



Comparative Evaluation of Three Methods of Vesicoureteral Reflux Induction in Rabbits

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Abstract

Objective- comparative evaluation of different surgical methods of vesicoureteral reflux induction in rabbits

Design- Elective experimental study

Animals- sixteen healthy male rabbits

Procedures- sixteen healthy NewZiland White male rabbits were randomly divided in four equal groups. All rabbits were preoperatively evaluated with retrograde cystography for the presence or absence of congenital or acquired vesicoureteral reflux. Rabbits of group1 (control) without any manipulation were kept for comparing with other three experimental groups. Rabbits of group 2, 3 and 4 undergone open surgeries with unroofing the ureter with surgical blade incision of 3-5mm in length. In group two incised ureter left without suturing ureteral mucosa, in group three incised ureter sutured with nylon material 5-0 and in group four ureter was incised with electrosurgical cutting blade without suturing mucosal layer to the bladder roof. Postoperatively on days 7th, 15th and 30th all animals in different groups undergone cystography for presence or absence of vesicoureteral reflux.

Results- All the animals of group 1, 2 and 4 didn't show any reflux in double contrast cystography but in group 3 reflux in the form of bilateral and unilateral were observed clearly.

Conclusion and Clinical Relevance- Unroofing the ureter with surgical blade incision and suturing the incised margins can induce the successful vesicoureteral reflux for undergoing an experimental study of vesicoureteral reflux treatment.

Key Words- Vesicoureteral Reflux, Cystography, Rabbits.

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Introduction

Vesicoureteral reflux (VUR) is characterized by the retrograde flow of urine from the bladder to the kidneys¹. VUR is an anatomic and functional disorder with potentially serious consequences that may be associated with urinary tract infection (UTI), hydronephrosis, and abnormal kidney development or renal dysplasia. Primary reflux is VUR in an otherwise normally functioning lower urinary tract and occurs in approximately 1% of infants, while secondary reflux is VUR that is associated with or caused by an obstructed or poorly functioning lower urinary tract, such as that observed with posterior urethral valves or a neurogenic bladder^{1,2}. In both conditions, the ureterovesical junction (UVJ) fails to function as a one-way valve, giving lower urinary tract bacteria access to the normally sterile upper tracts^{1,3}. Infection is the most common symptom of VUR. As the child gets older, other symptoms, such as bedwetting, high blood pressure, protein in the urine, and kidney failure, may appear⁴. Reflux nephropathy, the renal disease associated with VUR, accounts for up to 25% of cases in children⁵. The biologic basis for this disorder has not yet been defined¹. Some patients with VUR have an increased risk of developing pyelonephritis, hypertension, and progressive renal failure. However, VUR occurs with a spectrum of severity and thus may affect patients differently^{1,6}. A voiding cystourethrogram (VCUG) or radio-nuclear cystourethrogram (RNC) is used to confirm the diagnosis. Galen and Asclepiades described the valve action of the ureterovesical junction as early as the second century AD⁷. For the first time in 1893, Pozzi reported urinary reflux in lab animals and human⁷. In 1903, Sampson and Young described the functional flap-valve mechanism at the level of the ureterovesical junction, which is created by the oblique course of the ureter within the intramural portion of the bladder wall⁷. In 1913, Legueu and Papin described a patient with hydronephrosis and hydroureter in whom urine was shown refluxing through a widely patent ureteral orifice. In his report on cystography in 1914, Kretschmer demonstrated that reflux was present in 4 of the 11 children he studied. In 1929, Gruber noted that the incidence of VUR varied according to the length of the intravesical ureter and muscularity of the detrusor backing.

Paquin reported that the tunnel length-to-ureteral diameter ratio should be approximately 5:1 to prevent reflux. In the mid-to-late 1950s, Hutch postulated the causal relationship between VUR and chronic pyelonephritis in a cohort of patients with spinal cord injury and in 1959, Hodson demonstrated that renal parenchymal scarring occurs more commonly in children with VUR and UTIs. Ransley and Ridson Confirmed the studies of Tanagho in 1975 by showing that reflux could be experimentally created in animals by modifying the ureterovesical junction; in subsequent studies, they were able to show the correlation between reflux, renal papilla anatomy, pyelonephritis, and renal injury⁷.

