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ORIGINAL ARTICLE

Male Health

The effect of the degree of left renal vein constriction on the development of adolescent varicocele in Sprague–Dawley rats

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Experimental models have allowed inquiry into the pathophysiology of varicocele (VC) beyond that possible with human patients. A randomized controlled study in rats was designed to clarify the influence of the degree of left renal vein constriction on the development of adolescent VC. Fifty adolescent male Sprague–Dawley rats (*Rattus norvegicus*) were randomly assigned to five groups of 10: the experimental groups (I–IV) underwent partial ligation of left renal veins with 0.5-, 0.6-, 0.7-, and 0.8-mm diameter needles, respectively. The control group (V) underwent a sham operation. The diameter of the left spermatic vein (LSV) was measured at baseline and 30 days postoperatively. In addition, the lesion of the left kidney was examined with the naked eye and assessed by Masson's trichrome staining. VC was successfully induced in 2 (20%), 4 (40%), 7 (70%), and 10 (100%) rats in groups I–IV, respectively. The other rats failed to develop VCs primarily due to left renal atrophy. No VC was observed in group V. The postsurgical LSV diameters in VC rats in groups III and IV were 1.54 ± 0.16 and 1.49 ± 0.13 mm, respectively ($P > 0.05$), and their increments were 1.36 ± 0.10 and 1.31 ± 0.10 mm, respectively ($P > 0.05$). These results suggest that suitable constriction of the left renal vein is critical for adolescent VC development. In addition, the 0.8-mm diameter needle may be more suitable for inducing left renal vein constriction in adolescent rat models.

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INTRODUCTION

Varicocele (VC) is one of the most interesting and controversial topics in andrology, due to its high prevalence and as yet unclear relation to fertility. In the adolescent population, this disease had previously received relatively scant attention. In fact, however, the incidence of VCs in pubertal males can reach 15%, approximately equal to that of adults, with a marked left-sided predominance of 90%.[1,2] A large study from the World Health Organization (WHO)[3] showed that 25.4% of adults with VCs have fertility problems. These results raised questions about the risk of future infertility in adolescents with VC that can likely be answered with a greater understanding of the pathophysiology of adolescent VC.

Many theories developed from human studies need to be tested in animal models. The rat experimental left varicocele (ELV) model, induced by constriction of the left renal vein, is the most commonly used model due to its low cost and the anatomical similarities with humans. The left pampiniform plexus of the rat coalesces into the left spermatic vein (LSV) and then drains into the left renal vein. Unlike in humans, 90% of the LSVs in rats have irregular collaterals before draining into the left renal veins. However, the venous anatomy and distribution of blood flow in modified rat models, in which the collaterals are additionally ligated, is similar to those of VC patients.[4]

Although the validity of rat ELV model has been supported by a considerable number of studies,[4,5] it is far from perfect. First, most studies using the model have focused on adulthood,[6–8] since adolescent models are immature.[9] Second, the surgical procedures used to create this model are different, especially regarding the degree of left renal vein constriction. These differences may account for the varied success rates and research results.[7,10,11]

Third, a fidelity evaluation of the VC model has not been effectively implemented. The effects of the induction surgery on the left kidney and the LSV are two essential factors for determining whether ELV has been successfully induced.[6,12] However, they are seldom mentioned in studies.[13–15] When there is no evaluation of whether a VC has developed, it calls into question whether a claimed effect of ELV or the absence of an effect is truly related to the presence or absence of the lesion. Thus, in order to create a consistent adolescent VC model in the Sprague–Dawley (SD) rat, we designed the first randomized trial using this model to compare the effects of different degrees of left renal vein constriction on the development of adolescent VC.

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MATERIALS AND METHODS

Animals

A total of 50 adolescent male specific-pathogen free (SPF) SD rats (*Rattus norvegicus*), aged 7 weeks and weighing 230–255 g, were selected. This study was approved by the Animal Care and Ethics Committee of the First Affiliated Hospital of Sun Yat-Sen University (certificate number: SCXK [Yue] 2011-0029) and was conducted in accordance with the Declaration of Helsinki and International Association for the Study of Pain guidelines. All rats were housed in a controlled environment (24°C with a 12:12-h light–dark cycle) with free access to standard rat food and water. They were numbered and randomly assigned by computer to five groups of 10. Rats in groups I–IV (the experimental groups) underwent partial ligation of the left renal veins with 0.5-, 0.6-, 0.7-, and 0.8-mm diameter metal needles, respectively. Rats in group V rats (sham group) underwent sham laparotomies in which ligatures were only placed in position and not tied. One rat each from groups I and III died from bleeding during the operation.

