Applied Cancer Research 2009;29(2)65-68

Original Article

Adaptation of an Evaluation Method of 5 Alpha-Reductase Type 1 and Type 2 Enzyme Activities in Prostate Tissue

Emanuel Burck dos Santos; Walter Jose Koff; Osmar Luiz Magalhaes de Oliveira; Marcelo Quintanilha Azevedo; Francine Muraro; Rodrigo Rodrigues; Vera Maria Trindade

Programa de Pós-Graduação em Medicina: Cirurgia da Universidade Federal do Rio Grande do Sul,
Programa de Pós-Graduação em Medicina: Clínica Médica da Universidade Federal do Rio Grande do Sul, Serviço de Urologia do Hospital de Clínicas de Porto Alegre Departamento de Bioquímica da Universidade Federal do Rio Grande do Sul

Abstract

Objectives: We are proposing the adaptation of a method for the assessment of the activity of 5 alpha-reductase type 1 and type 2 isoenzymes in human prostate tissue to be used in studies of the relationship of enzymatic activity and cancer. **Material and Methods**: We have been developing a method, based on Thomas et al., 2003 which consists of collecting human prostate samples and performing 5 alpha-reductase activity assessment. We are proposing a method based on samples obtained from prostate biopsies, according to a pilot study developed by Oliveira et al. 2006. We have obtained two samples of the same prostate area, and our idea is to send one of these samples for pathological examination and another for 5 alpha-reductase evaluation. Herein we have showed the feasibility of this assay. Through a thin layer chromatography with 14C-testosterone and NADPH (nicotinamide adenine dinucleotide phosphate) cofactor, we have obtained radioactive spots which were detected by autoradiography. The areas associated with testosterone and its metabolite DHT (dihydrotestosterone) were scrapped and counted with a scintillation chamber. **Results**: We believe that it is possible to demonstrate the activity of 5 alpha-reductase through the adaptation of this biochemistry method. **Conclusions**: This method is feasible and can be utilized to assess 5 alpha-reductase type 1 and type 2 activity in prostate tissue samples.
Keywords: Prostate; Prostate neoplasms; Androgens; 5α – reductase

Introduction

The relation between the androgens and cancer of the prostate is not completely established. Dihydrotestosterone (DHT) is the most powerful androgen in the prostate. DHT is the result of an irreversible reaction that occurs in the cytoplasm of the prostate cell, where the testosterone (T) is metabolized by the catalytic action of the enzyme 5 alpha-reductase with the presence of cofactor NADPH. T and DHT act on the same androgenic receptor (AR), since DHT has greater affinity for AR. The interaction of this hormone with the nuclear receptor triggers the transcription process that is translated to proteins that act in the cell-target.[1-3] The importance of DHT and the role played by 5 alpha-reductase was studied in masculine pseudohermaphroditism for deficiency of this enzyme. Actually, two isoenzymatic forms of 5 alpha-reductase exist: type 1, present in the skin, liver and in the prostate epithelium; type 2, present abundantly in stroma and in a smaller amount, in the prostate epithelium.[4-6] In masculine pseudohermaphroditism there is a mutation only in the responsible gene for the production of 5 alpha-reductase type 2 (gene SRDA52) present in the short arm of chromosome 2 (band p23). This deficiency is called pseudovaginal perineoescrotal hypospadia. In this genetic syndrome, the development of the internal male structures

Correspondence
Emanuel Burck dos Santos
Rua Ramiro Barcelos - Largo Eduardo Zacaro Faraco-Bom Fim
90035 003 - Porto Alegre - Brasil
Phone: 55 51 21018286
Email: mar781@hotmail.com

