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Androgen receptor assays in specimens of prostatic tissue obtained by transurethral resection and transvesical adenomectomy

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Summary. The main goal of this study was to ascertain whether routine transurethral resection (TUR) of prostate may provide useful material for the evaluation of androgen receptor (AR) status. At the same time, either intracellular distribution of binding affinity and capacity of receptor molecules were particularly taken into account. Based on our previous findings in breast and endometrial cancer, we suggest that a "functional" receptor status may correspond to the presence of type I (high affinity, low capacity) AR in both soluble and nuclear fractions. However, the precise significance of type II (lower affinity, higher capacity) binding sites remains to be clarified. Ten samples of large prostatic adenomas, obtained by transvesical adenomectomy (TVA), were compared with ten parallel specimens obtained by an in vitro TUR, whereby a pure cutting current was used. The AR assay was carried out with a standard competition method using tritiated mibolerone as the radioligand and Scatchard analysis for data processing. No significant difference between the TUR and TVA groups emerged concerning type I AR content of soluble, nuclear or soluble together with nuclear fractions; this was also true when the results were expressed either as fmol/ml homogenate or as fmol/mg DNA. Similarly, concentrations of type II AR in TVA and TUR samples did not differ significantly in either cell compartment, although they were widely scattered, especially in the soluble fraction. In the light of our findings, it is suggested that TUR specimens represent suitable material for receptor studies, provided that only cutting current is employed and that the use of coagulation current, to control bleeding from the prostatic bed, is confined to the final step of the TUR procedure.

Key words: Androgen receptors – Binding sites – Transurethral resection – Transvesical adenomectony – Prostatic adenoma

Steroid receptors are universally considered an essential requirement for achieving hormonal control of steroid – sensitive target tissues. The clinical value of oestrogen receptors has been clearly demonstrated in patients with breast or endometrial cancer.

During the past decades, extensive research has been carried on androgen receptors (AR) in human prostatic tissues. In contrast to the findings in patients with breast or endometrial cancer, the value of AR assay in predicting the prognosis and clinical response to endocrine therapy in prostate cancer (PCa) patients is still not clear. Differences in methodological approaches and techniques may be an important factor in explaining these discrepancies.

In particular, sampling conditions can critically influence the end-product of AR assays [20]. This is especially true in the human prostate, where varying sensitivity to both oestrogens and androgens in distinct anatomical regions has been suggested from embryological and comparative primate studies [3, 16]. This issue is further complicated in the cancerous prostate gland where normal, hyperplastic and neoplastic tissues often co-exist, in addition to the biological and biochemical heterogeneity common to most human neoplasias.

The presence of two different binding sites for steroid hormones has been documented in the rat uterus [10] and in human breast cancer tissues [21]. Type I receptors have high affinity but low binding capacity, whilst type II receptors show reduced affinity but high capacity for binding hormones. Recently, we were able to distinguish type I and II AR in both human benign prostatic hyperplasia (BPH) and PCa tissues (Castagnetta et al., in preparation), by using selected ligand range (10^{-10}–10^{-9} M) and dissociation constant (K_d) cut-off (1.0 nM) values for AR assay. Our previous findings in breast and endometrial cancer suggested that a "functional" receptor status corresponded to the presence of type I receptors in both soluble and nuclear fractions [8, 9]. This observation implies an unimpaired receptor system and is classified as receptor positive (+/ve). Conversely, when type I binding sites are absent (or below threshold values) in one or both cell fractions, we assume that receptor machinery may, in some way, be altered and thus we define this condition as receptor negative (−/ve). We have previously reported that only "functional" type I oestrogen receptors appear

to be associated with a favourable prognosis outcome in breast cancer patients [9]; by analogy, receptor studies on prostatic tissues should consider not only the relative amounts of AR in soluble and nuclear fractions, but also their behaviour as type I and II binding sites and, especially, their functional status.

In recent years, the use of suprapubic prostatectomy has decreased with respect to transurethral resection (TUR) for the removal of both BPH and PCa. However, needle biopsy usually provides insufficient amounts of tissue for AR assay. Furthermore, the tissue obtained by needle biopsy represents only a very limited portion of the diseased gland, whose composition is heterogeneous. Open surgery is rarely employed in advanced PCa, in which androgen deprivation is carried out.

