Expression of Hypoxia-Inducible Factor-1α in the Internal Spermatic Vein of Rats with Experimentally Induced Left Varicocele

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OBJECTIVE: Increased expression of hypoxia-inducible factor-1α (HIF-1α) in the internal spermatic vein (ISV) of patients with varicocele was recently reported. In this study we investigated whether the same result occurs in the ISV of rats with experimentally induced left varicocele (ELV).

MATERIALS AND METHODS: ELV was created by partial ligation of the left renal vein in 24 adult male Sprague-Dawley rats. An additional 16 rats that underwent a laparotomy and renal vein handling without ligation served as the sham control group. Rats were sacrificed at 2, 4, 8, and 12 weeks following varicocele creation. The ISV was harvested to measure the expression of HIF-1α by immunoblotting.

RESULTS: HIF-1α immunoblots from both the control and varicocele rats revealed a single band. The relative intensities of HIF-1α protein at 2, 4, 8, and 12 weeks were 51.1±9.53, 65.9±12.98, 70.3±21.29, and 86.0±15.29 in the control group, and 162.6±16.77, 245.2±37.02, 200.5±33.36, and 85.8±26.09 in the varicocele group, respectively.

CONCLUSIONS: Expression of HIF-1α in the ISV of rats with a varicocele was significantly higher than that of rats in the control group, especially at 2, 4, and 8 weeks after varicocele creation, but not in the compensatory stage which occurred in the 12th week. This suggests that hypoxic pathophysiological changes occur in the ISV of rats with ELV. This is also compatible with the same hypoxic occurrence in the ISV of patients with varicocele. Additional studies are needed to investigate whether the reduction in testicular tissue hypoxia and the inhibition of HIF-1α can decrease the chance of varicocele recurrence in patients in the future. (JTUA 17:8-13, 2006)

Key words: HIF-1α, internal spermatic vein, experimental left varicocele, rat.

INTRODUCTION

Varicocele, described as an abnormal tortuosity and dilatation of the gonadal veins within the spermatic cord, is a common anomaly in adolescent and adult males, with an incidence of 15%–20% of the general population [1-3]. Varicoceles have long been recognized as the most common cause of male infertility in adults: approximately 30%–40% of male infertility is reported to be associated with a varicocele [3-5]. The effect of varicocele on infertility remains unclear. The hypothesis that varicocele leads to testicular tissue hypoxia is widely accepted [1-5]. Recently, increased expression of HIF-1α in the ISV of patients with varicocele was
Fig. 1 Control group. 1. Left kidney; 2. renal vein; 3. adrenal vein; 4. internal spermatic vein.

reported [6]. In this study, we investigated whether the same result occurs in the internal spermatic vein (ISV) of rats with an experimentally induced left varicocele (ELV).

Regulation of hypoxia is partially mediated by transcription factors of the hypoxia-inducible factor (HIF) family [7-11]. Hypoxia-inducible factor-1 (HIF-1) is considered the master regulator of the response to hypoxia [7-13]. Active HIF-1 is a heterodimer that consists of 1α and 1β subunit. HIF-1 is activated by hypoxia, and its activity is mainly determined by HIF-1α [7-10]. It is one of the most important adaptive mechanisms that occur in tissues subjected to hypoxic conditions [7-13]. In this paper, we studied HIF-1α expression in the ISV of rats with ELV. This knowledge may help clarify the relationship of hypoxia and varicocele.

MATERIALS AND METHODS

Experimental design

The experiment included 40 adult male Sprague-Dawley rats of the same weaning age (10 weeks), each of which weighed about 300 g. All rats were fed the same food and were maintained in a constant environment with a 12-hour light-dark cycle. The rats were separated into 2 groups. Group 1 rats (sham control, n = 16) underwent a laparotomy except that ligatures were only placed in position and not tied off. Group 2 rats (study group, n = 24) underwent varicocele-inducing surgery. Six rats of the study group and 4 rats of the control group were each sacrificed at 2, 4, 8, and 12 weeks following varicocele creation. The ISV of the control group and varicocele rats was harvested for HIF-1α immunoblotting.

