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THE USE OF INERT BULKING AGENTS TO REDUCE BLADDER SPONTANEOUS CONTRACTIONS: A POSSIBLE THERAPEUTIC PARADIGM AGAINST OVERACTIVE BLADDER

Hypothesis / aims of study

'Injecting inert bulking agents below the sub-urothelium reduces bladder spontaneous contractions.' To test this hypothesis we measured changes to tonic and phasic contractions in the presence of inert injectable agents such as polyethylene glycol or coaptite.

Study design, materials and methods

Male and female pig bladders were obtained from a local abattoir and transported to the laboratory in ice-cold gassed Tyrode's solution for use after 30-60 minutes. Bladder wall (mucosa & detrusor) preparations were dissected from the dome in a Sylgard dish containing fresh Tyrode's. Preparations were clamped in a tissue bath and superfused (12.5 $ml.min^{-1}$) with Tyrode's solution at 37°C. Isometric contractions were measured using a force transducer. Preparations were equilibrated for 30 minutes, stimulated to contract with carbachol (CCh, 1 µM) for ten minutes and then washed out for one-hour over which time spontaneous contractions developed. The bladder was unmounted from the tissue bath, placed into a warm Sylgard dish of Tyrode's (~37°C) and injected into the sub-urothelium with polyethylene glycol (PEG) or coaptite® using a 23 gauge rigid needle. Control interventions included a similar injection with Tyrode's solution, insertion only of the needle (null injectate) or no procedure (time control). Preparations were then remounted and re-equilibrated. The effects of CCh and spontaneous contractions were recorded as before. Experimental variables were: i) the maximum tension achieved during superfusion with carbachol or ii) the integral of the spontaneous contraction record over baseline (area under the curve, AUC) over a ten-minute period 60 minutes after the washout of carbachol.

Histological comparisons of time-control vs injectate preparations were examined with haematoxylin and eosin (H&E) staining. Preparations were injected as before with either Tyrode's, PEG, or coaptite (mixed with undiluted methylene blue dye) or given no injection (time-control) and fixed in 10% neutral buffered formalin. Images stained with H&E were taken of the whole bladder wall using a CCD-attached wide-field microscope with a 5x objective.

Data sets were analysed as median values with interquartile ranges and values were compared to control (=100%), i.e. to those prior to the injection or no injection procedure. The effects of injectables on tonic and phasic contractions were compared against the time-control or its own control (before the remounting procedure). Statistical significances between data sets were obtained when $p<0.05$, using non-parametric Wilcoxon rank tests, or Mann-Whitney U-tests.

Results

The CCh contracture of time-control preparations significantly increased when normalised to its own control (before remounting procedure). The null-injectate i.e. piercing tissue with a needle did reduce CCh contractures albeit insignificantly when compared to itself. This trend was not seen for injection with Tyrode's but was significantly increased for also PEG and coaptite injections. In contrast, this effect was different when comparisons were made vs. time-control. A significant reduction was seen for PEG and null-injectate.

CCh contractures.

Time control: 156.2% [126.4%, 250.8%], n=11, $p<0.001$ vs. own control
Null-injectate: 80.9% [79.9%, 91.4%], n=6, $p<0.01$ vs. time-control.
Tyrode's: 115.1% [77.5%, 208.5%], n=7
PEG: 119.5% [102.6%, 130.3%], n=11, $p<0.01$ vs. own control; $p<0.05$ vs. time-control
Coaptite: 133.2% [118.0%, 216.6%], n=10, $p<0.01$ vs. own control

The process of removing and then re-mounting the preparation, during which injection or no injection occurred, may itself have caused changes to contractile activity. Spontaneous contractions were not significantly increased when no intervention was applied.

Null-injectate preparations also had no significant effect on spontaneous contraction. Injection with Tyrode's solution, PEG or coaptite did not demonstrate a significantly reduced AUC of spontaneous contractions when normalised to their own control, albeit values were reduced. These conclusion were the same for the null-injectate, Tyrode's solution and PEG injection if data sets were compared to time-control preparations. However, coaptite showed a significant reduction when data was compared to the time-control preparations.

AUC of spontaneous contractions.

Time control: 128.6% [65.2%, 228.4%], n=10
Null-injectate: 56.2% [23.5%, 107.4%], n=6
Tyrode's: 61.3% [31.8%, 104.8%], n=6
PEG: 68.6% [41.8%, 127.1%], n=9
Coaptite: 55.7% [17.6%, 92.9%], n=9; $p<0.05$ vs. time-control

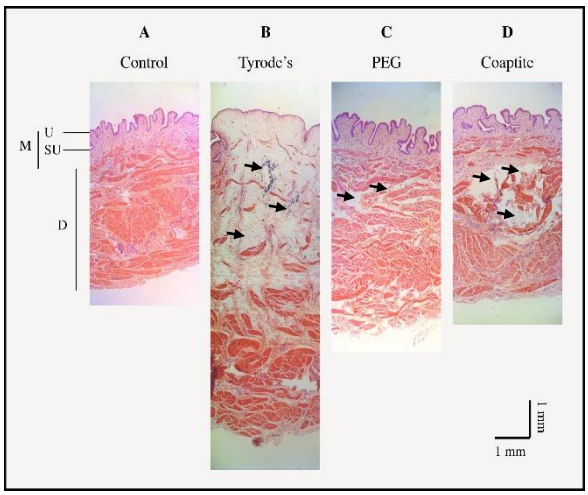

Figure 1. H&E staining for the comparison of injectate vs. no injection. A) No injection (time-control); B) Tyrode's injection; C) PEG injection; D) Coaptite injection. Arrows indicate injectate.

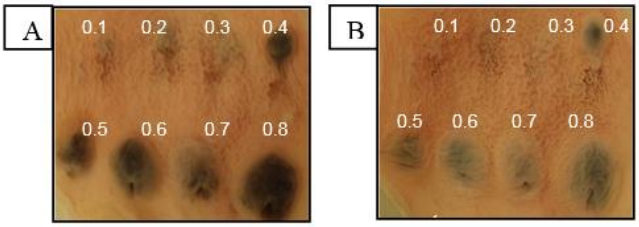

Figure 2. Spread of sub- urothelial PEG injection. Different volumes (numbers in mls) of injectate with carbon particles were injected into sub-urothelium. A) Time = 0 min; B) Time = 24 hours.

Interpretation of results

Injection of inert bulking agents into the suburothelium reduced the AUC of spontaneous contractions, but did not cause any significant decrease of CCh contractures. Coaptite was the most significant injectable in decreasing spontaneous contractile activity whilst leaving intact detrusor contractility as judged from the magnitude of the carbachol contracture. Both Tyrode's and PEG were ineffective at significantly reducing spontaneous contractions. Effects were seen when piercing the tissue with just the needle however these comparisons were different to those when the inert bulking agent was administered, i.e. bulking agents did not reduce CCh contractures but did spontaneous contractions.

Concluding message

The use of coaptite, compared to PEG or control injection procedures, to decrease spontaneous contractions suggests a treatment paradigm for reduction of overactive bladder contractions whilst leaving intact the voiding contraction mediated by acetylcholine release from efferent nerves. The time-course of the effect after a single injection of coaptite is the next step in evaluation of this material.

Disclosures

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