Routine TUR surgery would then represent a simple way to obtain adequate amounts of prostatic material for AR assay. However, it has been suggested that the passage of electric current and the heat generated by the cutting loop of the resectoscope might negatively affect the results of the assays, since AR are unstable at high temperatures [19, 20]. Some authors regard this as a disadvantage but not as a formal objection to performing receptor assays, particularly when fragments are large enough to permit removal of the most peripheral layers containing charred tissues [2, 14, 23].

In the present study we compared AR content in prostatic tissues obtained by either TUR or open surgical adenomectomy to determine whether these TUR specimens represent suitable material for receptor studies. Since AR can be found not only in PCa but also in BPH – so-called prostatic adenoma – the latter was employed in our investigations due to the ease in obtaining large amounts of tissue, so that not only intracellular localization of receptor molecules but also their affinity and capacity for binding hormones could be evaluated.

Materials and methods

Large prostatic adenomas were obtained from ten patients who underwent open suprapubic surgery. After removal, BPH samples were immedialely cut in two halves. One half was left intact; from the other half, several fragments (10–30 mg) were obtained using a standard resectoscope (24F sheath), whose loop was activated with the cutting current (set at 40 on the 0–100 scale) of a Bovie electrosurgical unit (Ritter Co. Inc., Cincinnati, USA). Urofluid solution (sorbitol 2.7% w/v, mannitol 0.54% w/v; Pierrel SpA, Milan, Italy) was used as the irrigating fluid. At least 200 mg tissue was used in the receptor assay. The hyperplastic nature of specimens was confirmed by histological examination.

AR assays

All samples were either processed immediately on the day of the surgical intervention or stored at −20 °C in a sucrose/glycerol (50% v/v) buffer system [11].

Fragments of tissues (20–50 mg/ml) were homogenized in buffer [HEPES 10 mM, EDTA 1.5 mM, ditiothreitol 5 μM, sodium molybdate 10 mM, glycerol 30% (v/v)] using a glass/glass homogenizer. The homogenate was then spun at 800 *g* for 5 min to separate the particulate (nuclear) from the supernatant (soluble) fraction.

Aliquots (150 μl) of each cell fraction were incubated overnight at 4 °C against 50 μl of the same buffer with increasing final concentrations (0.1, 0.2, 0.3, 0.5, 0.75, 1, 2, 3, 5 nM) of tritiated mibolerone (17α-methyl-[^{3}H]Mibolerone, Amersham International) as radioligand. A 100-fold excess of cold mibolerone was also used for competition studies. A constant concentration of unlabelled triamcinolone acetonide 10^{-7} M was used to prevent binding of mibolerone to both glucocorticoid and progesterone receptors.

Soluble fraction. After incubation, 0.5 ml dextran-coated charcoal suspension was added to each tube and mixed to remove the unbound radioligand. All samples were then centrifuged at 4 °C for 5 min. Finally, 1-ml aliquots of the resulting supernatant were placed in scintillation vials and counted using a β-counter.

Nuclear fraction. Aliquots (100 μl) of the nuclear pellet were placed in tubes containing 5 ml saline and filtered on pre-wetted Whatman GF/C glass fibre filters, using a Millipore apparatus. Filters were then placed in separate scintillation vials and dried overnight at room temperature. Finally, scintillation cocktail was added and the contents of the vials were counted for radioactivity.

Data analysis and processing. Both type I and II receptors were defined by means of the Scatchard graphical method using a least-square fit routine. The bound over free ratio values were plotted against the hormone-bound picomolar concentrations; the intercept on the abscissa yielded receptor concentration, whilst the slope indicated the affinity constant of the hormone for the receptor, the K_d being its reciprocal. As in a previous report [27], K_d values within 1.0 nM were used for defining type I AR. For type II AR K_d cut-off values were chosen arbitrarily. The K_d cut-off values are quite distinct from that reported for testosterone-oestradiol binding globulin [24]. Generally, type I and II AR were identified at ligand concentration ranges of 1.0×10^{-10} to 7.5×10^{-10} M and of 0.75×10^{-9} to 5.0×10^{-9} M, respectively.