occurs; however, without the development of the prostate or genital external with male phenotype. In addition, the patient does not develop benign prostate hyperplasia (BPH), prostate cancer or androgenic alopecia. [4,7,8] Based on this and also on studies where the administration of DHT in dogs provoked BPH, randomized clinical trials have been performed where a selective inhibitor to 5 alpha-reductase type 2 (finasteride) reduced the size of the prostate in approximately 25%. Additionally, finasteride, according to these trials, has modified the natural history of the BPH in voluminous prostates, improving International Prostatic Score Symptoms, urinary flow, and diminishing the possibilities of acute urinary retention and surgical treatment for BPH.[9,10] Due to the successful use of this medication in this pathology, the possibility of using finasteride to prevent prostate cancer was considered. To solve this question, the Prostate Cancer Prevention Trial (PCPT) was designed and performed.[11] Although finasteride has prevented the occurrence of prostate cancer in this study, the group that received the treatment with this drug has increased the possibility of high grade prostate cancer. Hypothetically, perhaps 5 alpha-reductase type 1 also needs to be blocked to improve the chemoprevention strategy in prostate cancer. In preclinical studies it was demonstrated by immunohistochemical methods that it is 5 alpha-reductase type 1 expression that is increased in prostate cancer and not 5 alpha-reductase type 2. Actually, the isoenzyme type 2 could have a lesser expression than in the normal prostate since the stromal component would be less preserved in cancer. In BPH, 5 alpha-reductase type 2 would have increased expression, explaining why the inhibitors of this isoenzyme (finasteride) have such a remarkable action in this disease.[12] As 5 alpha-reductase type 1 predominates in the epithelium modified by prostatic adenocarcinoma, while 5 alpha-reductase type 2 is increased only in the stroma, some studies have demonstrated that only 5 alpha-reductase type 1 is expressed in lymphatic node metastasis of prostate cancer.[6,12-17] However, there is a study that demonstrates through DNA amplification, using the Reversal Transcriptase Polymerase Chain Reaction (RT-PCR) and detected by probes (Southern blot), that there is an expression of the genes of both isoenzymes in metastasis of prostate cancer.[18] In the Clínicas Hospital of Porto Alegre and in the Federal University of Rio Grande do Sul, we adapted an evaluation method of the 5 alpha-reductase type 1 and type 2 isoenzymes in human prostate tissue. In the present study we are presenting the development of an assessment method for the evaluation of both isoenzymes in prostate tissue obtained from transrectal prostate biopsies.

Material and Methods

Patient and Samples

Thirty-eight patients submitted to transrectal ultrasound prostate biopsies due to suspicions of malignancy (hard nodules on digital rectal examination or PSA alterations) had their samples analyzed by pathological examination. Two patients did not agree to participate. Four patients were excluded due to prostatic intraepithelial neoplasia (PIN), and three patients were also excluded due to prostatitis. Fifty-eight samples were obtained during the study in order to analyze 5 alpha-reductase activity. Considering these 58 samples, [19] presented no cancer on the left lobe and 7 presented cancer. On the right lobe,[20] had no cancer whereas 8 presented prostatic neoplasm. We obtained samples to perform an adaptation of a method similar to the method demonstrated by Thomas et al.[13] to evaluate 5 alpha-reductase type 1 and type 2 activities. However, in our study, we used human prostate obtained from biopsies. The study was approved by the Research Ethics Committee of the Clínicas Hospital of Porto Alegre. Samples were put in formalin before pathological examination, but one sample was immediately frozen in liquid nitrogen and stored in a freezer at -80°C for later evaluation of the 5 alpha-reductase activity.

5 Alpha-Reductase Assay

The frozen prostate tissue was defrosted and homogenized with Polytron PT 1200 CL (manufactured by Kinematica). In the homogenized prostate tissue we added 0.3M Tris-Citrate for pH 5.0 (to 5 alpha-reductase type 2) and for pH 7.0 (to 5alpha-reductase type 1). Radioactive testosterone (14C-T) 1.4μM (2.1GBq / mmol, Amersham Biosciences) was added and then we included 0.5mM NADPH (nicotinamide dinucleotide adenine phosphate) (Sigma), cofactor, with a final volume of 500μl. The 14C-T added to the solution reacted with the 5 alpha-reductase of the homogenized tissue, forming radioactive dihydrotestosterone (14C-DHT). Afterwards, the solution was incubated at 37°C for zero, twenty, thirty, and sixty minutes. After that, the reaction was interrupted by the addition of frozen ethyl acetate. The solution was shaken for 5 minutes and, soon after, was centrifuged by an equal period to separate ethyl acetate with the steroids from the watery residue. The pH was controlled by the addition of Tris-Citrate to differentiate the action of isoenzymes type 1 and 2. The organic phase was separated