Surgical procedure

In this study, we induced ELV in adolescent rats using the method of Saypol *et al*.[7] with some modifications. Each animal was anesthetized by an intraperitoneal injection of 3% sodium phenobarbital (40 mg kg^{-1}). The left renal vein, inferior vena cava, and LSV and its collaterals were exposed and identified through a midline laparotomy incision. A tunnel was made around the left renal vein by blunt dissection, and the vein was cleared of connective tissues in a position medial to the insertion of the spermatic and adrenal veins. A 4-0 silk suture was placed around the vein and tied down over a metal needle at this site. The needle was then removed from the stricture, allowing the vein to expand to the limits of ligation. In addition, the collaterals of the LSV were fully occluded. Finally, the abdomen was closed in two layers with 3-0 silk sutures.

Assessment of pathological changes in the left kidney

After 30 days, each rat was anesthetized and subjected to laparotomy. Macroscopic changes in the left kidney were identified by careful observation and comparison to changes in the right kidney. The left kidney was then resected; fixed in cold, fresh, 4% neutral buffered formaldehyde; embedded in paraffin; and cut into 5-μm longitudinal sections. Sections were then deparaffinized, rehydrated, and stained with Masson's trichrome stain to distinguish smooth muscle (stained in red) and collagen (green). For histological evaluation, both renal cortex and medulla structures were observed and analyzed under a microscope (Nicon, Tokyo, Japan).

Analysis of the LSV

Prior to the surgical procedure in all animals, the external diameter of the LSV where it crosses the iliolumbar vein was measured by micrometer under light microscopy (Olympus, Tokyo, Japan). This measurement was repeated after 30 days. In addition, the LSV and pampiniform plexus were examined for marked dilation at this time.

Criteria for successful ELV

VC induction was regarded as successful when the diameter of the LSV was >1 mm, and the pampiniform plexus showed appreciable dilation without pathological changes in the left kidney.[6]

Statistical analysis

Data are presented as means ± standard deviation and were analyzed using SPSS (Version 13.0; SPSS Inc., Chicago, IL, USA). All values were verified to be normally distributed. Paired *t*-tests were applied to compare vein diameters within groups before and after the operation. Diameters between groups were analyzed with the unpaired *t*-test. $P < 0.05$ was considered statistically significant.

RESULTS

Macroscopic and histological findings of the left kidney

In groups I–III, respectively, there were 7/9, 6/10, and 2/9 rats showing atrophy in the left kidney at 30 days after the operation. Interstitial fibrosis, renal tubular atrophy, and even tissue necrosis were observed in these kidneys. There was no atrophic kidney in group IV or V. No pathological changes were observed in nonatrophic kidneys. The structures of the renal cortex and medulla were well-preserved in these kidneys (**Figure 1**).

Evaluation of VC

The status of VC development in each group 30 days after the operation is shown in **Table 1**. All 10 rats without renal atrophy in group IV showed obvious dilatation of the LSVs and pampiniform plexus. The diameters of their LSVs were >1 mm (**Figure 2**). The animals in the other three experimental groups (i.e., groups I–III) showed similar dilatations, except for the animals with left kidney atrophy (**Figure 3**). There was no VC in the animals in group V.

Measurement of the LSV

For the rats in which ELV was successfully induced, the postoperative LSV diameter was remarkably increased as compared with the baseline in both group III ([1.54 ± 0.16] *vs* [0.17 ± 0.07] mm, $P < 0.01$) and IV ([1.49 ± 0.13] *vs* [0.18 ± 0.08] mm, $P < 0.01$). But there was no significant difference in the postsurgical diameters between the two

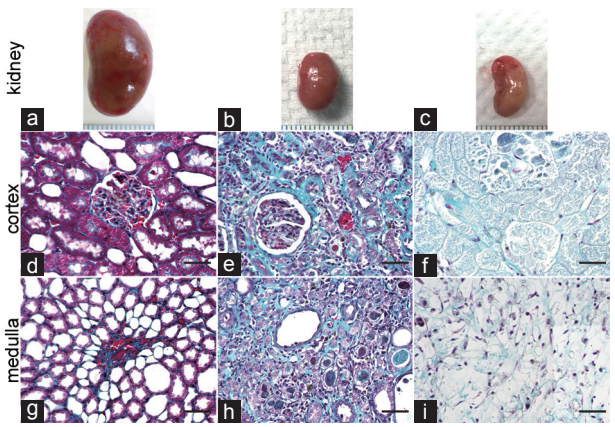

Figure 1: Macroscopic feature and Masson's trichrome staining of the left kidney 30 days postoperation. (**a**, **d**, **g**) The structures of the renal cortex and medulla were well preserved (**d**, **g**) in the nonatrophic kidney (**a**). (**b**, **e**, **h**) The atrophic kidney (**b**) showed diffuse interstitial fibrosis (green) and extensive renal tubular atrophy (**e**, **h**). Some tubules were dilated and filled with casts (**f**). (**c**, **f**, **i**) Though tissue outlines vaguely remained, the structures of the glomerulus and renal tubule disappeared (**f**, **i**) in the necrotic kidney (**c**). Calcium salt and lipofuscin deposits can be seen in necrotic tissue (**f**). Scale bar = 100 μm.

