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ORIGINAL PAPER

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An experimental model for stricture studies in the anterior urethra of the male rabbit

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Abstract In this study, an animal model was developed for the examination of urethral strictures (US). Through a resectoscope, a resection was made in the urethras of 15 male rabbits. After 30 days, the rabbits were evaluated with urethrography, impedance planimetry and either histology or the determination of collagen content. Fifteen rabbits serving as controls were evaluated in the same way. Three rabbits in the resection group and one in the control group died before evaluation. Urethrography demonstrated a stricture in the remaining 12 animals in the resection group. The urethras of the control animals were all normal. Impedance planimetry confirmed that the luminal cross sectional area (CSA) of the strictures was significantly smaller than the CSA of the corresponding part of the urethra in the control group. No difference in CSA was found 1 cm proximal to the stricture. The strictures consisted of densely woven collagen which sent tongues into the adjacent normal parts of the urethra. No difference in collagen content was found between the two groups either at the stricture site or 1 cm proximally. The described method of producing US in the rabbit model was very consistent with all operated animals developing a stricture. The model might prove valuable in evaluating new methods for the treatment of US.

Keywords Urethra · Stricture · Histology · Collagen · Animal

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Introduction

Urethral strictures (US) constitute a major problem among urological patients. In Denmark, approximately 2,000 US are treated every year (corresponding to an incidence of 1 per 1,250 male inhabitants) [12]. More than 50% of these strictures are iatrogenic, most frequently seen as a complication after instrumentation of urethra or to an indwelling catheter. US used to be secondary to infection (gonorrhoea) [3] but nowadays less than 10% are due to infection [1, 9, 10]. Multiple procedures are available for the treatment of US but none are completely satisfactory. The best results are obtained with open urethroplasty which is, however, a major operation associated with many complications and great costs [10]. Direct vision internal urethrotomy is a simple and initially effective treatment with only few complications. However, the recurrence rate is high with about 50% of the strictures recurring within the first year [1, 9, 10].

The pathogenesis of iatrogenic US is not known. A number of factors are thought to predispose to stricture formation: pre- or postoperative infection, indwelling catheter, the material of the catheter and urethral ischemia at the time of surgery. The relative significance of these factors is, however, not known [5, 7, 11, 14, 15, 17, 18, 19]. No doubt trauma is of importance. Lesion of the epithelial lining of the urethra, and maybe even the deeper parts of the wall, permits urine to leak into the urethral wall. This leads to an inflammatory reaction, scar formation and subsequently, if the reaction is severe enough, to a stricture.

There is a need to develop new procedures for the treatment of US. These should be simple and effective and, most importantly, not associated with the high recurrence rate seen today.

This study was undertaken in order to develop a standardized animal model for the study of US, a model which can be used to evaluate the use of drugs in the prevention of development and recurrence of strictures.

Material and methods

Thirty male, Danish, white land-race rabbits were randomly allocated into two groups: the resection group ($n = 15$) and the control group ($n = 15$). The rabbits in the resection group were 230 ± 2 days old (mean ± SEM) and weighed 4.04 ± 0.08 kg at the time of the resection. They were 262 ± 2 days old and weighed 3.92 ± 0.16 kg at the time of the evaluation. The rabbits in the control group were 239 ± 6 days old at the time of the investigation and weighed 4.17 ± 0.10 kg.

The study complied with the Danish regulations for care and use of laboratory animals.

Procedure

All procedures were performed on anaesthetized rabbits. Anaesthesia was induced by premedication with i.m. fentanyl citrate 0.055 mg/kg and fluanisone 1.75 mg/kg (Hypnorm, Janssen-Cilag, Beerse, Belgium) and midazolam 1.25 mg/kg (Dormicum, Roche, Basel, Switzerland) and maintained with i.m. fentanyl citrate 0.032 mg/kg and fluanisone 1 mg/kg and i.v. midazolam 0.625 mg/kg every 20–30 min. The rabbits were placed in a supine position. A resection was made in the urethra in the resection group as described below. At an average of 30 days after resection (28–35 days) the rabbits were evaluated with urethrography, impedance planimetry and either histology or the determination of collagen content. The rabbits in the control group were evaluated in the same way.