and evaporated under nitrogen, as ethyl acetate has high volatility, and the residues were dissolved again in a small amount of ethyl acetate sufficient to transfer them from the assay to the chromatography plate. After application in the chromatography plate (Kieselgel 60 F254, Merck), these residues underwent migration in ethyl acetate: benzene (2:1), separating 14C-DHT from the14 C-T. The radioactive points were detected by autoradiography and the areas corresponding to testosterone and its metabolite, 14C-DHT, were scraped and put inside of vials with scintillation liquid (4g/L POP; 50mg/L POPOP; toluene) and the activity was counted with scintillation counter Wallac 1409 Liquid Scintillation Counter. It is possible to tell that it is indeed 14 C-DHT because we also had a standard DHT that formed a band in the same position of the14 C-DHT obtained from the 14 C-T by Oliveira.[21] The radioactive vials with material were evaluated in a scintillation liquid counter and the enzymatic activity expressed as picomoles of dihydrotestosterone formed by 1mg of protein during 1 minute (pmol DHT/mg protein/min).[18-20,22-25]

Results

The processed samples of prostate through the method in question showed the presence of testosterone, DHT, diols and triols corresponding bands. Through DHT standard we could verify the position of the same in the autoradiography (Figure 1) and then we were able to make the counting in a scintillation chamber through the scraping of the respective bands from the chromatography plate. The activities obtained from Figure 1 were 0.03 and 0.065 picomoles DHT/mg protein/min for pH 5 and pH 7, respectively.

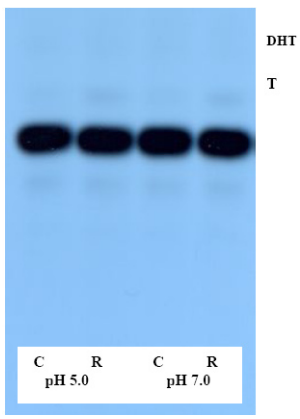

Figure 1 - Autoradiography of the thin layer chromatography analysis of radioactive lipid extracts from enzymatic system of 5α-R2 performed (R) at pH 5.0 and pH 7.0 with 100 µg of biopsy protein, incubated during 60 minutes, in presence of NADPH. Control reaction incubated without NAPDH (C).

Discussion

The evaluation of both 5 alpha-reductases could have been carried out through different methods. The choice of a certain method would take into consideration the feasibility, as well as the cost and usefulness in future research. Some methods would require the importation of antibodies or DNA or RNA probes. As the proposal was to evaluate the action of the prostate enzyme, we chose a biochemical method which evaluates the activity of the enzyme through the DHT formation. We differentiated isoenzymes 1 and 2 through the pH values of the homogenate solutions. Thomas et al.[13] had validated a new antibody to 5 alpha-reductase. For this validation the related researchers compared the immunohistochemical findings to the expression of the enzyme by the formation of 14C-DHT from 14C-T. The DHT migration in the chromatography plate was very similar to ours.[13]

The adaptation of a method for 5 alpha-reductase activity evaluation opens the possibility of innumerable studies related to the hormonal mechanisms of prostate neoplasm. It could also be used in studies with 5 alpha-reductase inhibitors, prognostic studies, just to name a few examples.

The method in question is relatively cheap, and the importation of antibodies or probes of nucleic acids is not necessary; only the necessity of acquisition of radioactive testosterone as well as the NADPH cofactor. But the incorporated technology, as the homogenizer and the standard of DHT, can permanently be used in further studies.