AR concentrations were expressed as fmol/ml homogenate (absolute values) or as fmol/mg DNA for both a single cell fraction and the sum of soluble together with nuclear fractions; the latter is, in our experience, the best way to present the overall receptor levels [7]. DNA tissue content was determined by a modification of the method of Burton [6]. The previous experience of our own and other research groups indicated that DNA is a better reference point, with respect to protein content, for expressing results of receptor assays [7, 28].

Statistics

A two-tailed (paired) Student's *t*-test (95% confidence limits) was used for the statistical comparisons of results.

Results

The distribution and content of both type I and II AR were ascertained in transvesical adenomectomy (TVA) and in in vitro TUR tissues obtained from the same ten surgical specimens.

Type I binding sites were almost equally distributed in both TUR and TVA groups (Table 1). We found that soluble type I receptors were present in six TUR and six TVA specimens, whilst nuclear type I AR were present in all but one of either TUR or TVA samples. The presence of both soluble and nuclear type I receptors was identified in only five of ten TUR and TVA specimens.

Type II AR were widespread in soluble and nuclear fractions of both TUR and TVA samples. Their presence was revealed in the soluble fraction in nine out of ten TUR and TVA specimens, whereas they were detected in the

Table 1. Concentration[a] and dissociation constant (K_d)[b] of soluble and nuclear type I androgen receptors in prostatic tissues obtained by transurethral resection (TUR) and transvesical adenomectomy (TVA)

Case no.	Soluble				Nuclear			
	TVA		TUR		TVA		TUR	
	fmol/ml	(K_d)	fmol/ml	(K_d)	fmol/ml	(K_d)	fmol/ml	(K_d)
1	83	(6.73)	139	(6.01)	105	(4.11)	95	(2.17)
2	–	–	–	–	92	(3.97)	100	(4.60)
3	152	(6.04)	116	(5.75)	80	(2.96)	93	(3.85)
4	157	(9.21)	125	(5.66)	93	(7.08)	85	(8.25)
5	139	(8.92)	–	–	119	(6.08)	77	(3.66)
6	–	–	9	(2.35)	69	(4.64)	59	(5.84)
7	–	–	56	(5.23)	80	(3.14)	108	(8.19)
8	–	–	–	–	52	(2.06)	57	(3.94)
9	91	(4.69)	60	(3.10)	–	–	–	–
10	65	(7.01)	–	–	41	(1.82)	56	(3.50)

[a] Values expressed as fmol/ml homogenate
[b] Values expressed as 1×10^{-10} M

Table 2. Soluble (S), nuclear (N) and S + N type I androgen receptor concentrations (fmol/ml)[a] in BPH tissues obtained by TUR and TVA

	S	N	S+N
TUR	50.5 ± 21.3	73.0 ± 10.1	123.5 ± 23.0
TVA	68.7 ± 20.9	73.1 ± 11.0	141.8 ± 25.6
Stat[b]	NS	NS	NS
(*P* value)	(0.344)	(0.987)	(0.431)

[a] Values are mean ± standard error of $n = 10$ cases
[b] No significant difference, paired Student's *t*-test

Table 3. Soluble (S), nuclear (N) and S + N type I androgen receptor concentrations (fmol/mg DNA)[a] in BPH tissues obtained by TUR and TVA

	S	N	S+N
TUR	499.9 ± 188.7	684.8 ± 107.1	1184.8 ± 262.6
TVA	693.5 ± 227.3	720.0 ± 147.5	1413.5 ± 320.3
Stat[b]	NS	NS	NS
(*P* value)	(0.458)	(0.823)	(0.565)

[a] Values are mean ± standard error of $n = 10$ cases
[b] No significant difference, paired Student's *t*-test

nuclear fraction of nine TUR and of all ten TVA specimens (data not shown).

