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 has 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)1 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.3

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,4,6 since adolescent models are immature. 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.8,9 However, they are seldom mentioned in studies.10,11 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.
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., 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 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.

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] mm, P < 0.01) and IV ([1.49 ± 0.13] mm vs [0.18 ± 0.08] mm, P < 0.01). But there was no significant difference in the postsurgical diameters between the two groups.
groups ($P > 0.05$). The LSV diameters in groups III and IV, respectively, increased by $1.36 \pm 0.10$ mm and $1.31 \pm 0.10$ mm, which were not significantly different from each other ($P > 0.05$). Baseline and postsurgical diameters were $0.16 \pm 0.06$ mm and $0.17 \pm 0.07$ mm in group V ($P > 0.05$) (Figure 4).

**DISCUSSION**

VC is a frequent abnormality in male infertility. Approximately, 40% of men with primary infertility and up to 80% of men with secondary infertility have suffered such disease. It is rarely seen in prepubescent males. As they enter adolescence, however, the prevalence increases rapidly and can even reach adult levels. 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. Choi et al. 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. Experimental models have allowed inquiry into VC-related consequences beyond that possible with human patients. Since Saypol et al. first established an ELV model using adult SD rats in 1981, this model has been widely used in different laboratories.

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. The venous anatomy and redistribution of blood flow in the modified rat model are similar to those in VC patients. 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. Thus, 7-week-old SD rats were selected as appropriate research subjects.

VC can be induced by constricting the left renal vein. 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, but also mimics the main driving force of VC development – increased venous pressure in the LSV. 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, 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. 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 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,
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.\textsuperscript{7,9} 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 varicoceles 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.\textsuperscript{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 dilatation. 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 the entire research. WLZ provided technical support and helped to review the manuscript. All authors read and approved the final manuscript.

**COMPETING INTERESTS**

The authors declare that they have no competing interests.

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**REFERENCES**

1. Serefoglu EC, Saltz TR, La Nasa JJ, Hellstrom WJ. Adolescent varicoceles: management controversies. Andrology 2013; 1: 109–15.
2. Skog SJ, Roberts KP, Goldstein M, Pryor JL. The adolescent varicocele: what’s new with an old problem in young patients? Pediatrics 1997; 100: 112–21.
3. The influence of varicoceles on parameters of fertility in a large group of men presenting to infertility clinics. World Health Organization. Fertil Steril 1992; 57: 1289–93.
4. Turner TT, Howards SS. The venous anatomy of experimental left varicocele: comparison with naturally occurring left varicocele in the human. Fertil Steril 1994; 62: 869–75.
5. Yu JJ, Xu YM, Tao Y. The comparison of two experimental rat varicocele models and their effect on sperm quality. Urol Int 2011; 86: 325–9.
6. Turner TT. The study of varicocele through the use of animal models. Hum Reprod Update 2001; 7: 78–84.
7. Saypol DC, Howards SS, Turner TT, Miller EJ. Influence of surgically induced varicocele on testicular blood flow, temperature, and histology in adult rats and dogs. J Clin Invest 1981; 68: 39–45.
8. Wang R, Chang JS, Zhou XM, Chen DY. Varicocele in the rat: a new experimental model. Effect on histology, ultrastructure and temperature of the testis and the epididymis. Urol Res 1991; 19: 319–22.
9. Tek M, Cayan S, Yilmaz N, Oguz I, Erdem E, et al. The effect of vascular endothelial growth factor on spermatogenesis and apoptosis in experimentally-induced adolescent rats. Fertil Steril 2009; 91: 2247–52.
10. Choi H, Kim KS, Kim KM. The effect of experimental varicocele on the testis of adolescent rats. J Urol 1990; 144: 499–501, 512–3.
11. Gurdal M, Kirecci S, Hui E, Karaman I, Turkeri L. Correlation between duration of varicocele and apoptosis in testicular tissue in an experimental model. Urology 2008; 72: 933–6.
12. Ko KW, Chae JY, Kim SW, Moon DG, Kim JJ, et al. The effect of the partial obstruction site of the renal vein on testis and kidney in rats: is the traditional animal model suitable for varicoceles research? Korean J Urol 2010; 51: 565–71.
13. Arena S, Minutoli L, Arena F, Nicotina PA, Romeo C, et al. Polyethyleneoxidebrucelotide administration improves the intra-testicular vascularization in rat experimental varicocele. Fertil Steril 2012; 97: 165–8.
14. Tanriveri G, Sari L, Tekcan M, Demir N, Gunel M, et al. Potential role of p38-mitogene-activated protein kinase and nuclear factor-kappa B expression in testicular dysfunction associated with varicocele: an experimental study. Andrologia 2012; 44 Suppl 1: 94–101.
15. Velez de la Calle JF, Racho J, Le Marteilot MT, Ducot B, Mulligner L, et al. Male infertility risk factors in a French military population. Hum Reprod 2001; 16: 481–6.
16. Blumer CG, Fariello RM, Restelli AE, Spaine DM, Bertolla RP, et al. Sperm nuclear DNA fragmentation and mitochondrial activity in men with varicocele. Fertil Steril 2008; 90: 1716–22.
17. Simsek A, Ozbek E, Ilbey YO, Cekmen M, Somay A, et al. Potential role of inducible nitric oxide synthase (iNOS) activity in the testicular dysfunction associated with varicocele. New Microbiol 2012; 35(3): 211–6.
18. Preston MA, Carnat T, Flood T, Gaboury I, Leonard MP. Conservative management of adolescent varicocele: an experimental study. Andrology 2010; 44 Suppl 1: 94–101.
19. Venes J, Kim HK, Chai BR, Zhao C, Lee SW, et al. Analysis of testicular-internal spermatic vein variation and the recreation of varicocele in a Sprague-Dawley rat model. Andrology 2014; 2: 466–73.
20. Yao B, Han DY, Deng CH, Ouyang B, Sun XZ, et al. [Anatomical variability of the left spermatic vein and establishment of the experimental left varicocele model in adolescent rats]. Zhonghua Nan Ke Xue 2014; 20: 505–9.
21. Chen XG, Pan XH. [Experimental Animals and Experimental Medicine]. Kunming: Yunnan Science and Technology Publishing Press, 2009. p. 47–52.
22. Nielsen ME, Zderic S, Freedland SJ, Jarow JP. Insight on pathogenesis of varicoceles: relationship of varicocele and body mass index. Urology 2006; 68: 392–6.
23. Turkeri KI, Erdogru T, Gulkesen H, Sezer C, Usta M, et al. The potential role of inducible nitric oxide synthase (iNOS) activity in the testicular dysfunction associated with varicocele: an experimental study. Int Urol Nephrol 2004; 36: 67–72.
24. Safari A, Zahrani M, Sadeghipour HR, Kajbafzadeh A, Sarrafnejad A, et al. Flow cytometric evaluation of sperm superoxide anion production in rats with experimental varicocele. Urology 2010; 75: 217–22.
25. Lee JD, Lee TH, Cheng WH, Jeng SY. Involves intrinsic apoptotic pathway of testicular tissues in varicocele-induced rats. World J Urol 2009; 27: 527–32.
26. Zhang Y, Gao X, Liu X, Wang K, Pang J, et al. A new experimental inbred Wistar rat varicocele model: anatomy of the left spermatic vein and the effect on histology. Andrologia 2008; 40: 13–7.
27. Takemura T, Ishida H, Yamamoto S, Hino S, Fukushima K, et al. Clinical and radiological features in four adolescents with nutcracker syndrome. Pediatr Nephrol 2000; 14: 1002–5.