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EXPRESSION OF INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) SPLICE VARIANTS AND BLADDER DISTENSION. DOES A REDUCED BLADDER VOLUME PREVENT DETRUSOR MUSCLE REGENERATION?

Hypothesis / aims of study

There is increasing evidence that different isoforms of the IGF-1 gene responding to mechanical stimuli are involved in smooth and skeletal muscle regeneration. Studies looking at average voided volumes in the presence of urinary frequency in OAB patients have shown a significant statistical reduction in voided volume (mean 170 ml) when compared with healthy asymptomatic volunteers [(1)]. We hypothesize that in the absence of normal physiological bladder distension leading to insufficient mechanical stimuli, the IGF-1 gene related muscle repair mechanism will fail to activate preventing further regeneration of the detrusor muscle.

Several studies to date have shown that during regeneration of adult muscle, the IGF-1 gene undergoes alternative splicing within the E domain generating two distinct isoforms in humans: IGF-1Ea and IGF-1Ec (also known as Mechano Growth Factor, MGF). Although both isoforms are directly involved in muscle regeneration, they also have very distinctive albeit complimentary physiological effects. MGF has a unique E domain that results in a reading frame shift and C terminal peptide which stimulates satellite cells (known as myoblasts) proliferation. IGF-1Ea acts as the main source of anabolic agent and promotes further differentiation of myoblasts and fusion with myotubes [(2)]. Whilst IGF-1Ea is the most abundant isoform with a longer bioavailability assisted by the same IGF-1 binding proteins, MGF displays a very short life span following mechanical strain and/or damage. Under physiological conditions, MGF expression peaks earlier than IGF-1Ea and it has been shown that both exert their action through a different set of receptors.

Study design, materials and methods

Ethical approval was obtained and patients provided written informed consent prior to enrolment in the study. Patients undergoing surgery for lower urinary tract symptoms (LUTS) or prolapse were recruited from the outpatients urogynaecology clinic. At the end of their scheduled procedure all patients underwent a saline diagnostic cystoscopy and bladder biopsy under general anaesthetic. All patients had residuals prior to cystoscopy of less than 100mls. Biopsies were obtained in 13 patients following standard bladder distension with 400 ml of saline solution. In five patients, biopsies were obtained following bladder distension only up to 200 ml of saline.

Two bladder biopsy samples using cold cup biopsy forceps were obtained from each patient and placed in RNA later. Total RNA was extracted using Trizol Reagent® and cDNA synthesized using an Omniscript RT kit (Qiagen). mRNA expression of IGF-1Ea and MGF was determined by quantitative reverse-transcriptase polymerase chain reaction (qPCR) using SYBR Green Chemistry (Bioline) on a RotorGene 6000 (Qiagen).Normalised data were expressed as target gene copy number/housekeeping gene (GAPDH) copy number.

Results

Eighteen patients were recruited for this pilot study. No intra or post operative complications were seen related to the bladder sampling procedure. As seen in Table 1, biopsies were obtained from patients with a wide variety of lower urinary tract symptoms. MGF was expressed in all patients who underwent cystoscopy at physiological bladder distension (400 ml of saline), but it could not be detected in samples from patients who underwent cystoscopy with limited bladder filling.

Interpretation of results

IGF-1 gene expression has already been demonstrated in several organs such as skeletal muscle, liver, myometrium, and ovary. Several studies have shown it plays a significant role in organogenesis and cell differentiation including malignant tumour development, prevention of apoptosis and may have a neuroprotective role in the presence of ischaemia. Amongst the different theories behind the pathophysiology of OAB, the myogenic hypothesis [(3)] supports that abnormal morphological features caused by reduced excitatory impulses to the bladder, results in histological alterations in bladder smooth muscle leading to enhanced coupling and fused tetanic contractions. Our results confirm that the above repair mechanism is also present in the bladder detrusor muscle and as seen in previous studies its expression is regulated by the presence of mechanical stimuli and/or damage. It cannot be established at this stage whether this repair mechanism is impaired as a result of an already damaged bladder when compared with the healthy bladder, or whether absence or deficient activation of MGF could be a contributory cause leading to the histological changes seen in the pathological bladder.

Concluding message

A better understanding between the interaction of this muscle repair mechanism and other predisposing factors to detrusor overactivity will potentially provide novel insight into the pathophysiology of OAB and may open the door to innovative treatment options.

Patient	Age	Presenting Problem	Urinary symptoms	Urodynamic results	Anti-Cholin treatment	MGF*	IGF-1Ea*
1	54	Menorrhagia	None	N/A	N/A	0.8848	1901.12
2	56	Prolapse	None	N/A	N/A	$2.4\ 10^{-3}$	$8.95\ 10^{-1}$
3	65	Prolapse	None	N/A	N/A	$5.04\ 10^{-3}$	$5.43\ 10^{-2}$
4	91	Prolapse	Mixed	USI / No DO	Nil	$2.3\ 10^{-3}$	$1.93\ 10^{-1}$

5	48	Prolapse	Mixed	USI / DO	YES	$5.4\ 10^{-3}$	$1.60\ 10^{-1}$
6	53	Prolapse	Mixed	USI / No DO	YES	$3.9\ 10^{-3}$	$1.45\ 10^{-1}$
7	64	Recurrent SUI	Mixed	USI / No DO	In the past	$4.35\ 10^{-2}$	95.105
8	52	SUI	Mixed	USI / No DO	YES	$2.58\ 10^{-3}$	$9.80\ 10^{-2}$
9	62	SUI	Mixed	USI / No DO	Nil	$3.54\ 10^{-3}$	$2.11\ 10^{-2}$
10	65	PBS	Pain	No USI / No DO	Nil	$1.95\ 10^{-2}$	2.281
11	91	PBS	Pain/SUI/Freq	USI/No DO	Nil	$1.78\ 10^{-2}$	$3.06\ 10^{-1}$
12	86	PBS	Pain	No USI / No DO	YES	$4.87\ 10^{-2}$	$5.52\ 10^{-1}$
13	26	VVF	Continuous leak	No USI / No DO	Nil	$7.18\ 10^{-3}$	$2.89\ 10^{-1}$
14	**31**	**Recurrent UTI**	**Mixed**	**USI/ No DO**	**Nil**	**Negative**	**9.184**
15	**36**	**Continuous leak**	**OAB**	**No USI/ No DO**	**Nil**	**Negative**	**$6.23\ 10^{-2}$**
16	**65**	**Failed TVT**	**SUI**	**USI/ No DO**	**Nil**	**Negative**	**$2.19\ 10^{-1}$**
17**	**53**	**Intractable OAB**	**OAB**	**No USI/ DO**	**YES**	**Negative**	**Negative**
18	**71**	**SUI**	**Mixed**	**USI/ No DO**	**Nil**	**Negative**	**$1.32\ 10^{-2}$**

Table 1:
Patients recruited for cystoscopy and bladder biopsy. *SUI*: stress urinary incontinence; *PBS*: painful bladder syndrome; *USI*: urodynamic stress incontinence*; DO*: detrusor overactivity; *N/A*: Not applicable; *VVF*: vesico- vaginal fistula;
*= copy numbers relative to GAPDH.
**; Patient 17: samples were obtained at the time of open abdominal surgery of a clam cystoplasty. The patient had a long history of intractable DO and treatment with Bottox in 10 occasions prior to surgery.
Patients in bold underwent filling cystoscopy with only up to 200ml of saline solution

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Is this a clinical trial?	**No**
What were the subjects in the study?	**HUMAN**
Was this study approved by an ethics committee?	**Yes**
Specify Name of Ethics Committee	**South East London Ethics Committee**
Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**