Concentrations and K_d values of soluble and nuclear type I AR are shown in Table 1. Adenomectomy specimens exhibited slightly higher concentration values, ranging between 65 and 157 (median 115.0) fmol/ml, as compared with those obtained by TUR (range of 9–139, median 84.2 fmol/ml) in the soluble fraction. However, the nuclear AR content was almost identical in the two groups, ranging from 41 to 119 (median 81.2) and from 56 to 108 (median 81.1) fmol/ml in TVA and TUR samples, respectively.

K_d range values, from 2.35 to 9.21×10^{-10} M for the soluble and from 1.82 to 8.25×10^{-10} M for the nuclear fraction, were well below cut-off limits (1.0 nM); this ensured that receptor was purely type I.

As regards AR content, no significant difference between TUR and TVA specimens emerged in either the soluble, the nuclear or in the soluble together with the nuclear fractions (Fig. 1). This was also true when type I AR content was expressed either as fmol/ml homogenate (Table 2) or as fmol/ml DNA (Table 3), as indicated by the respective *P* values (paired Student's *t*-test).

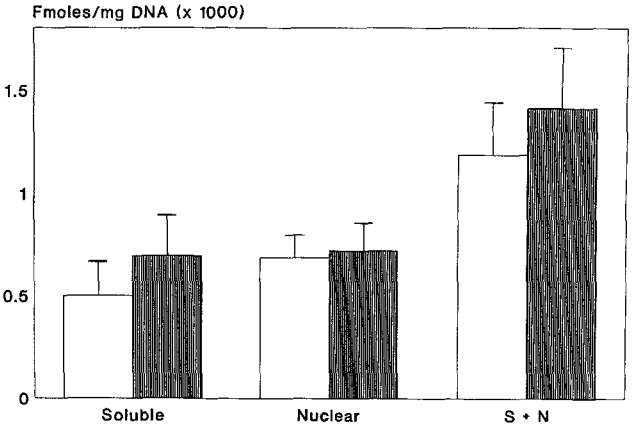

Fig. 1. Comparison of type I androgen receptor concentrations in soluble (*S*), nuclear (*N*) and S + N fractions of human prostatic adenoma tissues obtained by transurethral resection (TUR, □) and transvesical adenomectomy (TVA, ▥). Values are expressed as means ± SEM. No significant difference was found in S ($P = 0.46$), N ($P = 0.82$) and S + N ($P = 0.56$) fractions (paired Student's *t*-test)

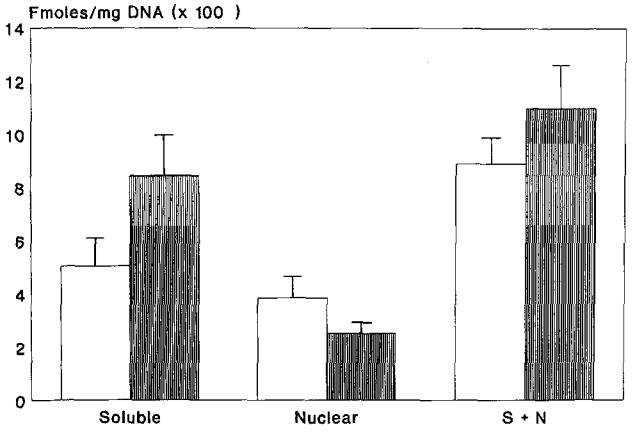

Fig. 2. Comparison of type II androgen receptor concentrations in soluble (*S*), nuclear (*N*) and S + N fractions of human prostatic adenoma tissues obtained by transurethral resection (TUR, □) and transvesical adenomectomy (TVA, ▥). Values are expressed as means ± SEM. No significant difference was found in S ($P = 0.10$), N ($P = 0.07$) and S + N ($P = 0.39$) fractions (paired Student's *t*-test)

Table 4. Soluble (S), nuclear (N) and S + N type II androgen receptor concentrations (fmol/ml)[a] in BPH tissues obtained by TUR and TVA

	S	N	S+N
TUR	567.7 ± 146.4	435.8 ± 91.1	1003.5 ± 129.2
TVA	953.9 ± 232.1	268.2 ± 21.9	1222.1 ± 234.4
Stat[b]	NS	NS	NS
(*P* value)	(0.275)	(0.105)	(> 0.5)

