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Microassays for androgen and progesterone receptor quantitation as compared with standard saturation analyses in human prostatic tissues

J. Brolin and P. Ekman

Department of Urology, Karolinska Institute, Stockholm, Sweden

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Summary. Simultaneous measurement of androgen and progesterone receptor content in cytosol and salt extractable nuclear subcompartments of benign hyperplastic prostatic tissue was carried out with various microassay techniques and compared to the results from analyses on bulky tissue from the same tissue specimens. The microassays were carried out as modified saturation analyses or as single concentration assays at various degrees of dilution with tris-EDTA-glycerol (TEG) buffer. Tissue samples for the standard assay weighed between 1.76 and 3.22 g, whereas the microassay samples weighed between 0.14 and 0.47 g. When considering the results of the standard assay as the "true" value, the microassays on the same tissue samples tended to underestimate both the androgen and progesterone receptor contents. Data from the microassays showed a wide variation of the androgen and progesterone receptor content in cytosol and nuclei. With the standard assay technique no detectable amount of progesterone receptor was found in the nuclei, whereas the microassays often indicated false-positive progesterone receptor content in this subcompartment. Therefore, the measurements of steroid receptors using biochemical microassays in prostatic tissue are unreliable and not suitable for clinical use, at least with the techniques available today. Reports in the literature based on such assays should therefore be interpreted with great caution.

Key words: Androgen receptors – Progesterone receptors – Microassay techniques – Human prostate

Assays that measure steroid receptor content in human prostatic tissue have been developed to increase our understanding of the endocrine dependency of the prostate and to develop techniques whereby the endocrine sensitivity of a prostate cancer can be estimated.

In previous publications we have reported on the simultaneous measurement of androgen, progesterone and oestrogen receptor content in normal, benign hyperplastic (BPH) and malignant prostatic tissue [3, 8]. The androgen receptor (AR) was most abundant in the nuclear compartment and the progesterone receptor (PR) was only detected in the cytosol. There was a tendency, however, for the AR content to be higher in nuclei of BPH and cancer of the prostate as compared with that of normal prostatic tissue.

All these studies were carried out on bulky tissue pieces, each weighing around 2 g. To develop a technique which might be introduced into the clinical routine, one has to rely upon biopsy material, with a total weight of up to 0.2 g only. We have developed such a technique and have described it previously [6]. However, more recently, using this technique, we obtained highly conflicting data with a wide variation of the AR content and also, in several cases, considerable amounts of intranuclear progesterone receptors, in sharp contrast to results obtained with the standard saturation analysis technique [3]. Data from the literature, which are based on microassay techniches, also report conflicting results [1, 7, 9, 11, 13]. It has been suggested by van Aubel et al. [1] that four biopsy specimens should be taken from each patient and processed separately, the mean value of the receptor content representing the "true" steroid receptor content. However, using such a technique, the authors also found a poor relationship between steroid receptor content and response to endocrine manipulations.

The purpose of this study was to assess the reliability of microassay techniques described earlier [1, 10] and to study further the effect of dilution of the tissue suspensions. As a measure of the "true" receptor content of each case, a bulky tissue from the same specimen was processed in parallel.

Materials and methods

Tissue samples

BPH were recovered from 13 patients undergoing transvesical adenoma enucleation for obstructive symptoms. The adenomas were immediately sliced into pieces and the major parts were sent

Table 1. Microassays used on tissue samples from human benign prostatic hyperplasia (BPH) in analyses of cytosol/nuclear androgen and progesterone receptor content

Microassay	Cytosol/nuclei	Ligand		TEG dilution
		Volume	Concentration	
250 µl Saturation analyses	200 µl	50 µl	0.15-10 nM	4 times
60 µl Saturation analyses	50 µl	10 µl	0.15-10 nM	None
250 µl Single concentration undiluted	200 µl	50 µl	10 nM	None
250 µl Single concentration diluted	200 µl	50 µl	10 nM	2.5 times
60 µl Single concentration undiluted	50 µl	10 µl	10 nM	None
60 µl Single concentration diluted	50 µl	10 µl	10 nM	2.5 times

for histological examination. Tissue samples for receptor studies were frozen to −80°C. The tissue used for standard saturation analyses ("controls") weighed between 1.76 and 3.22 g. From these, small pieces (0.14–0.47 g) were prepared and used for various parallel microassays. Fifteen samples were analysed for both "gross" receptor content (standard saturation analyses) and as various forms of microassays. A total of 29 microassays were evaluated.