Considering the 58 samples, a total of 15 presented cancer and 43 did not. These samples have been analyzed for 5 alpha-reductase activity and the results are the object of another study to follow. The aim of the present assay is to present the steps to the development of the technique herein described as well as the initial pathological evaluation. From the present study we then move to lead another where we are evaluating the enzymatic activity of 5 alpha-reductase in radical prostatectomy samples of patients with prostate cancer. Simultaneously, we are carrying out another comparative study between biopsies obtained by transrectal ultrasound in patients with and without diagnosis of prostate cancer. Presently we are going to evaluate the activities of the two isoenzymes in prostate cancer and in benign prostate hyperplasia. In the near future we will also be able to evaluate the enzymatic expression in androgenic blockade patients. Moreover, we intend to correlate this with tumor virulence and grade, and with PSA. DHT was the main inductor of

the PSA increasing according to a study that evaluated isoenzymes type 1 and 2 in lymph nodes with prostate cancer metastasis, where the methods used were PCR and Southern blot.[18] However, no study of correlation between PSA and the activity of the enzyme 5 alpha-reductase using a method similar to ours could be found in the literature. Therefore, we are exploring the possibility of studying these aspects from what we have developed so far. We are also planning to evaluate the activity of the enzyme in lymph node metastasis using the method developed by us. Later on, we will also be able to compare our findings to others obtained by immunohistochemistry and see if they are equivalent, as Thomas et al. did.[13] At the moment we did not make the comparison mentioned above due to financial limitations.

We believe that these studies are the beginning of a new and promising research line that we are developing.

Conclusions

We suggest a cheap and replicable adaptation of a biochemist method for evaluation of the 5 alpha-reductase type 1 and type 2 activities in human prostate tissue samples.