Technique to experimentally induce left varicocele (ELV)

Each animal was anesthetized with an intraperitoneal injection of sodium phenobarbital (50 mg/kg) [7,14]. An abdominal midline incision was made. The left renal vein, inferior vena cava, and left spermatic vein were identified (Fig. 1), and a clamp was positioned behind the left renal vein just distal to the spermatic vein insertion. A 4-0 silk ligature was loosely placed around the left renal vein at this site, and a rigid hydrophilic guide wire of 0.64 mm in diameter was placed on the left renal vein [7,15]. The ligature was tied around the vein over the top of the guide wire. The guide wire was then withdrawn and the vein was allowed to expand to the limits of the ligature, causing the vein diameter to be decreased to approximately half of its original diameter [7,15]. The renal vein and spermatic vein in each rat were immediately diluted. The midline incision was closed in 2 layers with 4-0 silk sutures. When rats were sacrificed, the external diameter of the spermatic vein was measured with a ruler where it crosses the anterior to the iliolumbar vein. Creation of varicocele was considered successful when the diameter of the spermatic vein was greater than 1 mm [16] in the varicocele group (Fig. 2).
HIF-1α in the ISV of Rats with Experimentally Induced Left Varicocele

![Graph showing relative intensity of HIF-1α expression in control and varicocele groups](image)

**Fig. 3** Hypoxia-inducible factor (HIF)-1α expression in the varicocele group compared to that in the control group (p < 0.05) at 2, 4, and 8 weeks after varicocele creation.

**HIF-1α, Immunoblotting**

Two centimeters of the ISV was sampled from both groups at the time of sacrifice. A rabbit polyclonal antibody (Santa Cruz, sc-10790, Santa Cruz, CA, USA) against HIF-1α was used as the primary antibody. The secondary antibody was an alkaline phosphatase-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch, West Grove, PA). Proteins within the membrane fractions (100 µg of protein/mg) were separated by electrophoresis on sodium dodecyl sulfate (SDS)-containing 10% polyacrylamide gels. The separated proteins were then transferred to PVDF membranes (Millipore, Charlestown, MA) by electroblotting. After incubation for 2 hours in PBST buffer (containing 5% (w/vol) nonfat dried milk to minimize nonspecific binding), blots were incubated for 2 hours in the primary antibody diluted in 1% BSA and 0.05% sodium azide in PBST (1: 500 dilution), washed in PBST, and incubated for 2 hours with the secondary antibody (1: 6000 dilution). Blots were developed by incubation with a BCIP/NBT kit (Zymed, South San Francisco, CA). A-tubulin mouse monoclonal antibody (Santa Cruz, sc-5286) was used as an internal control in this study. Immunoblots were photographed and imported as TIF files into an ID image analyzer (Kodak Digital Science, 1998). Blot images were converted into numerical values in order to compare the relative intensities of the immunoreactive bands. Statistical analysis

Data were analyzed by one-way ANOVA and Fisher’s comparison. P < 0.05 was considered statistically significantly. Micrcol version 6 software was used.

**RESULTS**

Varicocele was successfully induced in 4, 5, 6, and 5 rats at 2, 4, 8, and 12 weeks after surgery (as the varicocele group), respectively. The rate of successful varicocele creation was 83.3% (20/24) in this study. Immunoblots of HIF-1α from both control and varicocele rats revealed a single band. The relative intensities of HIF-1α protein (HIF-1α/α-tubulin; %) at 2, 4, 8, and 12 weeks were 51.14 ± 5.3, 65.94 ± 12.98, 70.38 ± 21.29, and 66.07 ± 15.29 in the control group, and 162.63 ± 16.77, 245.24 ± 37.02, 200.52 ± 33.36, 58.2 ± 26.09 in the varicocele group, respectively (Table 1). HIF-1α expression in the varicocele group was significantly higher than that in the control group (p < 0.05) at 2, 4, and 8 weeks after creation of the varicocele (Fig. 3). In the 12th week during the compensatory stage, the difference between groups was not statistically significant.

