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PARALLEL STUDY OF MOLECULAR CHANGES IN THE PROSTATE AND BLADDER OF MEN WITH BENIGN PROSTATE ENLARGEMENT (BPE) AND OVERACTIVE BLADDER (OAB) SYMPTOMS AND IN COMPARISON WITH CONTROLS

Hypothesis / aims of study

The lower urinary tract (LUT) is a functional unit controlled by a complex interplay between the central and peripheral nervous system and local regulatory factors. Peripherally, the lower urinary tract is dependent on the concerted action of the smooth and striated muscles of the urinary bladder, urethra and pelvic floor. In conditions of bladder outlet obstruction (BOO), the overactive bladder (OAB) syndrome is commonly thought to be associated with detrusor overactivity (DO) generated to overcome the BOO. In men with benign prostatic hyperplasia (BPH) and LUTS/OAB, however, approximately 30% will have remaining OAB symptoms following prostatectomy. Thus, although LUTS, BPH and OAB are clearly causally related, the extent to which they are and the mechanisms linking them are ill understood. Further, the molecular changes associated with OAB symptoms in BPE patients have not been determined.

In this study, we explored in parallel the molecular changes in the prostate and bladder of BPE patients and in association with their LUTS. In order to do that, we studied the expression of muscarinic receptors in ~~the~~ enlarged prostate(s) and bladder(s) of men with OAB symptoms and in comparison with control tissue. Furthermore, we studied the bladder and prostatic expression of adrenergic and androgen receptors, as well as the TRPV1 receptor, whose role in the obstructed human bladder and the prostate remain unclear, in comparison with control tissue.

Study design, materials and methods

In this cohort study, we included men over 50 who fall into one study group and two controls. The study group (group 3) included treatment-naïve patients with BPE, presenting with storage LUTS as defined by IPSS score (storage subscore ≥ voiding subscore and score ≥3 in the urgency question). Bladder and prostatic tissues were obtained either from prostatectomy patients or patients undergoing transrectal ultrasonographic guided biopsy (TRUSgbx) of the prostate for elevated PSA. Controls were asymptomatic for LUTS including OAB with total IPSS score ≤7 and score ≤1 in the urgency questions of IPSS. In the bladder control group (group 1), the tissue was sampled during check cystoscopy from macroscopically healthy bladder mucosa. In the prostate control group (group 2), prostatic tissue was obtained from TRUSgbx from the transitional zone. Control tissues that were found positive for bladder or prostatic malignancy were excluded. The Central Ethics Committee approved the study and all patients gave written informed consent.

Real–time polymerase chain reaction (RT-PCR) and Western Blotting techniques were employed to examine the mRNA and the protein expression of the muscarinic receptors M1, M2, M3, the adrenergic α1A and α1D receptors, the androgen and the TRPV1 receptor. Expression levels of transcripts were normalized to GAPDH (for gene expression) or actin (for protein expression) as endogenous control.

The paired t-test and Mann-Whitney test were used for intra- and intergroup variability of examined parameters. Pearson and Spearman correlation coefficients were used depending upon the uniformity of distribution. The statistical analysis was performed on SPSS 21.0 software (Armonk, NY: IBM Corp).

Results

Clinical characteristics: Fifteen (15) patients were included in each group. Mean age was no different between the three groups (72.7, 71.2 and 72,6 years for groups 1, 2 and 3 respectively. total IPSS score was 4.9, 8.8 and 14.9 for groups 1,2 and 3, respectively. The average prostate volume for groups 2 and 3 were 59ml (range 28-80ml) and 80ml (range 25-180) respectively.