References

1. Veltri R, Rodriguez. Molecular biology, endocrinology, and physiology of the prostate and seminal vesicles. In Wein AJ, Kavoussi LR, Novick AC, Partin AW, Peters CA. Campbell-Walsh Urology. 9th Edition. Volume 3, Section XVI, Chapter 85, Pages 2677-2726. Saunders - Elsevier Internacional Edition, 2007.
2. Wilson JD. The role of 5 alpha-reduction in steroid hormone physiology. Reprod. Fertil. Dev. 2001;13:673-678
3. Steers W. 5 alpha-reductase activity in the prostate. Urology. 2001;58(6A):17-24.
4. Russell DW, Wilson JD. Steroid 5 alpha-reductase: two genes/two enzymes. Annu. Rev. Biochem. 1994; 63:25-61.
5. Jenkins EP, Andersson S, Imperato-McGinley J, Wilson JD, Russell DW. Genetic and pharmacological evidence for more than one human steroid 5 alpha-reductase. J. Clin. Invest. 1992;89:293-300.
6. Iehlé C, Radvanyi F, Medina SGD, Ouafik L, Gérard H, Chopin D, Raynaud JP, Martin PM. Differences in steroid 5 alpha-reductase iso-enzymes expression between normal and pathological human prostate tissue. Journal of Steroid Biochemistry and Molecular Biology. 1999;68:189-195.
7. Andersson S, Berman DM, Jenkins EP, Russell DW. Deletion of steroid 5a-reductase 2 gene in male pseudohermaphroditism. Nature. 1991;354:159-161.
8. McConnell JD, Stoner E. 5 alpha-reductase inhibitors. Advances In Protein Chemistry. 2001; 56:143-180.
9. Gormley GL, Stoner E, Bruskewitz RC, Imperato-McGinley J, Walsh PC, McConnell JD, Andriole GL, Geller J, Bracken BR, Tenover JS, Vaughan ED, Pappas F, Taylor A, Binkowitz B, Ng J. The effect of finasteride in men with benign prostatic hyperplasia. The Journal of Urology. 2002;167:1102-1107.
10. Kaplan SA. 5 alpha-reductase inhibitors: what role should they play? Urology. 2001;58(6A):65-70.
11. Thompson IM, Goodman PJ, Tangen CM, Lucia MS, Miller GJ, Ford LG, Lieber MM, Cespedes RD, Atkins JN, Lippman SM, Carlin SM, Ryan A, Szczepanek CM, Crowley JJ, Coltman CA Jr. The influence of finasteride on the development of prostate cancer. The New England Journal of Medicine 2003;349(3):215-224.
12. Kaefer M, Audia J, Bruchovsky N, Goode RL, Hsiao KC, Leibovitch IY, Krushinski JH, Lee C, Steide CP, Sutkowski DM, Neubauer BL. Characterization of Type I 5 alpha-reductase activity in DU145 human prostatic adenocarcinoma cells. J. Steroid Biochem. Molec. Biol. 1996;58(2):195-205.
13. Thomas LN, Douglas RC, Vessey JP, Gupta R, Fontaine D, Norman RW, Thompson IM, Troyer DA, Rittmaster RS, Lazier CB. 5 alpha-reductase type 1 immunostaining is enhanced in some prostate cancers compared with benign prostatic hyperplasia epithelium. The Journal of Urology. 2003;170:2019-2025.
14. Habib FK, Ross M, Bayne CW. Factors controlling the expression of 5a-reductase in human prostate: A possible new approach for the treatment of prostate cancer. European Urology. 1999; 35: 439-442.
15. Bonkhoff H, Stein U, Aumüller G, Remberger K. Differential expression of 5alpha-reductase isoenzymes in the human prostate and prostatic carcinomas. The Prostate. 1996;29:261-267.
16. Negri-Cesi P, Poletti A, Colciago A, Magni P, Martini P, Motta M. Presence of 5 alpha-reductase isozimes and aromatase in human prostate cancer cells and in benign prostate hyperplastic tissue. The Prostate. 1998;34:283-291.
17. Bjelfman C, Söderström TG, Brekkan E, Norlén BJ, Egevad L, Unge T, Andersson S, Rane A. Differential gene expression of steroid 5 alpha-reductase 2 in core needle biopsies from malignant and benign prostatic tissue. Journal of Clinical Endocrinology and Metabolism. 1997; 82(7):2210-2214.
18. Zhu YS, Cai LQ, You X, Cordero JJ, Huang Y, Imperato-McGinley J. Androgen-induced prostate-specific antigen gene expression is mediated via dihydrotestosterone in LNCaP cells. Journal of Andrology 2003 Sep-Oct;24(5):681-687.
19. Hirosumi J, Nakayama O, Fagan T, Sawada K, Chida N, Inami M, Takahashi S, Kojo H, Notsu Y, Okuhara M. FK143, a novel nonsteroidal inhibitor of steroid 5 alpha-reductase: (1)in vitro effects on human and animal prostatic enzymes. J. Steroid Biochem. Molec. Biol. 1995;4:357-363.
20. Pratis K, O´Donnell L, Ooi GT, McLachlan RI, Robertson DM. Enzyme assay for 5a-reductase type 2 activity in the presence of 5a-reductase type 1 activity in rat testis. Steroid Biochemistry & Molecular Biology. 2000;75:75-82.
21. Oliveira OLM. Activity assessment of 5 alpha-reductase em prostate tissue [PhD Thesis]. Porto Alegre: Universidade Federal do Rio Grande do Sul; 2006.
22. Furuta S, Fukuda Y, Sugimoto T, Miyahara H, Kamada E, Sano H, Takei M, Kurimoto T. Pharmacodynamic analysis of steroid 5 alpha-reductase inhibitory actions of Z-350 in rat prostate. European Journal of Pharmacology 2001;426:105-111.
23. Grisenti P, Magni A, Olgiati V, Manzocchi A, Ferraboschi P, Villani V, Pucciariello R, Celotti F. Substrate interation with 5 alpha-reductase enzyme: influence of the 17ß-chain chirality in the mechanism of action of 4-azasteroid inhibitors. Steroids. 2001;66:803-810.
24. Weisser H, Ziemssen T, Krieg M. In vitro modulation of steroid 5 alpha-reductase activity by phopholipases in epithelium and stroma of human benign prostatic hyperplasia. Steroids. 2001;66:521-528.
25. Span PN, Bokhoven AV, Smals AGH, Sweep CGJ, Schalken JA. Partial sequencing and tissue distribution of the canine isoforms of steroid 5 alpha-reductase type I and type II. The Prostate. 2000;44:233-239.