Production of urethral strictures

In the resection group, a 2–3 mm wide resection was made under sterile conditions at the transition from the spongious to the bulbous part of urethra from 2–10 o'clock using the electrical sling in a paediatric resectoscope Ch 13. The resection was deep enough to expose the periurethral tissue in order to allow urine to leak from the lumen. All resections were done by the same urologist (J.B.N.). I.v. ampicillin 300 mg (Anhypen, Yamanouchi Pharma, Leidersdorp, Holland) was administered before and after the resection and an additional 300 mg was administered twice daily i.m. for 2 days after the operation.

Urethrography

The tip of a 5 F catheter was passed through the external urethral orifice and contrast medium (Urografin, Schering, Berlin, Germany) was injected into the urethra under X-ray direction in order to visualize the configuration of the lumen and the possible presence of a stricture (Fig. 1). Two distension sites in the anterior urethra for biomechanical investigation were defined from the urethrography: the distal site at the transition from the spongious to the bulbous part of urethra, the location of the resection in the resection group, and the proximal site 1 cm further up in the bulbous urethra.

Impedance planimetry

The principles of measurement of CSA during balloon distensions using impedance planimetry have been described in detail elsewhere [4, 6, 8]. The probe for the biomechanical measurement and validation data for the measurement system have been described previously [2]. In short, a four electrode impedance measuring system located inside of a balloon on a 7 F probe was constructed for measurement of the CSA according to the field-gradient principle. The electrodes were connected to an impedance planimeter (GateHouse, Noerresundby, Denmark). Through the lumen of the probe, the balloon was connected to a level container with 0.09% saline solution. Under X-ray supervision, the probe was placed in the urethra with one detection electrode on each side of the distal distension site. The tissue was preconditioned by increasing and decreasing the pressure in the balloon in steps every 1.5 min (0, 1, 2.5, 4, 5 kPa) twice. In the third series of pressure increase, steady state CSA was awaited at each pressure step (0, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 5 kPa). Related values of steady state CSA and pressure were registered. The balloon was emptied, the probe moved to the proximal distension site and the same preconditioning and measuring procedure accomplished. The probe was removed and the animals sacrificed by i.v. pentobarbital 200 mg (Mebumal, Nycomed DAK, Roskilde, Denmark). The urethras were harvested as described below.

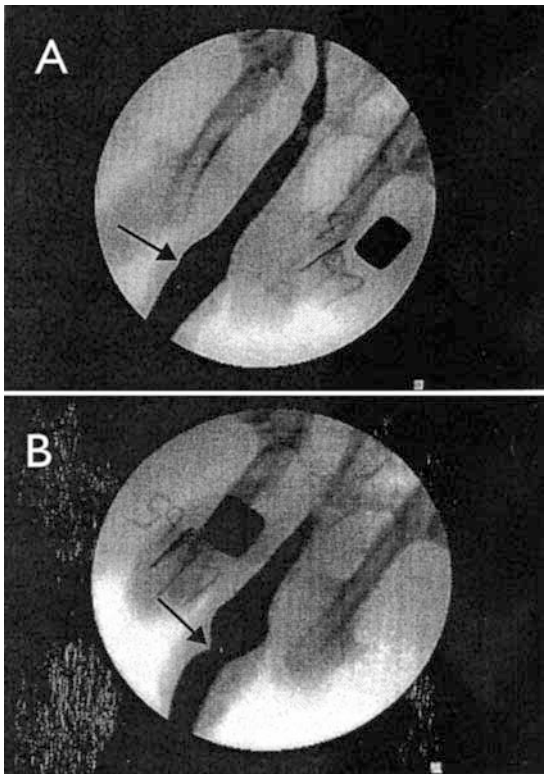

Fig. 1 **A** Urethrography of a normal male rabbit urethra. The passage from the spongious to the bulbous part of urethra is marked with an *arrow*. **B** Urethrography of a urethra with an induced stricture marked with an *arrow*. The *square* is 1 cm^2

Histology

The urethras from four rabbits from each group were randomly selected and processed for histology. The procedure has been described previously [2]. Formaldehyde was injected into the urethra under a pressure of 1.8 kPa for 30 min. The urethra was removed, further fixed in formaldehyde for 24 hours and then embedded in paraffin. Sections were cut corresponding to the two distension sites longitudinally from the posterior wall of the urethra, perpendicularly to the wall and stained with Sirius red.

