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THE EFFECT OF IMATINIB MESYLATE ON SPONTANEOUS CONTRACTIONS OF GUINEA-PIG BLADDER IN FRESH TISSUES AND ORGANOTYPIC CULTURES

Hypothesis / aims of study

Since the discovery of interstitial cells of Cajal (ICC) in the urinary bladder, investigations to elucidate their functional role in bladder activity have not yet been conclusive. It is known that several subtypes of ICC exist in bladder wall, including ICC within the lamina propria (ICC-LP), ICC running alongside detrusor smooth muscle bundles (ICC-IM) and in the interbundle spaces (ICC-IB). There is emerging evidence that ICC sub-populations may carry out distinct physiological roles. For example ICC-LP respond to purinergic but not cholinergic agonists, whereas detrusor ICC fire Ca^{2+} transients in response to cholinergic stimulation (1).

ICC express the proto-oncogene, *c-kit*, and signalling via the receptor tyrosine kinase gene product, Kit, is essential for development and maintenance of gut ICC phenotype (2). Tyrosine kinase inhibitors such as imatinib mesylate (Glivec), currently used as a treatment for Philadelphia chromosome-positive chronic myeloid leukaemia (CML) and Kit-positive gastrointestinal stromal tumours may be a useful tool to study bladder ICC function. Previous investigations of the effect of imatinib on spontaneous activity in guinea-pig bladder indicated that this drug may reduce activity but cautioned that when used at concentrations that exceed plasma serum levels a non-specific inhibition of Ca^{2+} channels may occur (3).

The aim of the present study was to examine the effect of imatinib on spontaneous activity of fresh guinea-pig bladder strips and organotypic cultures.

Study design, materials and methods

Bladders were removed from adult guinea-pigs of either sex (250-600g), opened longitudinally and the mucosa either left intact or removed to leave the underlying detrusor. *In vitro* tension recordings were made from bladder strips (10mm x 2mm x 2mm) after equilibration. Drugs were acutely applied via the perfusion system. In a separate series of experiments, strips of bladder were cultured with or without 1μM imatinib for up to four weeks before measuring mechanical responses.

Results

Guinea-pig bladder strips exhibited spontaneous phasic contractions whether the mucosa was present or absent. Application of imatinib to mucosa-free strips had no significant effect on the frequency of spontaneous phasic contractions over the concentration range 100nM to 10μM (n=19, 5 animals, $p>0.05$). Interestingly, in mucosa-intact preparations, imatinib (100-300nM) had no effect on contractile frequency (n=20, 5 animals, $p>0.05$), however, frequency was significantly reduced in a concentration-dependent manner over the range 1μM to 10μM (n=20, 5 animals, $p<0.05$).

In organotypic cultures, spontaneous contractile activity was observed in strips which had been cultured as far as four weeks. Cultured tissues maintained agonist-evoked responses when stimulated with carbachol (1μM) or ATP (5mM). Neurogenic stimulation of cultured tissues by 10s trains, 70V, 0.3ms duration, over the frequency range from 0.5 to 16 Hz produced frequency-dependent contractions which were reduced by atropine (1μM) and further attenuated by PPADS (100μM). No differences were observed between tissues cultured in the absence or presence of imatinib.

Interpretation of results

The c-Kit inhibitor imatinib, acutely applied to guinea-pig bladder strips, reduced contractile activity at therapeutic doses only when the mucosa was intact. This may be explained by the fact that intact strips contain the mucosal ICC-LP population in addition to the detrusor ICC and may imply a role for ICC-LP in modulating detrusor smooth muscle activity. Chronic application of imatinib does not appear to affect bladder contractility suggesting detrusor ICC are insensitive to inhibition of Kit during long-term organotypic culture. This may be explained by reduced plasticity of ICC in adult tissues.

Concluding message

A different approach utilizing tissues cultured from infant animals where Kit-positive cells have yet to fully differentiate may provide further insights into the role of ICC in bladder function. These studies are ongoing.

References

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2. Torihashi et al. (1995). Cell Tissue Res. 280, 97-111.

3. Kubota et al. (2004). Auton Neurosci. 115, 64-73.

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What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**The animals had been sacrificed by cervical dislocation in accordance with Schedule 1 United Kingdom Animal Scientific Procedures Act (1986) and were approved by local University animal welfare and ethics committee.**