EFFECTS OF INTRAVESICAL RESINIFERATOXIN AND ETHANOL ON UROTHELIALMEDIATOR RELEASE & CONTRACTILE RESPONSES

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K. Smith, C. McDermott, D. Sellers & **R. Chess-Williams**
Centre for Urology Research, Bond University, Australia

BACKGROUND

Interstitial cystitis/bladder pain syndrome (IC/PBS) is a chronic condition that presents with bladder urgency, frequency and pain on bladder filling. Resiniferatoxin (RTX) has been used to treat IC/PBS(1) by desensitizing sensory nerves via the TRPV1 receptors found on nociceptive c-fibres(2). RTX is usually dissolved in 10% ethanol (v/v) and is delivered intravesically. The aim of this study was to investigate the effects of intravesical RTX and its vehicle ethanol, on bladder function using voiding pattern analysis and whole bladder functional studies.

METHODS

Female C57/BL6J ARC mice (10-12 weeks) were treated intravesically with RTX (50nM), 10% ethanol (Ethanol control) or 0.9% saline (Saline control) for 30 minutes. Twenty-four hours later mice were sacrificed and a whole bladder preparation **(Figure 1)** was used to assess intravesical pressure changes in response to distension (30µl/min), electrical field stimulation (EFS) (1,5,10, 20Hz) and pharmacological agent (ATP, carbachol, isoprenaline). Luminal contents were collected for analysis of ATP and ACh. Voiding behavior was also assessed before and 24 hours following treatment using voiding pattern analysis.

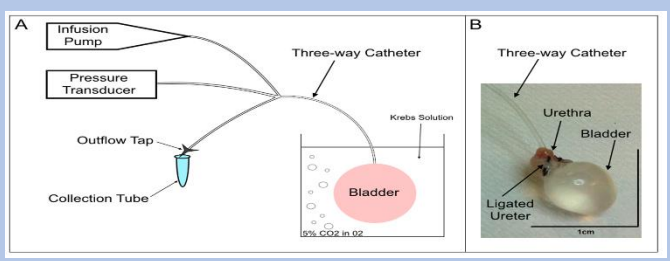

Figure 1: *A- Isolated mouse bladder setup, depicting the bladder connected to a three-way catheter. B- Photograph of a mouse bladder connected to a three-way catheter.*

RESULTS

Intravesical RTX significantly increased voiding frequency by 2-fold, while the volume of the primary voided area was significantly reduced **(Figure 2).** This corresponded to an increase in the number of small voids, with no change to the total volume voided 24-hours after treatment with RTX (Data not shown). Voiding behaviour was not altered by intravesical ethanol or saline.

No significant change in bladder compliance or spontaneous phasic activity was observed.

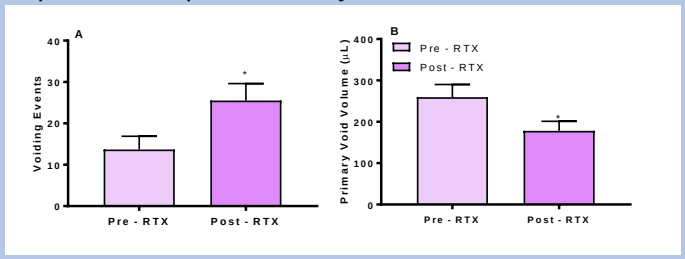

Figure 2: *The effect of intravesical RTX on voiding frequency and volume of primary voids in mice 24-hours following treatment. *p<0.05 Pre vs Post RTX treatment.*

CONCLUSIONS

Intravesical treatment with RTX causes a number of changes in bladder function that may contribute to its clinical effectiveness, however these appear to be produced by the vehicle ethanol rather than direct actions of RTX. Thus ethanol may contribute to its clinical effects on the bladder following treatment.