Chemicals

[17-methyl-^{3}H]Methyltrienolone (MT) (sp. act., 3200 MBq/mmol) was purchased from New England Nuclear (Boston, mass.). Radioinert steroid (MT) was obtained from Steraloids (Pershing, N.Y.). Activated charcoal, dextran (mol. wt. 73,200), phenylmethylsulphonyl fluoride, triamcinolone acetonide, disodium ethylenediaminotetra acetate (EDTA), and Triton X-100 were purchased from Sigma (St. Louis, Mo.).

Radioligand binding assays

Tisue homogenization, subcellular fractionation and saturation analyses were carried out as described earlier [5, 8]. For saturation analyses [4, 12], six different concentrations of the radioactive ligand [^{3}H]MT were used, ranging from 0.30 to 10 nM, and all these analyses were done in duplicate.

The microassays were carried out as described by Hicks and Walsh [10], using a cytosol/nuclear suspension of 200 µl, adding 50 µl ligand suspension or (as described by van Aubel et al. [1]) using a cytosol/nuclear suspension of 50 µl mixed with 10 µl ligand suspension. For saturation analyses the cytosol/nuclear suspension had to be diluted by approximately 4 vol of TEG buffer (10 mM TRIS, pH 7.4; 1.5 mM EDTA, 10% w/v glycerol) to achieve an incubation volume of 200 µl for each tube. When the 50 µl suspension was used, saturation analyses could be carried out without further dilution.

To evaluate further the effect of dilution on the samples, some microassays were carried out as single concentration analyses. Those microassays were run either undiluted, protein concentrations varying between 2 and 4 mg/ml, or diluted to "critical" protein concentrations between 0.75 and 1.0 mg/ml [8]. All single concentration assays were performed in triplicate, using a ligand concentration of 10 nM, and the receptor content was measured as the mean value from three different tubes containing cytosol or nuclear subfractions.

The different microassays with incubation volumes, ligand concentrations and the degree of dilution are listed in Table 1.

Other methods

The protein content of soluble extracts was assayed by the method of Bradford [2] using bovine gamma globulin as a standard. The receptor content was expressed in fmol/mg protein.

Results

When the results of the saturation analyses on the bulky tissues were regarded as the "true" receptor content, a wide variation was seen in the results of the corresponding microassays. In general, the microassays underestimated the AR content in cytosol as well as in the nuclei. The underestimation of AR content was greater in the nuclei than in the cytosol. In several microassays the AR was almost undetectable. The mean underestimation of the AR content in cytosol was 24%, whereas the mean underestimation of the nuclear AR content was 59%. All the microassays underestimated the PR content in cytosol, in some cases no PR was detected. The mean underestimation of the cytosol PR content was 27%. With the standard saturation analysis technique used, no detectable amount of PR was found in the nuclei, whereas the microassays often indicated the presence of nuclear PR.

No major differences were found in the tendency to underestimate the AR and PR content between the different microassay methods used. The mean underestimation of the AR content in the cytosol was greater in microassays using the saturation analysis technique as compared with those based on single concentration assays.

The results from the various microassays concerning AR and PR content expressed in fmol/mg protein are presented in Tables 2–5 and can be summarized as follows. With the 250 µl saturation analysis microassay technique used, the mean underestimation of the cytosol AR and PR content was close to 60% (Table 2) and the nuclear AR content was underestimated by 75% (Table 3). This microassay also showed false-positive values with regard to nuclear PR content (Table 5).

