Establishment of a stable urethral stricture model in New Zealand rabbits

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Abstract

Objective: To explore the method of building a stable urethral stricture (US) model in New Zealand white rabbits.

Methods: Through 10× magnification optical microscope, a resection of 1.0 cm urethral mucosa was made in 6 male rabbits and other 6 male rabbits were controlled. After 60 days, the rabbits were evaluated with urethrography, urethral pressure profile (UPP) and histology.

Result: Urethrography demonstrated a stricture with narrow lumen and discontinuous mucosa in the resection group. The urethras of the control animals were all normal. UPP showed that the urethral pressure on operative site in the controlled group was 14.67 ± 2.16 cm H2O, and 27.83 ± 3.71 cm H2O in the resection group. There was significant statistical difference between the two groups (p < .01). The urothelium was well-distributed, covered without any inflammatory cells in the controlled group, which had 3–4 layers of the epithelial cells. And the urothelium was unequally covered with neutrophils and lymphocytes in the resection group.

Conclusions: We establish the way to build a stable urethral stricture model of New Zealand rabbits by the microsurgical technique, which is a good laboratory model to research all kinds of urethral stricture. Urethrography and histology combined UPP are the reliable methods to identify the urethral stricture.

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PALABRAS CLAVE

Estenos uretral; Modelo animal; Perfil de presión uretral; Urodinámica

Establecimiento de un modelo estable de estenosis uretral en conejos de Nueva Zelanda

Resumen

Objetivo: Explorar el método de construir un modelo de estenosis uretral estable en conejos blancos de Nueva Zelanda.

Métodos: A través de un microscopio óptico de 10 aumentos se realizó una resección de 1 cm en la mucosa uretral de 6 conejos machos y se controló a otros 6 conejos hembras. Después...
Establishment of a stable urethral stricture model in New Zealand rabbits

Introduction

Urethral stricture (US) is one of the common diseases among urological patients. In Denmark, approximately 2000 USs are treated every year.\(^1\) US has a close relation between age and gender. The prevalence of US is low in children. However, it rises by aging and it does not become lower until 55 years old.\(^2\) The US prevalence in male patients is higher than that in female patients.\(^3,4\) More than 50% of these strictures are iatrogenic, most frequently seen as a complication after instrumentation of urethra or to an indwelling catheter. 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.\(^5\) The relative significance of these factors is not known.\(^6-8\) US used to be secondary to infection,\(^9\) but nowadays less than 10% are due to infection.\(^10-12\) Multiple procedures are available for the treatment of US but none is completely satisfactory. Direct endourethrotomy is a simple and initially effective treatment for the short stricture segment. However, the recurrence rate is high with about 50% of the strictures recurring within the first year.\(^10,11\) For the long stricture segment, the open urethroplasty is a major operation which, however, has many complications and great costs.\(^11\) There is a need to develop new procedures for the treatment of US. These should be simple and effective and, most importantly, no high recurrence rate is seen today. To research the treatment of US, especially for US of the long stricture segment, developing a stable animal model of US is very important.

Material and methods

Twelve male New Zealand rabbits were randomly allocated into two groups: the US group (\(n = 6\)) and the control group (\(n = 6\)). The rabbits in the US group weighed 2.88 ± 0.14 kg and those in the control group weighed 2.80 ± 0.16 kg. The laboratory animals were provided by the Laboratory Animal Centre of Fudan University. The study complied with the Chinese regulations for care and use of laboratory animals.

Procedure

All procedures were performed on anaesthetized rabbits. Anesthesia was induced by premedication with i.v. 3% pentobarbiton sodium 2 ml/kg (0.1 ml/s). The rabbits were placed in supine position. A resection was made in the urethra in the US group as described below. After 60 days, the rabbits were evaluated with urethrography, urethral pressure profile (UPP), and histology. The rabbits in the control group were evaluated in the same way.

Production of urethral strictures

In the US group, the F8 catheter was pulled to the bladder of rabbits through the urethra under sterile conditions. The bladder was washed by 5.0 ml Gentamicin (160,000 U/500 ml) through the catheter 5 times. A 1.5-cm length resection was made at the transition from the spongy to the bulbous part of the urethra on the abdominal side of the penis in a 10-magnification optical microscope. The resection was deep enough to expose the periurethral tissue in order to resect a 1.0-cm-length urethral mucosa. All resections were done by the same urologists (Z.J. and S.C.Y.). I.v. 10% ceftriaxone 30 mg/kg was administered before and after the resection and an additional dose (30 mg/kg) was administered once daily i.m. for 7 days after the operation.

Urethrography

The F8 catheter was pulled through the urethra and 20 ml of the contrast medium were injected into the bladder. After the catheter was taken away, the suprapubic region of the rabbit was pressed in order to visualize the configuration of the lumen and the possible presence of a stricture under X-ray direction. All the procedures were carried out by the same urologists (Z.J. and L.B.K.) and radiologists.

Urodynamics

The F7 urethral pressure-measurement catheter was placed to the bladder. After the tubes and the junctions were checked and the air bubble was emptied from the tube, the external zero was performed, in which the tube was placed on the suprapubic level of the rabbits. The urethral pressure profile (UPP) was measured 3 times in each rabbit under the constant removal rate (1 mm/s) and the constant perfusion rate (1 ml/min). All the dynamic devices were provided by...
Laborie Medical Technological Ltd. And all the urodynamic procedures were performed by the same urologists (Z.J. and S.C.Y.).