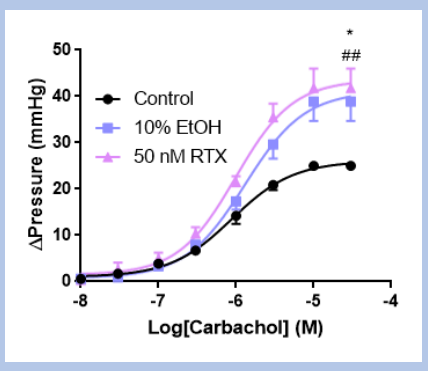

Figure 3: *The effect of intravesical treatment with 0.9% saline, 10% ethanol and 50 nM RTX on the contractile response to carbachol. Mean ± SEM, n=6. (* p<0.05 Ethanol vs control, ## p<0.01 RTX vs control)*

A significant increase in the maximal response to carbachol occurred in both ethanol (46.1 ± 2.49 mmHg, p<0.05, n=6) and RTX treated bladders (46.5 ± 4.99 mmHg, p<0.01, n=6) when compared to control (27.7 ± 2.49 mmHg, n=6) (**Figure 3**). No significant alteration to the relaxation response by isoprenaline was observed.

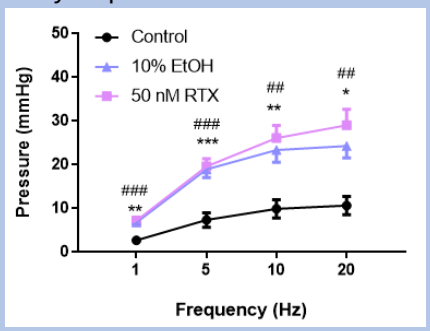

Figure 4: *The effect of intravesical treatment with 0.9% saline, 10% ethanol and 50 nM RTX on the nerve evoked response to EFS. Mean ± SEM, n=6. (* Ethanol vs control, # RTX vs control)*

Similarly, efferent nerve evoked contractile responses were significantly enhanced at all frequencies **(Figure 4)** Addition of LNNA to block nitric oxide synthase and atropine to block muscarinic receptors did not significantly affect nerve evoked responses. However, desensitization of the purinergic system with αβ-mATP significantly reduced the response to EFS by 85% (Data not shown). ATP was the dominant neurotransmitter in all treatment groups.

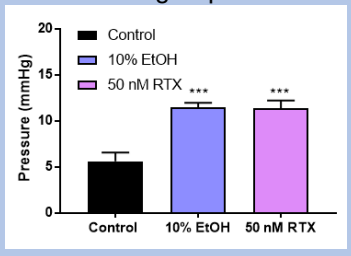

Figure 5: *The effect of intravesical treatment with 0.9% saline, 10% ethanol and 50 nM RTX on contractile response to ATP. Mean ± SEM, n=6. (*** vs control)*

Contractile response to purinergic stimulation with ATP (Figure 5) or αβ-mATP (data not shown) was significantly enhanced in ethanol and RTX treatment groups. Following intravesical treatment, luminal ATP levels increased significantly from 0.45 ± 0.23nM (n=6) at full distension in controls to 5.96±1.39nM in ethanol (p<0.05, n=6) and 8.43 ±1.91nM (p<0.01,n=6) in RTX treated mice **(Figure 6A).** However, intravesical ethanol or RTX did not significantly affect luminal ACh **(Figure 6B).**

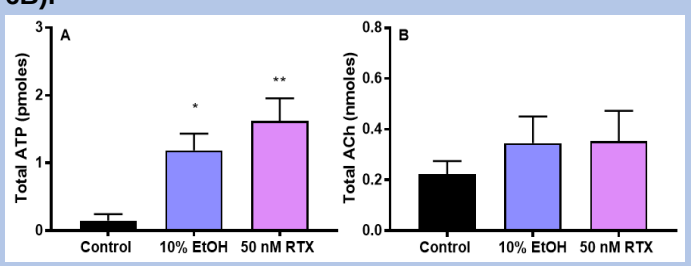

Figure 6: *The effect of intravesical treatment with 0.9% saline, 10% ethanol and 50 nM RTX on luminal distension induced ATP (A) and ACh levels. Analysed by a one-way ANOVA with Dunnet post hoc test (*p<0.05, **p<0.001 vs control).*

REFERENCES

1. Payne et al. J Urol. 2005;173:1590–1594.
2. Raisinghani, et al. J Physiol 567.Pt 3 (2005): 771–786.