Analysis of collagen content

The urethras from six rabbits from the resection group and ten from the control group were processed for the analysis of collagen

content. The urethra from one rabbit in the control group was not properly weighed and had to be disregarded in the analysis. The tissues from the two distension sites in the urethra were separated, chopped, defatted in two parts of chloroform and one part of methanol for 24 hours and freeze-dried. The hydroxyproline content was determined by the method of Stegeman and Stalder [16]. The total collagen concentration was estimated and expressed as μg/mg of dry, defatted tissue.

Statistical methods

The results are expressed as mean ± SEM unless otherwise stated. The data distribution was tested for normality by inspection of probability plots and for variance homogeneity by Bartlett's test. Only those parts of the pressure-CSA plots showing homogeneity of variance were tested using two-way analysis of variance. The collagen content of the tissue was tested using the paired *t*-test (within groups) or the unpaired *t*-test (between groups) in case of normal distribution of data, otherwise the Wilcoxon signed rank test was used. Correlations were performed using Pearson product moment correlation. The results were considered significant if $P < 0.05$.

Results

One rabbit in each group died during anaesthesia before any experiments could be carried out. Two rabbits in the resection group were killed during the follow-up period: one the day after the resection as it failed to thrive. The other developed diarrhoea and was killed 16 days after the resection.

Urethrography

The urethrogram of the remaining 12 rabbits in the resection group confirmed a stricture of the urethra (Fig. 1B), but in two rabbits the stricture was distal to the intended site at the transition from the spongious to the bulbous part of urethra. These two rabbits were disregarded in the analysis. The urethrogram of the 14 rabbits in the control group were all normal, the transition from the spongious to the bulbous part of urethra was always easily identified (Fig. 1A).

Impedance planimetry

In two of the ten rabbits in the resection group, the stricture was so tight that even though it was possible to pass the probe through it the maximal applied pressure, 5 kPa, was not enough to distend the urethra. At a pressure of 5 kPa, X-ray investigation with radiodense contrast media injected into the urethra in the vicinity of the balloon showed the balloon to be shaped like an hourglass with the narrow part at the stricture site. In one of the two rabbits, it was not possible to advance the probe to the proximal distension site. Therefore, the study comprises the pressure-CSA relation from eight rabbits at the distal and nine rabbits at the proximal distension site in the resection group and from 14 rabbits both distally and proximally in the control group.

At all pressure levels, the response to pressure increase was a phase of rapid CSA increase followed seconds later by a phase of slow CSA increase. The relation between the increasing distension pressure and steady state CSA was nonlinear at both the distal and the proximal distension sites in both groups (Fig. 2). At the distal distension site, the CSA for pressure-steps 2–5 kPa of the resection group was significantly smaller than the CSA of the control group ($P < 0.01$). No difference in CSA was found at the proximal distension site ($P > 0.4$). In the resection group, the CSA at the proximal distension site for pressure-steps 2–5 kPa was significantly larger than the CSA at the distal site ($P < 0.001$). In the control group, the CSA at the proximal distension site for pressure-steps 2.5–5 kPa was significantly larger than the CSA at the distal site ($P < 0.01$).

In the control group, a positive correlation was found between the CSA at the distal distension site at the maximum applied pressure 5 kPa and the age and the weight of the animals (age: $r = 0.571$, $P < 0.05$, weight: $r = 0.682$, $P < 0.01$). At the proximal distension site, no correlation was found between the CSA at 5 kPa and the age of the animals ($r = 0.062$, $P > 0.8$) but a positive correlation with weight was found ($r = 0.644$, $P < 0.05$). No correlation was found between the age and the weight of the animals ($r = 0.2$, $P > 0.4$).