**Histology**

The urethras from each rabbit were removed, further fixed in formaldehyde for 24 h and then embedded in paraffin. The tissue of the urethra was stained with HE and keratin antibody immunohistochemically for 24 h. The configuration and distribution of the urethral epithelium were observed by optical microscope. All the procedures were done by the same pathologist (Z.J. and S.Y.F.).

**Statistical methods**

The results are expressed as mean ± SEM unless otherwise stated. The pressure of the urethra in UPP was tested using the unpaired t-test. The results were considered significant if $p < 0.05$.

**Results**

One rabbit in the US group died during anesthesia before any experiments could be carried out. After one rabbit was added in the US group, all the rabbits were alive during the whole experiment.

**Urethrography**

The urethrogram of the remaining 6 rabbits in the US group confirmed a stricture of the urethra (Fig. 1b). And the urethrogram of the 5 rabbits in the control group were all normal (Fig. 1a), but in one rabbit, the stricture of the urethra was seen for unknown reasons. The rabbit was disregarded in the analysis. Comparing the two groups, the continuity of the urethra in the US group changed, in which the diameter of the urethra became thinner and discontinuous when the contrast agent passed under X-ray.

**Urodynamics**

The pressures of the urethra on the operation region in the US group were higher than those in the control group. The mean value of urethral pressure was 27.83 ± 3.71 cmH$_2$O and 14.67 ± 2.16 cmH$_2$O, respectively (Table 1). There was significant difference between both groups ($p < 0.01$). The figure of urethral operation region in the US group showed a dense and higher wave (Fig. 2b), and a sparse and lower wave was seen in the control group (Fig. 2a).

**Histology**

Histological sections stained with HE, in the control group, of the luminal part of the wall of the urethra were lined with the equal and regular epithelium, which has 3-4 layers. There were no inflammatory cells and lymphocytes in all layers of the epithelium (Fig. 3a). The urethras in the US group were lined with the irregular epithelium and somewhere there was no epithelium. Lots of neutrophils and lymphocytes were distributed into the epithelium (Fig. 3b).

Stained with keratin antibody immunohistochemically, the epithelium in the control group became brown with regular array (Fig. 3c), however, irregularly distributed in the US group (Fig. 3d).

**Discussion**

A stable and repeated animal model of US should be established for researching the treatment of US. The choice of animal is important. We used to choose big animals such as pigs and dogs to develop the animal model of US. The expense of big animals restricted the establishing of US animal model. Small animals such as rabbits and rats were rarely used for urethra experiments because the genitals...
were too small to work macroscopically. However, with the technique of microsurgery, more and more small animals were used for the urethral experiments. McAninch et al. found that the urethra of the male rabbit resembles the urethra of the male human histologically, with a thin epithelium supported by the spongy tissue which was full of blood vessels. In this study, New Zealand male rabbits were used for the animal model of US under the optical microscope.

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 bulbous part of the urethra. 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. 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.

The strictures produced were demonstrated by way of urethrography, pediatric cystourethroscopes, UPP, histology, etc. It is necessary to combine more than two ways to

Figure 2  (a) UPP of a normal male rabbit. The wave of the operation region relative to the US group was marked with an arrow. (b) UPP of a urethra with an induced stricture marked with an arrow. The figure showed a dense and high wave.

Figure 3  (a) Histological sections with HE stained of the control group. The luminal part of the wall of the urethra was lined with the equal and regular epithelium, which has 3–4 layers. There were no inflammatory cells and lymphocytes in all layers of the epithelium. (b) Histological sections with HE stained of the US group. The urethras were lined with the irregular epithelium and somewhere there was no epithelium. Lots of neutrophils and lymphocytes were distributed into the epithelium. (c) Histological sections with keratin antibody immunohistochemically stained of the control group. The epithelium became brown with regular array. (d) Histological sections with keratin antibody immunohistochemically stained of the US group. The epithelium was stained asymmetrically with irregular distribution.
identify US in male rabbits. The procedures of urethrography and histology have been previously described. Some previous experiments showed that the strictures produced were demonstrated by way of impedance planimetry. The luminal cross sectional area (CSA) of the urethra in male rabbits was measured under different urethral pressures. However, the procedures of the impedance planimetry were complicated and difficult to control, especially the position of the probe placed, which was easy to cause deviation. UPP can evaluate both the position of stricture and the extent of stricture directly. The pressure of the whole urethra was traced under the constant removal rate and the constant perfusion rate. In this study, the figure of urethral operation region in the US group showed a dense and higher wave, which was significantly different from the normal figure of urethra. And the value of urethral pressure on the operation region in the US group was higher than those in the control group.

The establishment of this model has the novelty of examining UFR in rabbits, which is an objective tool to evaluate stability. Due to the variety of clinical presentations of US, a stable model would be of great value to simulate a specific clinical scenario.

Conclusions

We establish the way to build a stable urethral stricture model of New Zealand rabbits by means of the microsurgical technique, which can provide the foundation of laboratory animals to research all kinds of urethral stricture. Urethrography and histology combined UPP are the reliable methods to identify the urethral stricture.

Conflict of interest

The authors declare that they have no conflict of interest.

References