The 60 µl saturation analysis microassay technique used underestimated the cytosol AR content by a mean value of 47% (Table 2), although there were wide interassay variations, some even overestimating the receptor

Table 2. Androgen receptor content in BPH cytosol: relation between the results of various microassays as compared with the results of the corresponding standard saturation analyses

Method	*n*	Mean under-/ overestimation %	Range %
250 µl Saturation analyses	4	−59.4	−39.7 to − 78.2
60 µl Saturation analyses	7	−47.2	+25.4 to −100
250 µl Single concentration undiluted	5	+ 3.2	+58.4 to − 39.4
250 µl Single concentration diluted	4	+ 5.5	+95.2 to −100
60 µl Single concentration undiluted	6	−27.0	+18.9 to − 69.2
60 µl Single concentration diluted	3	−20.5	+62.3 to − 71.0

Table 3. Androgen receptor content in BPH nuclei: relation between the results of various microassays as compared with the results of the corresponding standard saturation analyses

Method	*n*	Mean under-/ overestimation %	Range %
250 µl Saturation analyses	4	−75.8	−32.4 to −100
60 µl Saturation analyses	4	−65.8	−32.6 to −100
250 µl Single concentration undiluted	5	−50.2	−11.9 to − 81.6
250 µl Single concentration diluted	4	−32.8	+ 9.5 to − 84.6
60 µl Single concentration undiluted	5	−87.0	−72.6 to −100
60 µl Single concentration diluted	3	−39.9	+65.9 to −100

Table 4. Progesterone receptor content in BPH cytosol: relation between the results of various microassays as compared with the results of the corresponding standard saturation analyses

Method	*n*	Mean under-/ overestimation %	Range %
250 µl Saturation analyses	4	−59.8	− 27.6 to − 76.6
60 µl Saturation analyses	7	−35.6	+215.4 to −100
250 µl Single concentration undiluted	5	−27.1	+ 26.9 to − 80.8
250 µl Single concentration diluted	4	− 6.9	+ 68.0 to −100
60 µl Single concentration undiluted	6	−18.8	+119.5 to −100
60 µl Single concentration diluted	3	−11.4	+145.7 to −100

Table 5. Mean progesterone receptor content in BPH nuclei expressed in fmol/mg protein ± SD

Method	*n*	PR
Standard saturation analyses	14	ND
250 µl Saturation analyses	4	3.1 ± 2.1
60 µl Saturation analyses	5	12.4 ± 24.0
250 µl Single concentration undiluted	5	1.0 ± 0.9
250 µl Single concentration diluted	4	14.2 ± 12.2
60 µl Single concentration undiluted	5	1.4 ± 1.4
60 µl Single concentration diluted	3	ND

ND, Not detectable

content. The nuclear AR content, measured with this assay, was underestimated by a mean value of 66% (Table 3) and all assays showed varying degrees of underestimation of the AR content. The PR content in the cytosol was likewise underestimated by a mean value of 36% (Table 4). Again there were wide variations, some analyses even overestimating the receptor content by more than 200%. False-positive values for nuclear PR content were also found in some analyses (Table 5).

The 250 µl single concentration, *undiluted* microassay only slightly overestimated cytosol AR content (3%) (Table 2). However, we found that some of the assays underestimated cytosol AR content (Table 2). The nuclear AR content was unterestimated in all assays employed in this study (Table 3). The cytosol PR content was underestimated by a mean value of 27%, even though one analysis overestimated the receptor content (Table 4). The 250 µl single concentration assay tended to give false-positive values for nuclear PR content.

The 250 µl single concentration, *diluted*, microassay overestimated the cytosol AR content by a mean value of 5% (Table 2) but underestimated the nuclear AR content by 33% (Table 3). The receptor contents recorded varied from a 95% overestimation to a 100% underestimation. The cytosol PR content was underestimated by a mean value of 7% (Table 4). There were wide variations with

over- as well as underestimations. Detectable nuclear PR content was also found (Table 5).

The 60 µl single concentration, *undiluted*, microassay underestimated the cytosol AR and PR contents as well as the nuclear AR content in the range of 19–90% (Tables 2–4). The mean underestimation was most marked for the nuclear AR content (87%) (Table 3). Nuclear PR content was also detected with this assay (Table 5).

The 60 µl single concentration, *diluted*, microassay underestimated the cytosol AR content by a mean value of 20% (Table 2), the nuclear AR content by 40% (Table 3) and the cytosol PR content by 11% (Table 4). The results were highly variable with marked over-and underestimations. No nuclear PR content was detectable with the 60 µl single concentration, diluted, microassay technique.

