

## INVESTIGATION OF EARLY PROTEIN CHANGES IN THE URINARY BLADDER FOLLOWING PARTIAL BLADDER OUTLET OBSTRUCTION BY PROTEOMIC APPROACH

### Hypothesis / aims of study

Little is known of the origin of overactive bladder symptoms associated with benign prostatic hyperplasia (BPH). Many pathophysiological mechanisms have been proposed, which are included that there were acute increases in the expression of bFGF, the transcription factors, upstream binding factor, c-Jun, and cyclins E and C, nerve growth factor, N-ras and c-myc, and decreased expression of TGF-beta 1, but they do not adequately explain all pathophysiological storage symptoms exhibited following bladder outlet obstruction (BOO). A proteomic technology has recently been developed to facilitate protein profiling of biological mixtures. We investigated the utility of this approach for the detection of marker proteins associated with pathophysiological mechanisms responsible for lower urinary tract symptoms (LUTS) following BOO.

### Study design, materials and methods

Rats were randomized into three groups: controls ( $n = 10$ ), a sham operated group ( $n = 10$ ), and a BOO group ( $n = 30$ ). The BOO group was sub-divided into three groups of 10, from which bladders were excised on days 1, 3, or 5 after the operation. Whole urinary bladders of all rats were excised completely after the beginning of the experiment. Proteins were extracted from rat urinary bladder tissue according to the German Heart Center method. Protein concentration was determined using a commercial Bradford reagent and the samples were stored at  $-70\text{ }^{\circ}\text{C}$  until analysis. Conventional proteomics was performed using high-resolution 2-D gel electrophoresis. The images were analyzed using Melanie III program (Swiss Institute of Bioinformatics, Geneva, Switzerland). All samples were processed at least three times to confirm reproducibility. Proteins that are differentially expressed in the bladder tissue are identified by matrix assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis with the search programs called ProFound and MS-FIT.

### Results

Two-dimensional electrophoresis of bladder tissue produced about 1000 spots per gel.

The proteins shown in these figures of two-dimensional electrophoresis were not all identified because, for many, the expression in obstructed bladder tissue was similar to that in normal bladder tissue. A comparison of bladder of BOO group with control bladder showed that three proteins; optineurin (OPTN), thioredoxin and prehaptoglobin were over-expressed in the bladder of BOO group and prehaptoglobin of these was increased transiently at 24 hours. Five proteins were under-expressed in the bladder tissue of the BOO group: peroxiredoxin 2, transgelin (SM22 alpha), hippocampal cholinergic neurostimulating peptide (HCNP), Golgi-associated protein, and beta-galactoside-binding lectin.

### Interpretation of results

In our animal model, BOO resulted in an increase in the expression of three proteins and a decrease in the expression of five proteins. Of these, HCNP, OPTN and transgelin are of particular interest. Up-regulation of OPTN may protect against nerve injury. Down-regulation of HCNP may make detrusor muscle supersensitive to acetylcholine, and down-regulation of transgelin may decrease its contractility.

### Concluding message

It is thus possible that the overactive bladder symptoms experienced by patients with BOO are due to detrusor hypocontractility. However, more information on human bladder tissue is needed for clinical application.