Histology

In the control group, at the distal distension site, the urethras were lined with transitional epithelium on a thin layer of dense collagen (Fig. 3A). The epithelium was, however, in most places abraded. Beneath this, a collagen network with many thin-walled cavernous blood vessels was found. In this network, especially near

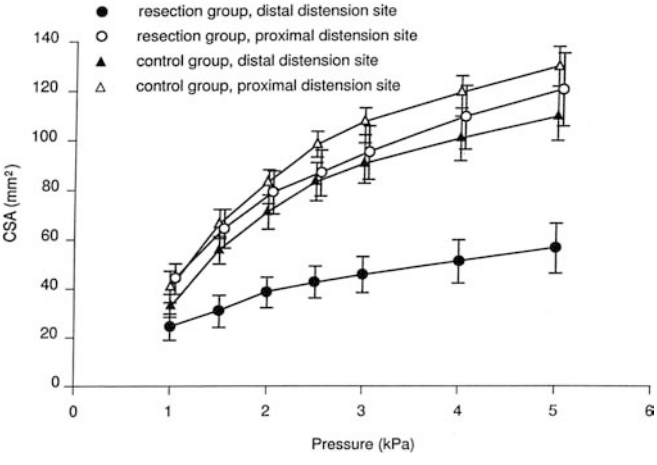

Fig. 2 Pressure-CSA relation for the distal and proximal distension site for the resection and control groups. Mean ± SEM for eight rabbits distally and nine proximally in the resection group and 14 rabbits both distally and proximally in the control group

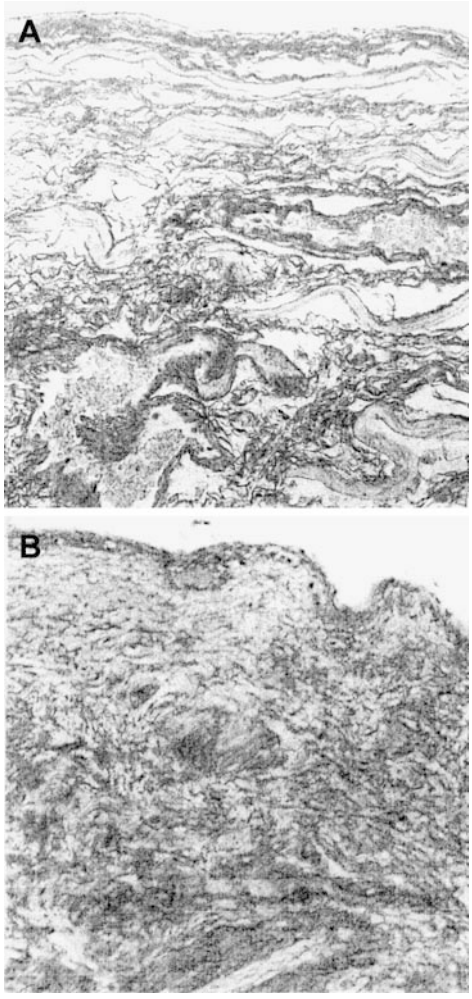

Fig. 3 Histological sections stained with Sirius red of the luminal part of the wall of the urethra corresponding to the distal distension site. The lumen is at the top of the histological sections. **A** The control group. Note longitudinally orientated collagen fibres and smooth muscle cells. **B** The resection group. Note the densely woven collagen network. Magnification ×50

the lumen, collagen fibres forming longitudinally wavy lines were seen. Mainly longitudinally orientated smooth muscle cells were interspersed between the collagen fibres. These were separated, suggesting oedema of the urethral wall, especially near the lumen. Extravasation in the wall was not pronounced but some erythrocytes were seen between the collagen fibres. In the deeper parts of the wall, the collagen became denser forming an interlacing network. In the resection group, epithelium was hardly ever seen in the strictures (Fig. 3B). When present, it was difficult to identify but did in some areas resemble transitional epithelium and in others squamous epithelium. The strictures consisted of densely woven collagen; longitudinally orientated collagen fibres were not seen. A few smooth muscle cells were found but these were not longitudinally orientated as in the control urethras. In the collagen network, large dark cell nuclei were seen which were probably fibroblasts. Small thin-walled vessels were embedded in the collagen. Neither acute nor chronic inflammatory cells were seen. Tongues of dense collagen projected into the adjacent normal parts of the urethra on each side of the stricture. The deepest part of the wall seemed normal and was a continuation of the normal tissue on each side of the stricture.