Table 1: The status of ELV development in each group after operation

Group	*n*	*ELV*	*Left renal atrophy*	*Bleeding*	*Success rate (%)*
I	10	2	7	1	20
II	10	4	6	0	40
III	10	7	2	1	70
IV	10	10	0	0	100
V	10	0	0	0	0

I: 0.5 mm; II: 0.6 mm; III: 0.7 mm; IV: 0.8 mm; V: sham. ELV: experimental left varicocele

groups ($P > 0.05$). The LSV diameters in groups III and IV, respectively, increased by 1.36 ± 0.10 mm and 1.31 ± 0.10 mm, which were not significantly different from each other ($P > 0.05$). Baseline and postsurgical diameters were 0.16 ± 0.06 mm and 0.17 ± 0.07 mm in group V ($P > 0.05$) (**Figure 4**).

DISCUSSION

VC is a frequent abnormality in male infertility.[16] Approximately, 40% of men with primary infertility and up to 80% of men with secondary infertility have suffered such disease.[17] It is rarely seen in prepubescent males. As they enter adolescence, however, the prevalence increases rapidly and can even reach adult levels.[18] Adolescence is a period of change. During this period, reproductive organs develop and mature quickly. Thus, VC beginning at this unique period may have different effects on male fertility than when it begins in the adult stage. Studies in adolescents have demonstrated that a VC can disrupt testicular growth, histology, and function.[19] Choi *et al.*[10] found that the onset of experimental VC in animal models at 7 weeks had more damaging effects on testis than when VC was induced at 4 weeks or 12 weeks.

However, whether early intervention is necessary to improve future fertility remains controversial, as the pathophysiology of VC has not yet been fully elucidated.[20] Experimental models have allowed inquiry into VC-related consequences beyond that possible with human patients. Since Saypol *et al.*[7] first established an ELV model using adult SD rats in 1981, this model has been widely used in different laboratories.[6,8,21]

Because most LSVs in rats have irregular collaterals before draining into the left renal vein, this traditional model has been modified by fully ligating the LSV collaterals in addition to constricting the left renal vein.[21,22] The venous anatomy and redistribution of blood flow in the modified rat model are similar to those in VC patients.[4] However, this modified method was based on adult data. Data from adolescent VC models are not well documented. Therefore, we attempted to create the first adolescent VC model using the modified method in SD rats. The stage of puberty in the male is about 7 ± 1 weeks postnatal.[23] Thus, 7-week-old SD rats were selected as appropriate research subjects.

VC can be induced by constricting the left renal vein.[21,22] The renal vein was ligated together with a metal needle, and the needle was then removed from the stricture, effectively reducing the lumen of the renal vein to the size of needle. This method not only imitates the "nutcracker" effect in human, which is one of the major causes of naturally occurring VC,[24] but also mimics the main driving force of VC development – increased venous pressure in the LSV.[4] However, different sizes of metal needles (or bars), ranging from 0.5 to 0.9 mm diameters, have been used to constrict the left renal vein in different laboratories,[25–27] which may account for the variable success rates and contradictory research results.

A standard surgical procedure with a high success rate for inducing VC is very important in the animal model for acquiring reliable and comparable results across studies.[28] Thus, a randomized, controlled study of rat models was designed to clarify the influence of the degree of left renal vein constriction on adolescent VC development. Turner and Howards[4] emphasized that excessive occlusion of the left renal vein would lead to pathological obstruction of the vein and eventually cause renal necrosis. As shown in the present study, left renal atrophy occurred when the luminal diameter of the left renal vein was reduced to <0.8 mm. In addition, the incidence rate was positively correlated with the degree of constriction. These findings show that the left renal vein can be reduced to a suitable size without causing renal lesions using a 0.8-mm needle in SD rats.

On the other hand, although it did not occur in our study, insufficient constriction of the left renal vein will fail to produce sufficient intravenous pressure to induce VC. Although the vein underwent the least reduction in size using the 0.8-mm needle,

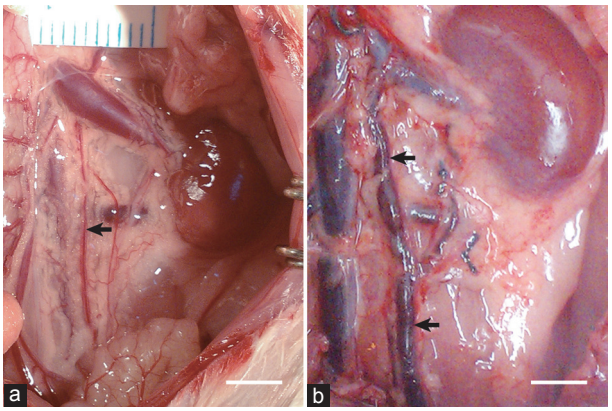

Figure 2: LSV varicosity. Arrows show the LSV. (**a**) Baseline. (**b**) 30 days postoperatively. The LSV showed obvious dilatation, and its diameter was >1 mm without atrophy of the left kidney after the operation. Scale bar = 5 mm. LSV, left spermatic vein.