Despite the large number of studies on VUR induction reported in the literature, some of the operated animals have either escaped the development of reflux or been spontaneously cured^{8,9}. Moreover the details, methods and operative techniques for VUR induction have not been illustrated¹⁰. It must be noted that the value of reflux induction in the animal model is mostly in the evaluation of different treatment modalities and assessment of tissue sensitivity or expansibility of the injected drugs in the site of ureteral submucosa. Accordingly the present study designed to induce VUR experimentally in rabbits with accuracy and success for further therapeutic evaluation.

Materials and Methods

Sixteen male NewZeland White rabbits about 1 year old, 3-3.5 kg weight were selected. Rabbits were randomly divided in four equal groups. Control group (group 1) and cases groups (group 2, 3, and 4). All rabbits considered healthy based on clinical examination, laboratory and radiographic findings. The absence of congenital or acquired vesicoureteral reflux was proved with preoperative retrograde cystography.

After preparation, general anesthesia was induced by IV administration of a combination of 35 mg/kg ketamin hydrochloride and 5 mg/kgbw xylazin hydrochloride. Rabbits were placed in dorsal recumbency and surgical site was prepared and draped aseptically.

In group 1, exploratory laparotomy was done and the abdominal wall was closed without any manipulation. In group 2, 3 and 4 all rabbits through midline laparotomy perefomed and the urinary bladder was incised ventrally about 2 cm, and the ureterovesical junction (UVJ) was identified microscopically and a plastic catheter was inserted into the ureter (Fig 1). This plastic catheter was made from approximately 1 cm of soft tubing (intramedic polyethylene tubing with inner diameter of 0.2 mm and outer diameter of 0.5 mm) placed on a hypodermic needle (Microlance 3 to 3.5 gauge; Becton Dickinson).

To ensure sterility, on the day before use, the catheter on the needle was placed for 20 min in 99.9% ethanol, which was then allowed to evaporate. The catheter was gently inserted through the ureter. To destroy the UVJ function and create VUR, the roof of each ureteral orifice was incised in group 2, 3 and 4. In group 2 unroofing of the ureter was done with a 3-5 mm incision through the midline of catheter and left unsutured. In group 3 after this procedure the incised margins of ureter was fixed to the same side of the bladder's wall (mucosa layer) with simple interrupted suturing using 5-0 nylon material (Fig 2). In group 4 unroofing the ureters were done with electrosurgical cautery blade. The catheter was removed immediately after procedure. Then the urinary bladder and abdominal wall were closed routinely.



Figure 1.
Insertion of a plastic catheter into the ureter.



Figure 2.
The ureter is fixed to the bladder wall in group 3.

Postoperative care was done routinely in all groups. Cystographic evaluation in all cases was performed on days 7th, 15th and 30th post operation. The 76% Meglamine compound as a positive contrast material was injected to the bladder through urethral catheter and VD and Lateral radiographs were taken immediately and 5 min after injection.

Statistical analysis and comparison were determined using student's T-Test. The p values less than 0.05 were considered statistically significant.

Results

There was no complication during and after anesthesia and all animals were recovered and began eating and walking about 6 to 8 hours after surgery. There were no statistically significant differences among clinical data such as heart rate, respiratory rate and also blood loss rating and body weight between the groups. The mean time of the surgery was about 1 hour.

There wasn't seen any reflux in the four rabbits of the group one as a control group in all radiographic and retrograde cystography were done. In the second group that the ureter's incision was not sutured there wasn't seen any ureterovesical reflux in all rabbits in this group. But in the third group that the incised ureters margins were sutured to the mucosal layer of the bladder's wall, bilateral vesicoureteral reflux in 3 rabbits (Fig 3) and unilateral reflux in the last one (Fig 4) were observed in the second and third radiographic and cystourethrographic evaluation on 15th and 30th postoperative days by the positive contrast agent. In the group 4 we used electrocautery blade to incise the UVJ along the ureter's canal which unfortunately all of the rabbits were died 2 to 4 days later to surgery.



Figure 3. Bilateral vesicoureteral reflux in 3 rabbits of group 3.



Figure 4. Unilateral vesicoureteral reflux in one rabbit of group 3.

Discussion

An abnormal intramural tunnel (short tunnel) results in a malfunctioning flap-valve mechanism and form VUR. When the intramural tunnel length is short, urine tends to reflux up the ureter and into the collecting system. Pacquin reports that refluxing ureters have an intramural tunnel length-to-ureteral diameter ratio of 1.4:1. To prevent reflux during ureteral reimplantation, the physician must obtain a minimum tunnel length-to-ureteral diameter ratio of 3:1². The most research of VUR induction is to find the best methods to treat this abnormality. There are a lot of studies to induce this disorder in some animals specially in pigs^{11,12}. A common method of VUR induction is ureteral incision and it's reimplantation to the bladder wall⁸. In this experimental study we tried to induce VUR in rabbits by some different methods to find the best and feasible method and apply it for later treatments.

