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THE CONTRACTILE AND HISTOLOGICAL PROPERTIES OF HUMAN BLADDER SAMPLES OBTAINED FROM CHILDREN WITH NORMAL AND PATHOLOGICAL BLADDERS.

Hypothesis / aims of study

Major congenital anomalies of the urinary tract have life long functional consequences and require long term urology follow-up. This study aims to demonstrate the detrusor physiology of these conditions. Direct comparison has been made between normal (control) tissue and bladder exstrophy, cloacal exstrophy, cloacal anomalies, neuropathic bladders and posterior urethral valves. The hypothesis is that major congenital anomalies result in measurable differences in the physiology of the bladder and thus an improved understanding of treatment targets and benefit.

Study design, materials and methods

Biopsy samples were obtained from children (neonatal to 10 years) undergoing surgical procedures and were grouped as those with normal or pathological bladders. The normal group included procedures for ureteric reimplantation, urachal cyst excision or localised bladder tumour excision. The pathological group included children with: bladder exstrophy (neonatal or paediatric cases); cloacal exstrophy; posterior urethral valves (PUV); neuropathic bladders; cloacal anomaly. Mucosa was dissected from the detrusor in the biopsy, and muscle strips (diameter < 1 mm) were tied to an isometric force transducer and superfused with Tyrode's solution (24 mM $NaHCO_3$, 5% CO_2, pH 7.4, 37°C). Nerve-mediated contractions (blocked by 1μM tetrodotoxin) were elicited by electrical field stimulation (3-s train of 0.1 ms pulses; 2-40 Hz); atropine (1 μM) was added subsequently and contractions again recorded. Contractures to the muscarinic agonist carbachol (0.1-30 μM) were elicited in unstimulated preparations. Frequency-response and dose-response curves were generated and the maximum tension, Tmax, at high frequencies or [carbachol], as well as the frequency or [carbachol] required to generate Tmax/2 were calculated from the Hill equation. For histological measurement of the ratio of smooth muscle to collagen tissue samples were cut from the main biopsy, stored in 10% formaldehyde and paraffin embedded. Sections (5 μm) were stained with Elastin van Gieson to stain with differential colour connective tissue and muscle and the ratio of the two tissues measured by colour image analysis with Image-J. Contractile data are given as medians [25, 75% interquartiles ranges] and differences between data sets were tested by Mann-Whitney U-test. Histological data are quoted as medians and ranges and differences tested with non-parametric ANOVA. The null hypothesis as rejected at $p<0.05$.

Results

Contractile data (Table 1) show that nerve-mediated contraction magnitude was reduced in samples from exstrophy bladders, neuropathic bladders and PUV bladders, but preserved in samples from cloacal anomaly bladders. The frequency required for half maximum contraction was similar in all groups. Qualitatively, similar data were obtained for the carbachol contractions. Atropine resistance was a significant feature of the nerve-mediated contractions. In the normal group about half of the contraction magnitude persisted in the presence of atropine. This was significantly less in the neonatal exstrophy, PUV and cloacal anomaly groups.

Table 1 also shows the smooth muscle (SM) to connective tissue (CT) ratio, as well as the range of values for the smooth muscle proportion. Samples from normal bladders and those with cloacal anomaly had a greater proportion of SM compared to CT, whereas the samples from the other pathological bladders showed varying decrease of the SM content and a decline of the SM/CT ratio. Figure 2 shows a good correlation between the magnitude of the maximum carbachol contraction and the ratio of smooth muscle to connective tissue (SM/CT).