Discussion

The present study highlights the unreliability of biochemical microassays for the measurement of steroid receptors. The results, when compared with the "true" receptor contents, varied from a three-fold overestimation to zero. Even though the 60 µl single concentration diluted assay seemed to perform slightly better than the other microassays, the range of variation of the results discourages the recommendation of this technique for extended studies. Indeed, it does not seem logical that the diluted assay technique should perform better compared to the non-diluted parallel sample [3, 8].

There are many reasons why microassays perform poorly. We have reported previously, that the available receptor content drops markedly when the protein concentration of the cytosol, or nuclear extract, falls below 1.5 mg/ml [8]. In the diluted samples, the protein concentrations varied between 0.75- and 1.0 mg/ml, hence underestimating receptor levels.

When using single concentration estimates, albeit in triplicate, variable data on total binding and non-specific binding are probably a result of laboratory errors. In saturation analyses, these varations will be "corrected" by the resulting plot. In single concentration estimates each result will be decisive. Therefore, it was surprising that single concentration estimates seemed to perform slightly better than the "micro"-saturation analyses described herein.

A factor contributing to the unreliability of microassays for biochemical steroid receptor analyses is the risk of denaturation when small amounts of tissue samples, like biopsy materials, are processed and compared with bulky material which is processed separately. Biopsy material may be more susceptible to protein denaturation as compared with bulky tissue samples.

The present study seems to shed some light on the conflicting data in the literature on steroid receptor assays using microassay techniques. In conclusion, steroid receptor assays using biochemical techniques are not recommended when only minute amounts of tissue are available. Reports in the literature, based on such techniques, should therefore be interpreted with caution.

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References

1. Aubel O van, Vries JB, Blankenstein MA, Schröder FH (1988) Prediction of time to progression after orchiectomy by the nuclear androgen receptor content from multiple biopsy specimens in patients with advanced prostate cancer. Prostate 12:191
2. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248
3. Brolin J, Andersson L, Ekman P (1991) Steroid receptor profile and receptor stability in subfractions of human prostatic tissues. Critical aspects on microassays. Urol Res 19:327
4. Chamness GC, McGuire WL (1975) Common errors in correction and interpretation. Steroids 26:538
5. Ekman P, Brolin J (1991) Steroid receptor profile in human prostate cancer metastases as compared to primary prostatic carcinoma. Prostate 18:147
6. Ekman P, Snochowski M, Zetterberg A, Högberg B, Gustafsson JÅ (1979) Steroid receptor content in human prostatic carcinoma and response to endocrine therapy. Cancer 44:1173
7. Ekman P, Dahlberg E, Gustavsson JÅ, Högberg B, Pousette Å, Snochowski M (1980) Present and future clinical value of steroid receptor assays in human prostatic carcinoma. In: Iacobelli S, King RJB, Lindner HR, Lippman M (eds) Progress in cancer research and therapy. Hormones and cancer. Raven Press, New York, p 361
8. Ekman P, Barrack ER, Walsh PC (1982) Simultaneous measurement or progesterone and androgen receptors in human prostate: a microassay. J Clin Endocrinol Metab 55:1089
9. Emtage LA, Dunn PJS, Rowse AD (1989) Androgen and oestrogen receptor status in benign and neoplastic prostate disease. Br J Urol 63:627
10. Hicks LL, Walsh PC (1979) A microassay for the measurement of androgen receptors in human prostatic tissue. Steroids 33:389
11. Kirkali Z, Moffat LEF, Leake R, Cowan S, Patel M, Kirk D (1990) Androgen receptors – a method to measure functional behaviour in human prostatic cancer. Eur Urol 17:66
12. Scatchard G (1949) The attraction of proteins for small molecules and ions. Ann NY Acad Sci 51:660
13. Widdowson SM, Ostrowski JL, Dangerfield VJM, Harris SC, Ingleton PM, Underwood JCE, Williams JL, Parsons MA (1989) Microassay for prostatic androgen receptors correlated with quantitative histological assessment. J Clin Pathol 42:322

Jan Brolin, MD
Department of Urology
Karolinska Hospital
Box 60500
S-10401 Stockholm
Sweden