Abstract. Background and Aim: The objective of this study was to establish a rat model to develop hypertrophic fibrosis for subsequent safe application of ureteral stents in order to investigate new treatment options for ureteral strictures. Materials and Methods: Thirty-two male Sprague-Dawley rats were used. Group 1: Sham surgery; group 2: surgery with uretero-ureteral anastomosis and stenting. Histopathological evaluation was carried out using 5-bromo-2-deoxyuridine administration before the animals were sacrificed. Results: A total of thirty-one animals reached the final end-point. The most common surgical complications were urine extravasation and stent dislocations. Histological examination showed full regeneration of urothelium after 28 days and development of a scarring process. With stent insertion, moderate hypertrophia was seen. In contrast, the sham group had no evidence of significant scarring or stricture formations. Conclusion: Our rat model allows for investigation of the wound healing processes of urothelium of the ureteral wall and the study of the application of new miniature stents as drainage and drug carriers.

Several methods have been used for ureteric reconstruction, but complications following this procedure, such as leakage or obstruction are frequently encountered, leading to various modifications of the surgical technique (direct anastomosis of ureter to re-configurated bladder, transposition of one ureter onto the contralateral one). The non-splinted uretero-ureterostomy has allowed for routine ureteric reconstruction to be performed with an acceptable rate of complications. This has been described by several authors in recent studies (1-8).

Materials and Methods

We used 32 male Sprague-Dawley rats (Charles River Laboratories Research Models and Services, Germany GmbH, Sulzfeld, Germany) with an initial weight between 300 and 320 g in the test series in two groups. In group 1, 16 rats underwent sham surgery without uretero-ureteral anastomosis but with intraoperative dissection and manipulation of the ureter with a blunt instrument (reference group); in group 2, 16 animals underwent surgery with transection of the left ureter, insertion of a ureteral stent and an end-to-end uretero-ureteral anastomosis by suturing.

For preoperative analgesia, buprenorphine (Temgesic®) in a dose of 0.1 mg/kg body weight was administered s.c. 1 h before starting the procedure. Anesthesia was induced by intramuscular injection of ketamine (dose 100 mg/kg body weight), (Ketanest S 25®) plus xylazine (dose 5 mg/kg body weight) (Rompun®). Approximately 10 min after injection, anesthesia level was sufficient for further preparation and commencement of surgery. All surgical procedures

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were performed with conventional surgical instruments under sterile conditions.

The abdomen was opened by a midline incision with extension from the suprapubic area to the xiphoid process. In the control animals (group 1), the left ureter, with a medium outside diameter of 0.4-0.6 mm, was dissected-free and manipulated with a blunt instrument at ×20-40 magnification under a surgical microscope. Closure of the abdominal wall was performed in two layers with separate running sutures continuously.

In group 2, the left ureter was dissected-free at ×20-40 magnification under a surgical microscope, diagonally transected at the level of the common iliac vessels. A Grilamid® L25 Natur stent with a caliber of 0.40/0.20 mm and 12 mm length (eucatech AG, Rheinfelden, Germany) was inserted into the ureter and fixed with a suture of 10-0 nylon to avoid dislocation. Subsequently, ureteral anastomosis was performed with six single sutures to avoid urine leakage and the stent was fixed by an additional suture cranial to the anastomosis [Ethilon®, black, monofil, non absorbable, CS-44C+CS44C, USP 10-0/0,30m, Ethicon].

Closure of the abdominal wall was performed in the same way as for the control group in two layers with separate running sutures (Marlin®, violet, monofil, absorbable, DS24, USP 3-0/0,7m, Catgut).

For all animals, blood tests were performed pre- and postoperatively (white blood cells, red blood cells, hemoglobin, platelets and creatinine) (9,10).

For postoperative histology, 5-bromo-2-deoxyuridine (BrdU, B5002-5G, Sigma-Aldrich, Steinheim, Germany) (100 mg/kg body weight BrdU or 5 ml/kg at a concentration of 20 mg/ml) was injected intraperitoneally on days 1 to 8 postoperatively, at the same time every morning.

For surveillance of the animals, a vigilance score (see appendix) was counted and evaluated on every postoperative day. This score included behavior, body weight, eating behavior, mobility, appearance and serum creatinine. Points were scored as 0.0, 0.2 or 0.4 per criterion to the termination criterion end-point. At ≥1.2 points, additional measures were considered if necessary (11,12).

The animals were sacrificed on day 28 and a histological examination of the left ureter was done (13).

The study had been approved by the Ethics Committee of the University Medical Center of Rostock University and the State Office of Agriculture, Food Safety and Fisheries, Rostock, Germany (AZ 7221.3-1-026/11).

**Histology and immunohistochemistry.** Histopathological examination of the ureter was analyzed by light microscopy using the urothelium in the area of the anastomosis with respect to its structure and...
preservation of the top cell layer. In addition, an examination of the kidney on the operated left side was made in comparison to the contralateral kidney. Histological quantification was carried out by the application of BrdU (BrdU, B5002-5G, Sigma-Aldrich, Steinheim, Germany), a thymidine analogue capable of replacing thymidine in DNA synthesis during the cell cycle. By pulse labelling with BrdU, cellular DNA synthesis can be analyzed by detection with antibodies to BrdU. Antibodies against BrdU recognize BrdU and thus form an antibody-antigen complex. With the help of a secondary antibody, an enzyme reaction and a dye, the bound antibodies are visible under microscopy.