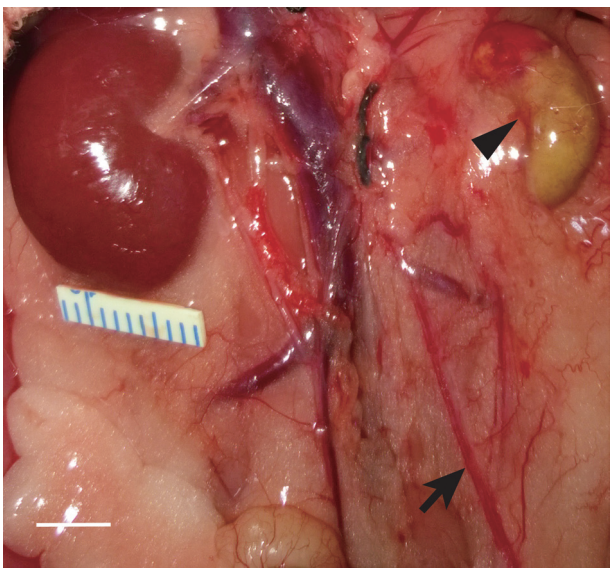

Figure 3: The change in the LSV in a rat with renal atrophy after the operation. Arrow shows the LSV, and arrowhead shows the left kidney. When the left kidney showed shrinkage and damage, the LSV failed to develop varicosity. Scale bar = 5 mm. LSV, left spermatic vein.

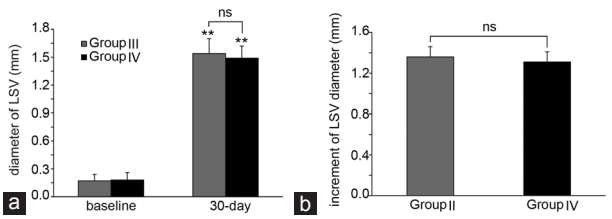

Figure 4: Statistical analysis of the diameters of LSVs in rats with VC in groups III and IV. (**a**) The diameters of LSVs at baseline and 30 days postoperatively. (**b**) The increments of LSVs diameters. The increments were calculated as postsurgical diameters minus their baseline ones. **: $P < 0.01$; ns: $P > 0.05$; group III: $n = 7$; group IV: $n = 10$; LSV: left spermatic vein; VC: varicocele.

increased intravenous pressure was sufficient to force the development of spermatic varicosity. The 0.5–0.8-mm diameter range of the needles used in this study was determined according to the common range used in previous reports. Thus, it not possible to foresee that we would gain the highest success rate using the 0.8-mm needle before the study. If we had used needles larger than 0.8 mm, insufficient constriction would have been more likely to occur.

From the two aspects of venous constriction, it can be seen that the changes in the left kidney and LSV are directly related to the fidelity of the adolescent VC model. Any rat presenting with a pathological lesion of the left kidney should be excluded because renal lesion is rarely seen in naturally-occurring VC in humans.[29] In addition, if this surgery had any effect on the left kidney, the pathophysiological results might not be due solely to the effect of experimental VC.

LSV diameter is an objective factor for evaluating varicose degree. In adult SD rats, LSVs are ~ 0.15–0.2 mm in diameter and can increase to more than 1 mm by 30 days after inducing surgery and stabilize.[6] In our study, the LSV diameter of adolescent rats ranged from ~ 0.1 to 0.2 mm. Postsurgical diameters can reach ~ 1.5 mm in rats with VC, and their LSVs and pampiniform plexus showed obvious dilation. Postsurgical diameters and their increments in rats with VC showed no significant differences between the 0.7-mm group and 0.8-mm group.

In conclusion, a suitable degree of left renal vein constriction is critical for the development of adolescent VC. A 0.8-mm needle may be more suitable for left renal vein constriction in adolescent rat models. Further research using the optimized model will reveal the pathophysiology of adolescent VC.

AUTHOR CONTRIBUTIONS

BY participated in the design of the study, performed experiments, and drafted the manuscript. XZS conceived of the study and coordinated the entire research. WLZ provided technical support and helped to analyze and interpret the data. DYH and SFC helped to establish models. BO participated in the design of the study and performed the statistical analysis. XC helped to draft the manuscript. CHD gave conceptual advice and helped manuscript revising. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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