

## **ANIMAL MODEL FOR BLADDER DYSFUNCTION FOLLOWING LONGTERM INCOMPLETE EMPTINESS**

### **Hypothesis / aims of study**

Patients with end stage renal failure have the amount of urine production usually strongly reduced which implicates major or total loss of normal bladder storage and voiding function. After renal transplantation LUT dysfunctions are not rare.

Different animal models have been developed to evaluate long term bladder dysuse and its effect on bladder function recovery (1, 2). Some authors have simulated bladder dysuse by urinary diversion. Others have, beside diversion, hemidissected the bladder. These techniques cause bladder emptiness but do not correspond to the functional problem of bladder filling impairment. Moreover bladder surgery will in itself influence bladder function. Also most patients waiting for renal transplantation still produce a certain amount of urine which can affect the afferent nerves in the bladder mucosal layer and thereby influence the micturition reflex (3).

We have searched for a rat model which closely simulates the situation in patients waiting for renal transplantation: a urinary bladder which continues to contain small amounts of urine.

### **Study design, materials and methods**

With permission of the local ethics committee female virgin wistar rats were used (225-250g). The rats were anaesthetised by a mixture of intraperitoneal ketamine (100 mg/kg) and xylazine (15 mg/kg).

We investigated different techniques to reduce urethral resistance and to create a bladder which continues to contain small amounts of urine over a period of 2 months.

Dilating the urethra by inserting a transurethral catheter of increasing diameter resulted in urinary retention because of urethral oedema. A suprapubic catheter (tunneled subcutaneously) showed a clear risk of infection and blockage.

Inserting a transurethral stent induced incontinence but only for a mean period of 9 days.

Finally we tried to reduce the urethral support. Through perineal approach the mostly horizontal urethra was surgically freed and transposed to a vertical position. But the urethral tissue was frail and not viable.

Last we developed following model which was successful (n=14). A transurethral catheter (22G) was inserted. A suprapubic skin incision (1cm) was made and the abdominal muscles were split to reach the urethra. The urethra together with the vagina were surgically freed and fixed to the lower abdominal skin. The incision were closed in two layers.

Analgesia (tramadol 5 mg/kg po) and antibiotics (enrofloxacin, 1ml 5%, sc) were given during 5 days. The entire study period was 8 weeks.

The urinary incontinence was evaluated weekly by objectivating urine loss when running on a special paper which colours in contact with urine. At 8 weeks leak point pressure (LPP) was measured as described before: manually increasing of the abdominal pressure until leakage at the urethral meatus. Urinary retention was daily evaluated by manual palpation of the rats' lower abdomen. Urinary infection was evaluated on microscopy.

### **Results**

Ten rats leaked urine continuously during the entire study period. In the other 4 rats incontinence became less starting at week 4, 5, 6, 7 respectively. LPP in incontinent rats (19.8 +/- 6 cm water) was significant less than in the control group (61.8 +/- 9 cm water, p<0.0001). Even the 4 rats who were less incontinent than the others had a very low LPP (15, 17, 23, 35 cm water respectively).

Urinary retention did not occur at any time. No signs of urinary infection were found. None of the sham group showed signs of urinary incontinence, urinary retention or urinary infection. Using this procedure all rats survived anaesthesia and surgical manipulation.

### **Interpretation of results**

We developed an animal model by mobilising the rat's horizontal urethra to an almost vertical position to reduce urethral support and create continuous leakage. Both the anterior vaginal

wall and the surrounding pelvic floor muscles contributes to the continence mechanism in rats. Mobilising both the urethra and vagina neutralises this continence mechanism and induces incontinence while keeping the thin urethra viable.

Our success ratio was high. LPP was statistically significantly reduced in the incontinent rats. Focussing on the actual incontinence when running, 10 out of 14 rats were abundantly incontinent during the period of 8 weeks. In 4 rats incontinence became less during the study period, despite a still low LPP. The induction of more than average growth of connective tissue around the urethra and vagina, would seem a possible explanation, creating a hammock-like support.

### **Concluding message**

We developed an animal model to study bladder function after long-term bladder defunctionalisation which enables us to study the pathophysiologic mechanism of LUT changes in chronic patients awaiting renal transplantation.

### **References**

- (1) Functional and pharmacological effects of ureteral diversion. J Urol 1989, 141: 403-407.
- (2) A unique new model to study the effects of urinary diversion in the developing rabbit bladder. J Urol 1998, 160: 1454-1458.
- (3) Control of bladder function by peripheral nerves: avenues for novel drug targets. Urology 2004, 63 (3 suppl 1): 24-31.