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DETRUSOR OVERACTIVITY IN WOMEN AND THE ASSOCIATION WITH CHANGES TO IN VITRO CONTRACTILE FUNCTION AND ALTERED GENOTYPE

Hypothesis / aims of study

Clinical objective and subjective measurements of detrusor overactivity are associated with changes to the in vitro contractile properties of detrusor smooth muscle and alterations to the genotype of isolated tissue.

Study design, materials and methods

Seventy women with lower urinary tract symptoms were recruited to the study. Urodynamic assessment was used to determine the presence (n=51) or absence (n=19) of detrusor overactivity (DO) - mean cystometric capacity (MCC) and detrusor pressure at peak flow (Pdet) were recorded. Patients also completed a scored form of the BFLUTS questionnaire (1) that contained symptom questions regarding the filling and voiding periods of the micturition cycle as well as bother associated with incontinence. Detrusor strips, with mucosa removed, were obtained from a subset of the recruitees, tied to an isometric force transducer and superfused with Tyrode's solution at 36°C. Contractions were elicited by: exposure to the muscarinic receptor agonist carbachol (0.1-10 μM); or electrical field stimulation (3-sec trains of 0.1 ms pulses at 1-40 Hz) to elicit nerve-mediated contractions. Contractile responses to maximum carbachol concentrations (T_{max},carb) and high stimulation frequencies (T_{max},EFS), as well as the carbachol EC_{50} (as pEC_{50} = -logEC_{50}) and $f_{1/2}$ (EFS frequency for 0.5*T_{max},EFS) were recorded. The ratio of EFS contractions generated at 4 Hz and 40 Hz (T_4/T_{40}) was also recorded. Finally, five samples from patients without DO and seven with DO were subjected to a genome-wide association study (GWAS) to identify single nucleotide polymorphisms (SNP) associated with DO. RNA was extracted using a Qiagen RNeasy Micro kit, quantified (>250 ng/sample) and quality assessed using NanoDrop analysers. Analyses used Illimina HumanHT-12 v4 Expression BeadChip Kits. Clinical and physiological data are expressed as median (25,75% interquartiles] values and differences between sets was tested using Mann-Whitney U-tests; the null hypothesis was rejected at $p<0.05$. GWAS data analysis is given in the Results.

Results

The age of patients in the stable and DO groups was similar –Table 1. In the DO group four patients also had urodynamic stress incontinence and five had low compliance bladders. In the control group two patients also had recurrent urinary tract infections. There was no difference in MCC between the two groups. Pdet was significantly greater in the DO group; however, it was not always recorded in the stable group. Histological examination of biopsy samples showed mild chronic inflammation in nearly all samples (stable 14 of 17; DO 45 of 46 tested). Filling and voiding symptom scores on the BFLUTS questionnaire were similar in both groups but the incontinence symptom score was significantly greater in the DO group.

Table 1. Age, urodynamic data (MCC, Pdet) and BFLUTS scores; stable and DO groups.

	Age, years *n*=19,51	MCC Ml *n*=15,46	Pdet cm H_2O *n*=9,38	Filling Total 15 *n*=17,41	Voiding Total 12 *n*=17,41	Incontinence Total 20 *n*=17,41
Stable	58 [45,71]	500 [418,504]	5 [3,8]	6 [3,10]	3 [2,3]	3 [1,9]
DO	54 [47,59]	450 [373,500]	20.5 * [13.5,31.5]	7 [5,9]	3 [1,5]	8 * [3,11]

Experiments with isolated tissues showed that responses to the muscarinic agonist carbachol was similar in both groups – i.e. maximum tension at high carbachol concentrations and the pEC_{50} values. However, contractions to electrical field stimulation were significantly different. Thus, the maximum tension to electrical field stimulation at high frequencies was reduced, the frequency for half maximum stimulation was decreased and the T_4/T_{40} ratio increased. The T_4/T_{40} ratio is a measure of the significance of ATP-dependent atropine resistant contractions; the larger the value the greater is the ATP-dependent (purinergic) component of contraction.

Table 2. Contractile characteristics of isolated detrusor preparations; stable and DO groups.

	T_{max} carb mN.mm^{-2} *n*=6,17	pEC_{50} *n*=6,17	T_{max} EFS mN.mm^{-2} *n*=9,12	$f_{1/2}$ Hz *n*=9,12	T_4/T_{40} *n*=9,12
Stable	19.9 [7.81,31.9]	6.62 [6.78.6.50]	31.0 [16.3,45.2]	16.8 [13.9,19.6]	0.34 [0.31,0.38]
DO	7.35 [4.07,17.8]	6.64 [6.89,6.59]	1.64 * [0.60,2,24]	6.4 * [5.9,9.3]	0.08 * [0.03,0.18]

Comparison of the clinical and *in vitro* data showed that neither MCC nor Pdet was significantly associated with any contractile variable. However, BFLUTS data for DO patients showed a significant association with several EFS contractile variables: In particular, filling, voiding and incontinence scores were positively associated with greater T_4/T_{40} ratios, the strongest association was observed with filling scores Table 3.

Table 3. Correlation coefficients between BFLUTS data and *in vitro* contractile variables for women with LUTS, *p<0.05

	T_{max} carb *n*=17	pEC_{50} *n*=17	T_{max} EFS *n*=12	$f_{1/2}$ *n*=12	T_4/T_{40} *n*=12
Filling	-0.349	-0.223	-0.829 *	-0.232	0.926 *
Voiding	-0.468	-0.591	-0.739 *	0.164	0.805 *
Incontinence	-0.522	--0.003	-0.491	-0.158	0.701 *

GWAS data analysis of 47,000 tests (probes) showed that no individual SNP was different in the DO and non-DO groups at log p<-6.0, the nominal significance level (p<0.05) divided by the number of probes and assuming each test is independent of each other. Pathway analysis (Ingenuity) revealed some significant upregulations in the DO group associated with intracellular signalling, cell hypertrophy and matrix deposition. The most significant were: akt-dependent pathways; calcineurin/calpain hypertrophy pathways; elastin, collagen, metalloprotease pathways and G-coupled intracellular signalling pathways.

Interpretation of results

In a group of LUTS symptomatic women with or without DO there was a poor correlation between quantified urodynamic data and either questionnaire data or *in vitro* contractile data. However, there was a significant association between BFLUTS filling and voiding components and nerve-mediated contractions data, but not with carbachol-derived data indicating that the BFLUTS scores correlate with disorders of bladder dysfunction associated with nerve-mediated contractions. GWAS data indicated that DO was associated with upregulation several pathways associated with cellular hypertophy, increased deposition of extracelluar matrix and intracellular signalling pathways associated with celluar contraction.

Concluding message

In women with or without DO, there is a poor correlation between objective urodynamic parameters and either scored questionnaires to assess severity of LUTS and in vitro functional data. However, questionnaire data correlates better with *in vitro* data. Whole genome studies indicate DO is associated with pathways that increase myogenic functional activity and increased extracellular material.

References

1. Brooks ST et al. Am J Obstetr Gynaecol 2004; 191: 73-82.

Disclosures

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