

## DISTRIBUTION OF INTERSTITIAL CELLS IN THE RAT BLADDER FOLLOWING SPINAL CORD INJURY.

### Hypothesis / aims of study

Overactive bladder (OAB) is defined as “urgency, with or without urge urinary incontinence” (1) and affects a significant proportion of the population. The normal function of the urinary bladder is to store and expel urine in a coordinated, controlled fashion which is modulated by the central nervous system. Several animal models have been developed for the study of OAB including bladder outlet obstruction (BOO) and the spinal cord injury (SCI) rodent models which exhibit OAB. Bladder interstitial cells of Cajal (ICC) have been proposed to act as modulators of bladder smooth muscle spontaneous activity, moreover, a recent study showed upregulation of ICC expression in guinea-pig bladder following pseudo-obstruction (2). Little is known of the expression of ICC in SCI bladders therefore the aim of the present study was to investigate their distribution in bladders from sham-operated and spinal cord injured rats.

### Study design, materials and methods

Bladders were removed from sham-operated control and spinal cord injured female rats sacrificed 5 weeks post-surgery. Bladders were opened longitudinally and pinned to a dissecting dish. Whole-mount preparations of bladder mucosa or bladder detrusor were processed for antibody labeling using standard immunohistochemical protocols and imaged with confocal microscopy. Cell counts were made by 2 independent, blinded researchers. Statistical comparisons were made with unpaired t-test. Several bladders (3 SCI and 3 sham-operated controls) were processed for transmission electron microscopy (TEM).

### Results

Whole-mount preparations of sham-operated and SCI bladder were labeled with vimentin antibodies. Vimentin filaments are found in ICC and other cells in the interstitium of mesenchymal origin but not in smooth muscle. Sham-operated bladders contained extensive networks of vimentin-positive cells which contained stellate and bipolar cells. These networks were found in the lamina propria and in the inter-bundle spaces in the detrusor, close to the smooth muscle bundles. SCI bladders contained strikingly fewer vimentin-positive cells with only small patches of cell groups apparent and cells were absent altogether in many samples. Vimentin-positive cells in SCI bladders had rounded, unipolar or bipolar morphologies and cell to cell contacts were disrupted. Cell counts for vimentin-positive cells/100mm<sup>2</sup> showed that the number of cells was significantly reduced from  $8.5 \pm 0.5$  in sham-operated control bladders (N=6) to  $1.4 \pm 0.2$  compared with SCI bladders ( $P < 0.0001$ , N=6).

Sham-operated control tissues labeled with anti-neurofilament (N=3) demonstrated a dense innervation which included cholinergic nerves, labeled with anti-vAChT (N=3). SCI bladders lacked these neuronal networks and nerve fibres appeared to be compromised or damaged (N=6).

The immunohistochemical findings were supported with TEM which demonstrated frequent nerve-smooth muscle interactions in sham-operated controls and cells with the ultrastructural profile of bladder ICC. In contrast, SCI bladders showed extensive nerve damage and cellular debris in the locations where ICC would typically be found.

### Interpretation of results

Vimentin-positive cells formed extensive cellular networks in sham-operated control bladders whereas spinal cord injury of 5 weeks duration resulted in the significant reduction or loss of vimentin positive cells within the rat bladder. Nerve networks in SCI bladders were severely compromised compared with sham operated controls.

### Concluding message

The findings suggest that 5 weeks post-SCI, the urinary bladder has significant defects in innervation and a significant reduction in the expression of vimentin-positive cells. Ongoing collaborative work will reveal whether these morphological changes are associated with defects in bladder physiology.

### References

1. Wein AJ, Rovner ES (2002). *Urology*. 60, 7-12.
2. Kubota Y et al. (2008). *Neurourol Urodyn*. 27, 330-40.

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<i>Is this a clinical trial?</i>	No
<i>What were the subjects in the study?</i>	ANIMAL
<i>Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?</i>	Yes
<i>Name of ethics committee</i>	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.