. The homogenate was centrifuged at 5000*g* with cooling for 10 min (Optima TM TLC centrifuge, Beckman). Supernatant was collected for measuring enzyme activity and protein concentration (by the method of Lowry).

Indicator solution (10 μl) of purified [^{3}H]A4 (about 8×10 cpm/sample) and 10 μl ethanol solution of unlabeled A4 of required concentration (selected so that the final concentration of the substrate in the incubation sample was 10^{-7} mol/liter) were put into 12×70 mm glass tubes. Ethanol solution of progesterone (30 μmol/liter) was added into the samples for inhibiting activity of 5α-reductase [1]. The solvent was evaporated on a water bath at a temperature <60°C. The homogenate (50 μl) was put into tubes on ice (50 μl phosphate buffer into control sample), and 50 μl solution (in phosphate buffer) containing 100 μg D-glucose, 25 μg NADH, and 25 μg NADPH was added. After incubation in a water bath at 37°C for 30 min, 10 μl 1 N hydrochloric acid, 10 μl ethanol solution of testosterone, and A4 in a concentration of 1.0 mg/ml were added to each sample, after which the samples were lyophilized. Benzene-ethanol (absolute) mixture (9:1 v/v; 100 μl) was added to the dry residue, 70-80 μl solution of each sample was applied to plates, chromatographed, and developed in iodine vapor. The spots of the corresponding androgens were cut, put into scintillation vials, incubated with 0.5 ml methanol for 30 min, and 5 ml scintillation fluid was added. Radioactivity was measured on an LS 6500 spectrometer (Beckman), the content of androgen metabolites formed by 1 mg total homogenate protein was estimated. Enzyme activity was expressed in picomoles of the corresponding metabolite formed by 1 mg total homogenate protein over 30 min. Protein concentration in the homogenate was measured by the method of Lowry on a DU 650 spectrophotometer (Beckman).

Serum concentrations of testosterone, DHT, A4 were measured on an ACS 180 plus biochemical analyzer (Bayer), concentration of testosterone was measured using reagent kits for chemiluminescent analysis (Bayer), and concentrations of DHT and A4 using enzyme immunoassay kits (DRG Instruments GmbH).

RESULTS

Reductase activity of 17β-HSOR was detected in all prostatic tumor biopsy specimens and varied within a wide range (Fig. 1). The lowest activity of 17β-HSOR was detected in soluble fraction of benign prostatic hyperplasia, but in both types of malignant tumors 17β-HSOR activity was by one order of magnitude higher ($p<0.0001$ for benign prostatic hyperplasia *vs.* prostatic cancer and prostatic intraepithelial neoplasia, according to Spearman's test). Activity of 17β-HSOR in prostatic intraepithelial neoplasia and cancer virtually did not differ. Hence, reductase activity of 17β-HSOR in soluble fraction of biopsy specimens can be ranked as follows: prostatic cancer>prostatic intraepithelial hyperplasia>>benign prostatic hyperplasia.

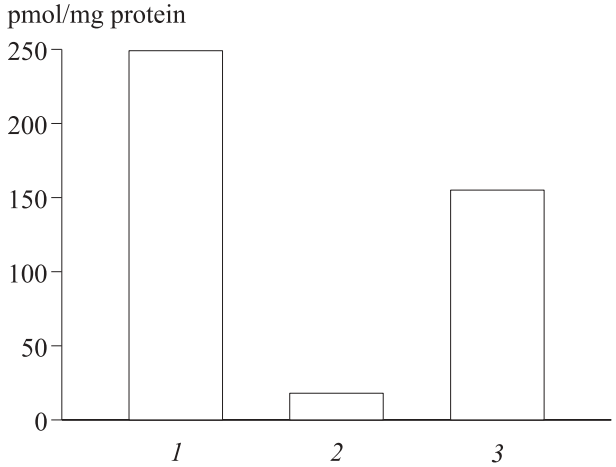

Fig. 1. Reductase activity of 17β-hydroxysteroid oxidoreductase in soluble fraction of prostatic tumors of different histological structure. *1*) prostatic cancer; *2*) benign prostatic hyperplasia; *3*) prostatic intraepithelial neoplasia.

TABLE 1. Serum Concentrations of the Main Androgens in Patients with Prostatic Tumors of Different Histological Structure

Group	Parameter		A4, ng/ml
	testosterone, nmol/liter	DHT, pg/ml	
Control	14.5 (1.7-22.4)	654.2 (187.4-1356.0)	1.6 (0.2-3.9)
Benign prostatic hyperplasia	15.1* (1.2-24.3)	684.7 (194.3-1408.7)	1.7 (0.2-4.9)
Prostatic cancer	17.8 (10.9-29.9)	637.6 (211.5-946.7)	1.4 (0.3-3.2)
Prostatic intraepithelial neoplasia	15.3 (9.9-19.8)	868.9 (453.2-1443.1)	1.9 (0.5-3.6)

Note. *Mean values. Range of values is given in parentheses.

No differences in the serum concentrations of testosterone, DHT, and A4 were detected in patients of all three groups, the mean serum concentration of these androgens in patients with prostatic tumors did not surpass the threshold normal values for men (Table 1).

Great attention is now paid to 17β-HSOR enzyme, highly prevalent in many tissues. This enzyme plays an extremely important role in mutual transformations of estrogens and androgens in steroidogenic and in many peripheral tissues, particularly in sex steroid target tissues [10]. There are good grounds to consider that this enzyme plays an exceptional role in the maintenance of physiological concentrations of the main sex effector steroids in the corresponding target cells. Presumably, activity of 17β-HSOR is associated with the mechanism of prostatic regulation by androgens after castration, which is important in prostatic cancer [4]. The concentration of A4 in prostatic tissue appreciably increases in cancer because 17β-HSOR activity increases (in parallel with decrease of 5α-reductase activity), while the concentration of DHT does not change (or decreases) [2, 8,9].

Published data and our findings suggest that high reductase activity of 17β-HSOR can be associated with the pathogenetic mechanisms of malignant prostatic tumors in humans.

REFERENCES

1. T. Anono, Y. Kitamura, S. Fukuda, and K. Matsumoto, *Steroid Biochem.*, **14**, No. 12, 1369-1377 (1981).
2. W. Bartsch, H. Klein, U. Scheimann, *et al. Ann. N. Y. Acad. Sci.*, **595**, 53-66 (1990).
3. N. Bruchovsky, *Cancer Medicine*, Eds. J. F. Holland *et al.*, 4th ed., Baltimore (1997), pp. 1133-1148.
4. K. Griffiths, M. S. Morton, and R. J. Nicholson, *Eur. Urol.*, **32**, Suppl. 3, 22-40 (1997).
5. F. K. Habib, *Eur. Surg. Oncol.*, **23**, No. 3, 664-668 (1997).
6. J. M. Hakimi, M. P. Schoenberg, R. H. Rondinelli, *et al.*, *Clin. Cancer Res.*, **3**, No. 9, 1599-1608 (1997).
7. R. F. Morfin, S. Di Stefano, J. F. Charles, and H. H. Floch, *J. Steroid Biochem.*, **13**, 529-532 (1980).
8. P. Negri-Cesi, A. Colciago, A. Poletti, and M. Motta, *Prostate*, **41**, No. 4, 224-232 (1999).
9. C. M. Smith, S. A. Ballard, M. G. Wyllie, and J. R. Masters, *J. Steroid Biochem. Mol. Biol.*, **50**, Nos.3-4, 151-159 (1994).
10. J. D. Wilson, *Endocr. Rev.*, **20**, No. 5, 726-737 (1999).