[a] Values are mean ± standard error of $n = 10$ cases
[b] No significant difference, two-tailed signed rank test

Figure 2 reports soluble, nuclear and soluble plus nuclear content of type II binding sites. They ranged widely in both cell compartments. Soluble AR ranged between 117 and 2300 (median 845.5) fmol/ml in TVA specimens. The corresponding figure was 179–1436 (median 404.5) fmol/ml in TUR specimens. The nuclear fraction displayed ranges of 172–395 (median 260.5) and 143–861 (median 361.5) fmol/ml in TVA and TUR samples, respectively. Type II AR levels did not differ significantly in TVA and TUR groups (two-tailed Student's *t*-test), in soluble, nuclear and soluble plus nuclear fractions (Table 4).

Discussion

It has been previously reported that cytoplasmic AR of prostatic specimens obtained by TUR may be altered or even destroyed by heat in the resected chips [1, 18, 19, 26]. However, some studies have pointed out that the operating current setting may be critical [1].

It should be stressed, however, that our in vitro TUR was carried out using only the cutting current, which produces a negligible amount of heat, whereas the coagulation current, which has a strong diathermic effect, was not employed. Therefore, if any alteration in AR concentrations, shown in previous studies using TUR material, was due to a rise in temperature, this would not have occurred in our model. On the other hand, in the clinical situation, surgeons experienced in transurethral procedures use cutting current alone for the resection phase, resorting to coagulation current only in the final step, when haemostasis of bleeding vessels is achieved.

Some investigators have used cold punch (CP) resection as an alternative procedure to remove prostatic tissues endoscopically [12, 17]. This technique does not produce any heat-mediated injury of the resected chips. Previous studies have also compared results of AR assays on prostatic tissues obtained by either TUR or CP resection. Using both techniques in obstructive prostatic cancer, Fentie et al. [13] found no significant difference in nuclear AR content. Only cytosolic AR differed significantly between TUR and CP tissues. These authors hypothesized that nuclear AR of TUR chips was stabilized against heat-mediated inactivation by its binding to endogenous dihydrotestosterone, while the soluble AR, which was steroid free, may more easily undergo inactivation. Ligand-bound steroid receptors are, in fact, more stable than steroid-free receptors [22]. However, Bowman et al. [4] reported that TUR did not differ from CP, in so far as no difference in cytoplasmic AR content of prostatic tissues was observed, provided that only large uncharred samples were employed. This finding is in agreement with other reports [15, 25]. These data substantiate our findings that no significant difference in AR status exists between TUR or TVA material, either in soluble or in nuclear fractions.

However, it should be emphasized that two main issues have been neglected so far in research on AR in prostatic tissues. First, AR have been found to be unevenly distributed across benign and malignant human prostate gland [5]. Soluble AR is more likely to be present in the outer prostate than in the inner (periurethral region) or in specimens taken from the peripheral limit of the resection. This is of great importance, since it is conceivable that different areas may be sampled during TUR, namely the periurethral region at the beginning of the resection and the peripheral (outer) prostate at the end of the resection. Second, the presence of two different androgen binding sites in PCa tissues has been disregarded in most studies. In our experience, it can be hypothesized that, in keeping with results obtained in breast and endometrial cancer, a "functional" receptor status may correspond to the presence of type I AR only, in both soluble and nuclear fractions, but no evidence supporting this assumption has been obtained so far. A better understanding of this problem could be achieved by prospective investigations in which patients with androgen withdrawal are followed with regard to objective response, time to progression and survival. If prior to treatment the AR status is routinely established, a correlation could be investigated between AR status and clinical response. In so far as hormonal therapy is generally given to patients with metastatic disease or those otherwise unsuitable for radical prostatectomy, adequate material for AR determination can

only be obtained, for ethical reasons, by needle biopsy. However, such material is usually insufficient and, in particular, may not be representative of the bulk of the tumour.

Therefore, we conclude that TUR specimens are suitable for AR assays. We also recommend that pure cutting current without coagulation should be employed. The possibility of using TUR material for AR studies may offer a new impetus for research on AR in hormone-dependent prostatic diseases, namely BPH and PCa.

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