Table 1 Collagen content of the distal and the proximal distension site in μg/mg of dry, defatted tissue. Mean ± SEM for six rabbits in the resection group and nine rabbits in the control group

	Collagen content (μg/mg)	
	Distal distension site	Proximal distension site
The resection group	534 ± 30	299 ± 65[1]
The control group	523 ± 45[2]	283 ± 38[1, 2]

[1]The collagen content of the distal distension site was significantly higher than the content of the proximal site
[2]no significant difference between the two groups

At the proximal distension site in both groups, the collagen beneath the transitional epithelium formed a network. Longitudinally orientated collagen fibres were present in this network. Some separation of the collagen fibres was seen but this was not as pronounced as at the distal distension site. Longitudinally orientated smooth muscle cells and thin-walled cavernous blood vessels were embedded in the collagen network. The network was denser and the wall thinner at the proximal than at the distal distension site. The wall of the urethra was surrounded with striated muscles.

Analysis of collagen content

The collagen content of that part of the urethra corresponding to the distal distension site was larger than the content of the proximal site in both groups ($P < 0.05$ and $P < 0.01$, resection and control group, respectively) (Table 1). No difference was found between the two groups in collagen content of either the distal or the proximal distension site ($P > 0.5$ at both sites).

Discussion

This study describes an animal model of US in the anterior urethra of the male rabbit. The strictures were produced by a resection at the transition from the spongious to the bulbous part of urethra. This method is highly efficient: four rabbits were killed or died in the two groups. All 12 surviving animals in the resection group developed a stricture. The resection made is indeed a large trauma to the urethra but in our experience smaller injuries, electrocoagulation or smaller resections, will not always result in a stricture. Meria et al. [13] described a method of producing US in rabbits by circumferential electrocoagulation of the bulbar urethra, however, only 50% of the animals developed a stricture within 1 month. As the purpose of the model developed

in this study is to evaluate new methods of treatment of US, it is necessary that a large percentage, if not all the animals, should develop a stricture.

For the animal model, 4 kg male rabbits were used because the anatomical conditions of this animal permits transurethral instrumentation with standard paediatric cystourethroscopes. Furthermore, the urethra of the male rabbit resembles the urethra of the human male histologically, with a thin epithelium supported by spongious tissue rich in blood vessels.

The strictures produced were demonstrated by way of impedance planimetry. Sources of error for this method have previously been described in detail [4, 6, 8]. Probe characteristics and in vitro tests of the system used in this study corresponded well with previously described validations of this method [2]. Of special concern when using the system in strictures is the slope of the wall of the urethra between the detection electrodes [8]. Measurements in ''stricture-dummies'' (a 1–2 mm long tube of known diameter put into a 5 cm long tube with a larger diameter) have shown the error due to slope of the wall to be 8–21%, depending on the difference between the diameters of the large and small lumina in the dummies [2], the recorded CSA being larger than the actual CSA of the small tubes. The CSA of the strictures will thus be overestimated. In spite of this error, the method is found suitable for the evaluation of induced strictures in the male rabbit anterior urethra.

This study showed that the luminal CSA of the strictures was reduced to about half the size of the CSA of the corresponding part of the urethra in the control group, whereas no difference in CSA was found 1 cm proximally in the bulbous urethra. In the control group, a positive correlation was found between the CSA at the maximum applied pressure and the age and the weight of the animals. As the rabbits in the resection group were older at the time of evaluation than the animals in the control group, this might have influenced the results. The difference in age and weight of the rabbits used could explain some of the large variation in CSA of the urethra. It is therefore important when using this model to match the rabbits very carefully in terms of both age and weight.

Even though no difference in the content of collagen was found between the two groups, the structure of the collagen was completely different: the collagen network rich in blood vessels seen in the normal urethras was replaced with densely woven collagen with only a few thin-walled vessels. This might indicate that not only the total amount, but also the structure of the collagen determines the biomechanical properties of the tissue.

In conclusion: this study describes a method for producing US in the anterior urethra of the male rabbit by a resection at the transition from the spongious to the bulbous parts of urethra. The strictures were demonstrated by urethrography. Impedance planimetry substantiated that the luminal CSA of the strictures was significantly smaller than the CSA of the corresponding part of the urethra in the control group, whereas no difference was found 1 cm proximal to the stricture. As this method of producing US was highly efficient, the model might prove valuable in evaluating new methods of treatment of US.

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