Puri induced VUR given incision to intramural part of ureters in pigs¹¹. Schimberg with similar method made unroofing the ureter induced vesicoureteral reflux by cystoscopy in pigs.¹³ Mcdougall induced VUR with laparoscopic method¹². We used three methods of unroofing the

ureters surgically. VUR is graded based on appearance of contrast in the ureter and upper collecting system during the voiding phase of the cystogram. In our study in group 2 we didn't see any sign of reflux in radiographic surveys and we guess the early healing. But we found VUR in all rabbits of the third group, 3 bilateral and 1 unilateral which the incised margins of ureter sutured to the roof of the bladder. In the group 4 unroofing the ureters was done with electrocautery that was not successful because all the rabbits were died and we guess the monopolar system can provide some side effects in the necropsy of these animals.

Because of thin diameter of the ureter, we designed a special catheter with a hypodermic needle as a guide to incise the ureter through this guide correctly. With this method we inhibited more injury and swelling of the ureters and bladder.

In the group 3 we found the VUR signs 15 days post operation. This findings was comparable with the findings of Puri after 6 weeks, Atala after 4 weeks, Schimberg after 1 week and Mcdougall after 6 weeks of operation were seen VUR in their cystograms^{10,11,12,13}.

We concluded that the unroofing of the ureters giving incision about 3-5 mm in VUJ and suturing the incised margins could be induced vesicoureteral reflux successfully. Using suitable catheter as a guide to identify the correct incision line in the VUJ through ureter could help to get good results.

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ارزیابی مقایسه ای سه روش ایجاد ریفلاکس ادراری در خرگوش

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هدف- در این مطالعه به منظور ایجاد ریفلاکس ادراری با روشهای مختلف جراحی و مقایسه آنها، از ۱۲ خرگوش استفاده شد. ریفلاکس ادراری به برگشت ادرار از مثانه به داخل حالبها وسیستم ادراری فوقانی اطلاق می شود که یک مشکل رایج در بین انسان و حیوانات می باشد و هر ساله بیش از ۱۵۰ مقاله با موضوع ریفلاکس ادراری در تحقیقات و انتشارات علمی به چاپ می رسد.

طرح مطالعه- مطالعه تجربی

حیوانات- دوازده خرگوش نژاد نیوزلندی

روش کار- خرگوش ها به طور تصادفی در چهار گروه سه تایی قرار گرفتند. در تمام خرگوشها قبل از جراحی عدم ابتلا به ریفلاکس ادراری مادرزادی و یا خودبخودی بوسیله سیستوگرافی رتروگرید مورد تأیید قرار گرفت. گروه ۱ به عنوان گروه کنترل بدون مداخله جراحی به عنوان گروه مقایسه با سایر گروهها (آزمایش) قرار داده شد. گروههای ۲، ۳ و ۴ به عنوان گروههای آزمایش تحت عمل جراحی باز به منظور ایجاد ریفلاکس ادراری قرار گرفتند که در گروه ۲، برش سقف حالب به طول تقریبی ۵-۳ میلیمتر بدون بخیه رها شد. در گروه ۳، برش سقف حالب به طول تقریبی ۵-۳ میلیمتر به وسیله نخ نایلون پنج صفره به دو طرف لبه برش بخیه شد و در گروه ۴ برش سقف حالب به طول تقریبی ۵-۳ میلیمتر توسط الکتروکوتر و بدون انجام بخیه ایجاد شد.

نتایج و کاربرد بالینی- روز ۱۵ و ۳۰ پس از عمل حضور ریفلاکس ادراری توسط سیستوگرافی رتروگرید در تمام گروهها مورد ارزیابی قرار گرفت. نتایج به دست آمده حاکی از آن است که در هیچیک از خرگوشهای گروه ۱ و ۲، ریفلاکس ادراری مشاهده نگردید ولی در گروه ۳ ریفلاکس ادراری به صورت یکطرفه و یا دوطرفه مورد تأیید قرار گرفت.

کلید واژگان- ریفلاکس ادراری، سیستوگرافی، خرگوش