Statistical analysis. 16 animals per group were expected to be sufficient for the statistical evidence targeting hypertrophic fibrosis of the ureter (normal distribution with standard deviation, error \( \alpha \) set at 5\%, the difference between the true value of null hypothesis and alternative hypothesis being \( \delta=1 \) with standard deviation \( s=1 \) (15, 16).

Results

The sham surgery group animals started with a median weight of 302.5 g and developed a median increase of weight of 3.9 g per day until the end of the study on day 28 with a median end-of-study weight of 409.5 g. In contrast, the rats of the ureteric surgery group had a median starting weight of 315.0 g, but a lower daily weight increase of only 1.1 g per day. Thus, they finished the study with a median weight of 360 g (Table I).

The vigilance score showed no differences between the two groups and no significant deterioration in either group. Similarly, the median serum leukocyte counts were comparable in both groups (6.6×10^9/l).
In the ureteric surgery group, some surgical complications were observed. These were urine extravasation in one animal and stent dislocation in two animals. However, despite these complications, we were able to successfully complete the investigations in the ureteric surgery group of animals. A total of 31/32 animals reached the final end-point of the study with end-of-study sacrifice and histological analysis on day 28. One animal with a urine leak from the anastomosis and weight loss was euthanized earlier, but was also examined histologically.

Histological examination showed full urothelial regeneration at day 28 with development of scar formation in the deeper layers of the ureteral wall (Figure 2). The verification of the presence of cell proliferation in this process was confirmed by using antibodies against BrdU. The insertion of the miniature stent resulted in the development of moderate hypertrophia of the urothelium. In contrast, the ureters of the sham group showed no histological evidence of significant scar formation, stricture development or distension of the upper urinary tract. The median serum creatinine values were 53 μmol/l for the sham group compared to 48.5 μmol/l in the ureteric surgery group (not significant). Thus, there was no change of overall renal function, as expressed by serum creatinine levels following ureteric anastomosis with stenting.

**Discussion**

In ureteric surgery, repair tissues following uretero-vesical, uretero-ureteral or uretero-ileal anastomoses are formed by granulation tissue and scarring. Wound contraction and leaking urine promote hypertrophic fibrosis (1), which may lead to a relative excess of scarring and resulting stenosis of the ureteric lumen. The main causes of such a stricture of a ureteric anastomosis are postoperative anastomotic edema, microscopic urine extravasation, ischemia in the area of the dissected ureter and infection in the region of anastomosis (17).

A stenotic ureteric anastomosis results in further clinical problems and constitutes a failure of the primary reconstructive procedure. End-ourological procedures of low invasiveness can be used to solve the problem by endoscopic incision of the stricture, but this carries a recurrence rate of up to 50%. Also, the resulting new scar is usually longer than the previous stricture. In cases of recurrent stricture after such endoscopic treatment, secondary open surgery should be performed with a ureteral anastomosis or more extensive procedures such as ileal replacement of part of the ureter.

In our study, the surgical complications observed postoperatively in our small number of animals were urine extravasation and stent dislocation in the stented group which corresponds to clinical experience in urology. Therefore, the surgical procedure was ameliorated during the course of the study. The anastomosis was performed using six sutures to successfully avoid urine leakage and the stent was fixed by a special suture cranial of the anastomosis. Thus, a reliable stent positioning was achieved.

The results reported here, indicate that we have established a successful and reliable animal model in adult male Sprague-Dawley rats for the stenting of a ureteral anastomosis as a basic model for future studies investigating ureteral anastomotic healing with differently coated and/or drug-eluting stents.

**Conclusion**

We have developed and tested a rat model of ureteral surgical anastomosis showing the feasibility of ureteral splinting of the anastomotic area with an acceptable rate of ureteric

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**Table I.** Development of body weight, creatinine levels and vigilance score in the sham group and in the surgical group pre- and postoperatively. Data are given as range with minimum and maximum. Reference levels according to (10, 22, 23).

<table>
<thead>
<tr>
<th></th>
<th>Sham OP</th>
<th>With stent</th>
<th>Reference level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight on day 1 (g)</td>
<td>300-308</td>
<td>300-320</td>
<td></td>
</tr>
<tr>
<td>Creatinine on day 1 (μmol/l)</td>
<td>44.2-67.8</td>
<td>44.2-64.7</td>
<td>44.2-53.0</td>
</tr>
<tr>
<td>Weight on day 28 (g)</td>
<td>391-437</td>
<td>314-412</td>
<td></td>
</tr>
<tr>
<td>Weight increase on day 28 (g)</td>
<td>91-127</td>
<td>10-112</td>
<td>25 g/week</td>
</tr>
<tr>
<td>Best score 0-2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Worst score 0-2</td>
<td>0</td>
<td>0.4</td>
<td>0</td>
</tr>
<tr>
<td>Leukocytes (10⁹/l)</td>
<td>6.2-7.1</td>
<td>4.7-7.3</td>
<td>10.09-14.01</td>
</tr>
<tr>
<td>Creatinine day 28 (μmol/l)</td>
<td>44.2-93.3</td>
<td>44.2-88.3</td>
<td>44.2-53.0</td>
</tr>
</tbody>
</table>
complications. The model enables the investigation of ureteric wound healing and the study of new miniature ureteral stents as drainage and as a potential drug carrier. The model can be used for investigations of new technologies of drug applications as well as for drug dose finding trials.

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References

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