

HISTOCHEMICAL AND MOLECULAR LOCALIZATION OF C-KIT-POSITIVE CELLS IN PIG URINARY BLADDER, AND CHARACTERIZATION OF THEIR FUNCTIONAL ROLE IN THE BLADDER USING C-KIT-INHIBITOR, IMATINIB MESYLATE (GLIVEC)

Hypothesis / aims of study

It is believed that interstitial cells (ICs), analogous to the interstitial cells of Cajal (ICC) of the gut, may generate phasic activity (PA) in smooth muscle tissues including the bladder. ICs express the proto-oncogene c-kit and signalling via the tyrosine kinase gene product, kit, is essential for development of the ICC phenotype. Imatinib mesylate (Glivec®), a c-kit tyrosine kinase inhibitor has been used widely for studying the role of ICs in generating PA in various smooth muscle types, including detrusor (1). The aim of this study was to identify c-kit positive cells in porcine urinary bladder by using molecular, immunohistochemical and functional techniques. The effect of imatinib on spontaneous and cholinergic induced PA of both bladder strips and isolated whole pig bladder was also investigated.

Study design, materials and methods

Female pig (≈6months old) bladders were obtained from the local abattoir with an average warm ischemic time of 30±5 min.

Molecular studies:

Primers were designed for the *Sus scrofa* c-kit mRNA and polymerase chain reaction (PCR) carried out on the c-DNA synthesized from total cytoplasmic RNA isolated from female pig bladders. PCR products were separated by electrophoresis and sequenced.

Immunohistochemistry:

For immunohistochemical studies, formalin-fixed paraffin-embedded bladder tissue was analysed using immunohistochemical staining for c-kit.

Functional studies:

For whole organ experiments (n=6), the bladder and its associated vasculature were surgically excised and maintained under physiological conditions, perfused with Krebs' solution as previously described (2). The effect of intravascular administration of increasing concentrations of imatinib (1-50µM added cumulatively, 15-20 min exposure for each concentration) or drug vehicle on carbachol-induced (0.1µM) whole bladder PA was monitored by recording the intravesical pressure (cmH₂O).

For bladder strip experiments, longitudinal strips of denuded detrusor (n=16) or mucosa (n=17), were mounted in perspex microbaths, superfused with Krebs' solution and maintained at 37°C. Denuded detrusor strips were superfused constantly with 0.1µM carbachol (CCh) solution to induce PA. The effect of increasing concentrations of imatinib (1-50µM) or drug vehicle on basal PA in mucosal strips and CCh-stimulated PA in denuded detrusor strips was investigated by measuring the amplitude and frequency of phasic contractions. All data are expressed as the mean±SEM and statistical analysis was carried out using repeated measures ANOVA followed by Dunnett's post-hoc test.

Results

Molecular studies:

Sequencing of the PCR product confirmed the expression of c-kit mRNA in both the mucosa and detrusor layers of the pig bladder.

Immunohistochemistry:

Expression of c-kit-antigen was detected in the sub-urothelial and muscle layers of pig bladders by positive immunoreactivity to c-kit antibodies.

Functional studies:

Isolated whole pig bladders developed an increase in baseline pressure (tonic contraction) with superimposed PA in the presence of 0.1µM CCh. Intravascular imatinib had no effect on PA frequency and only the 50µM concentration significantly inhibited (p<0.01) the amplitude of PA (Fig 1A). However, imatinib (5µM-50µM) significantly reduced (p <0.05-0.001) the tonic contraction of the isolated whole bladder (Fig 1B). In bladder strips, spontaneous basal PA was detected in mucosal but not denuded detrusor strips. Imatinib did not have a significant effect on the amplitude or the frequency of basal PA in the mucosal strips at all concentrations, but 50µM imatinib significantly inhibited the amplitude of CCh-stimulated PA in detrusor strips with no effect on the frequency. The drug vehicle had no effect on PA.

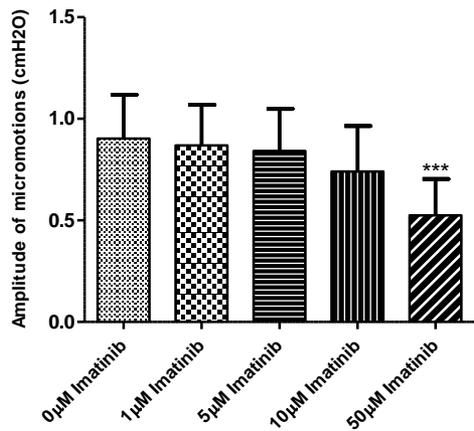
Interpretation of results

We have demonstrated c-kit expression in pig bladders using both PCR and immunohistochemistry. The absence of PA in denuded detrusor strips suggests an important role of the mucosa in driving such activity. Imatinib only reduced the amplitude of cholinergic-induced PA in the detrusor strips and the whole bladder preparations at the highest concentration with no effect on the frequency of PA. Imatinib did however reduce the tonic contraction in the whole bladder.

Concluding message

c-kit positive cells were detected in pig urinary bladder and may play an important role in modulating the phasic contractions and the tone of the bladder in this species.

A



B

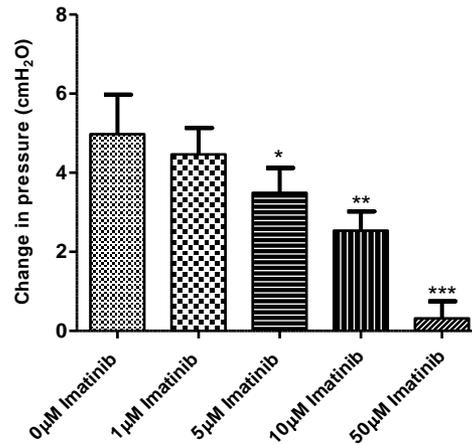


Figure 1) The effect of intravascular imatinib (1-50μM) on A) the amplitude of CCH-induced (10μM) PA and B) CCH-induced (10μM) tonic contractions of whole pig urinary bladders (n=6). *p<0.05, **p<0.01 and ***p<0.001 versus paired response with imatinib omitted. Data is presented as mean±S.E.M.

References

- 1) Kubota Y, Biers SM, Kohri K, Brading AF (2006). Effects of imatinib mesylate (Glivec) as a c-kit tyrosine kinase inhibitor in the guinea-pig urinary bladder. *Neurourology and Urodynamics*; 25: 205–10
- 1) Parsons, BA, Drake, MJ, Gammie, A, Fry, CF, Vahabi, B (2012) The validation of a functional, isolated pig bladder model for physiological experimentation. *Frontiers in Pharmacology*. 3:1-8

Disclosures

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