Protein expression

We found significant increases in bladder M3 protein expression in the BPE/OAB group compared to controls (0.79 ± 0.19 vs. 0.26 ± 0.11, p=0.014) as well as in the protein expression of the sensory receptor TRPV1 (1.59 ± 0.47 vs. 0.72 ± 0.17 respectively, p = 0.031). The protein expression of M3 was significantly increased also in the prostate tissue of the BPE/OAB group compared to the control (0.61 ± 0.12 vs 0.07 ± 0.02, p = 0.019). The prostatic expression of α1D receptor was significantly decreased in the BPE/OAB group (2.11 ± 0.88 vs 0.15 ± 0.09, p = 0.002) as was of the TRPV1 receptor expression (2.52 ± 0.33 vs. 1.29 ± 0.46, p = 0.018). There was no significant difference in the expression of the prostatic androgen, muscarinic M2 and adrenergic α1A receptors. In addition, there was no significant difference between the groups in the expression of M1 and M2 in the bladder tissues. Prostatic M1 receptor was detected in 5 samples from the study group but couldn't be detected in any tissue sample of the control group, thus no comparison could be performed.

Gene (mNA) expression

In bladder tissue, the TRPV1 expression was decreased in the study group in comparison to the controls (4.32±0.90 vs. 1.97±0.91, p=0.038). There was no significant difference among the groups in muscarinic, adrenergic and androgen receptors gene expression levels neither in bladder nor in prostatic tissue.

Correlations with clinical parameters

The gene expression of the α1A receptor in the prostatic tissue of the BPE/OAB group showed significant correlations with the total IPSS score and also with both storage and voiding subscores. Only marginal correlations were noted in the prostate control group. The prostatic gene expression of the M1, M2, M3 muscarinic receptors was found to correlate negatively with the total IPSS score. There was no significant correlation between the IPSS score and gene and protein expression in the bladder tissue.

Interpretation of results

Our findings suggest a possible role of muscarinic receptors, especially the M3, in the pathophysiological mechanisms associated with BPE/OAB. Among the other receptors studied, the sensory receptor TRPV1 seems to play a role in the pathophysiology of BPE/OAB since protein expression level was increased in their bladders. This finding is consistent with the increased sense of urgency and with previous literature demonstrating increased immunohistochemical receptor expression in idiopathic and neurogenic OAB. However, gene expression of the receptor appears to be reduced in patients with BPE/OAB. The mechanisms underlying these differences remain to be explored. Surprisingly, we found reduced protein expression level of the TRPV1 receptor in the prostatic tissue of patients with BPE/OAB, despite a (non significant) increase in gene expression. TRPV1 has been previously detected both in the human prostatic tissue and the prostatic urethra, but was not investigated quantitatively and in association with the presence of BPE and/or OAB. Our findings suggest a role for the receptor in the pathophysiology of BPE/OAB, and possible changes in expression levels need to be further determined in the prostatic stroma, glandular epithelium and prostatic urethra. Finally, the protein expression of α1D adrenergic receptor was found to be reduced in the prostate tissue of BPE/OAB patients. Although insufficiently studied, literature to date supports an important role for the α1A receptor in the prostate, and a more important role for the α1D in the bladder. Our findings suggest that a potential role of the α1D receptor in the pathophysiology of BPE/OAB cannot be excluded and needs to be further studied in larger patient samples. All results should be interpreted in conjunction with expression changes after treatment.

Concluding message

in this pilot study we detected gene and protein expression changes in particular of muscarinic, adrenergic and sensory receptors in prostate and bladder tissue of BPE/OAB patients compared to controls, suggesting a possible role in the mechanisms by which the enlargement of prostate gland may be associated with bladder dysfunction. Further experiments need to confirm these results, the importance of which needs to be assessed in the light of any change in expression after pharmaceutical treatment.

Disclosures

Funding: Research grant form the European Union (European Social Fund) and Greek national resources under the framework of the “ARISTEIA” project AVLOS code 2130 of the “Education & Lifelong Learning” Operational Programme **Clinical Trial:** No **Subjects:** HUMAN **Ethics Committee:** 1. Bioethics Committee, Aristotle University of Thessaloniki, Greece 2. Scientific Committee, Ippokration Hospital, Thessaloniki, Greece 3. Scientific Committee, Papageorgiou General Hospital, Thessaloniki, Greece **Helsinki:** Yes **Informed Consent:** Yes