Table 1. Contractile characteristics of detrusor samples from human paediatric bladders. Numbers in parenthesis refer to number of samples; *p<0.05 compared to normal. SM, smooth muscle; CT, connective tissue

	Nerve-mediated contraction		Atropine resistance	Carbachol contractures		Histology
	Tmax, mN/mm^2	f1/2, Hz	%, 16 Hz stim	Tmax, mN/mm^2	EC50 μM	SM/CT (% SM)
Normal bladder (18)	6.4 [3.0, 11.1]	15.9 [3.0, 26.9]	58 [36, 73]	19.3 [6.8, 44.8]	0.8 [0.7, 1.9]	2.02 (47-90 %)
Paediatric exstrophy (17)	1.3 [0.2,2.7] *	21.7 [14.3, 21.6]	42 [13,55]	6.5 [1.1, 11.2] *	0.8 [0.5, 1.4]	0.57 * (30-52 %)
Neonatal exstrophy (5)	0.8 [0.5, 1.0] *	17.2 [12.2, 18.6]	22 [0, 38] *	4.1 [3.9, 7.9] *	1.6 [1.5, 2.3]	0.22 * (18-42 %)
Cloacal exstrophy (7)	0.2 [0.1, 0.2] *	13.2 [11.9, 14.5]		0.8 [0.3, 3.0] *	2.0 [1.6, 2.9]	0.39 * (20-41%)
Posterior urethral valve (8)	1.8 [0.7, 3.2] *	18.0 [15.3, 19.3]	22 [3, 40] *	4.5 [2.3, 4.8]*	0.9 [0.8, 1.7]	1.07 * (38-75 %)
Neuropathic bladder (15)	0.5 [0.3, 2.9] *	11.9 [9.0, 15.2]	36 [10, 66]	6.1 [1.6, 13.4] *	0.8 [0.6, 1.8]	0.53 * (10-65 %)
Cloacal anomaly (6)	9.7 [6.5, 11.7]	13.5 [10.1, 17.5]	31 [17, 45] *	11.7 [8.2, 20.2]	0.8 [0.7, 0.8]	1.80 (48-82 %)

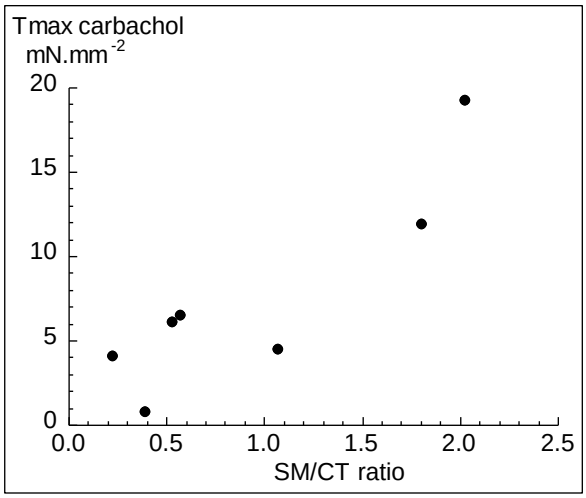

Figure 2. The association between median carbachol Tmax value and the SM/CT ratio for the different groups from whom detrusor samples were obtained. Pearson correlation coefficient = 0.86, $p<0.05$

Interpretation of results

Data show that detrusor muscle biopsies from children with normal and abnormal bladders are viable for in vitro experiments. For tissue from normal bladders the nerve-mediated and carbachol contracture response magnitude are similar to those for adult tissue. For the exstrophy, PUV and neuropathic bladders contractile force was significantly reduced. Similar conclusions were reached is nerve-mediated contractions were analysed, except for the PUV data. Contraction magnitude was strongly associated with the relative amount of smooth muscle in the tissue. We conclude therefore that infiltration of the tissue with connective tissue rather than a reduction of unit muscle contractility is responsible for the smaller contractile force developed by the pathological bladders. The significant atropine-resistant contractions in all groups is in marked contrast to adult human detrusor where such a component is absent in normal human tissue.

Concluding message

Poor bladder performance of children with major congenital anomalies can be explained by reduced contractile performance of the bladder wall. This is mainly due to replacement of muscle with connective tissue and less importantly due to degradation of muscle contractility.

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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	**UCL Research Ethics Committee**
Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**