**DISCUSSION**

Varicocele is the most common etiologic factor associated with male infertility [1-5]. The effect of varicocele on infertility remains unclear, and the hypothesis of hypoxia of testicular tissue is widely accepted [1-5]. To study the molecular changes in the ISV with varicocele is crucial for understanding the mechanism of varicocele formation.

In this study, we created an experimental left varicocele (ELV) model in Sprague-Dawley rats used the method from Saypol et al. [17] with some modifications. Researchers have hypothesized that poor venous return in the varicocele increases the volume of blood in the testis and results in venous stasis, which decreases
testicular oxygen levels and leads to microcircular dysfunction in testicular tissues with varicocele [2,3,5]. HIF-1α has clearly been shown to aid the adaptation of cells to the hypoxic milieu [7-13]. Moreover, HIF-1α has also been implicated in hypoxic cell survival and proliferation. In contrast, hyperbaric oxygenation effectively alleviates and even eliminates chronic ischemia and microcircular dysfunction in testicular tissues associated with a varicocele, and protected the functions of the testes in an animal study [5].

In this study, we found higher HIF-1α expression levels in the varicocele group than in the control group at 2, 4, and 8 weeks after varicocele formation until the compensatory stage which occurred in the 12th week. The spermatic veins in adult Sprague-Dawley rats are typically 0.15–0.2 mm in diameter, and reach 1.0–1.5 mm in diameter within 4–8 weeks after surgery, when they stabilize [15]. This result shows that a hypoxic reaction occurred in the ISV of rats with ELV and it was compatible to the increased HIF-1α expression in the ISV of patients with varicocele [6]. This evidence demonstrates the hypoxic pathophysiologic reaction occurs in the ISV of patients with varicocele and of rats with an experimentally induced left varicocele. Thus, testicular tissue hypoxia may be an important factor causing hypospermatogenesis in male infertility with varicocele because mammalian cells in low or zero oxygen concentrations undergo cell death through apoptosis [18]. In future studies, we will investigate whether the inhibition of HIF-1α and the reduction in testicular tissue hypoxia can decrease varicocele formation in this animal model.

CONCLUSIONS

This animal study showed that HIF-1α expression in the ISV of the varicocele group was higher than that in the control group at 2, 4, and 8 weeks after varicocele creation, but not during the compensatory stage in the 12th week. This suggests that hypoxia-related pathophysiological changes occur in the ISV of rats with ELV. It is also compatible with the same hypoxic occurrence in the ISV of patients with varicocele. Additional studies are needed to investigate whether the reduction in testicular tissue hypoxia and the inhibition of HIF-1α can decrease the varicocele recurrence in patients in the future.

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REFERENCES
以實驗誘導左側精索靜脈曲張的老鼠探討
缺氧誘發因子-1α (HIF-1α) 在內精索靜脈的表現

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研究目的：左側精索靜脈曲張的病人，已被發現在內精索靜脈中有較高的缺氧誘發因子-1α (HIF-1α) 的表現量；所以本文利用實驗誘導出左側精索靜脈曲張的老鼠，探討其內精索靜脈是否有與病人相同的情形發生。

材料與方法：以 24 隻 Sprague-Dawley 成鼠做左側部分精靜脈的結紮實驗組，另外 16 隻進行假手術當對照組，分別在術後的二、四、八及十二週取下兩組的內精索靜脈進行缺氧誘發因子-1α 的分析比較。

結果：精索靜脈曲張的老鼠（實驗組）在術後的第二、四及八週缺氧誘發因子-1α (HIF-1α) 的表現量均大於對照組，且有統計意義，直到第十二週的代償期來臨。

結論：缺氧誘發因子-1α 在內精索靜脈的表現量增加，在病人及實驗的老鼠均有這個現象，證明缺氧的病生理反應是存在精索靜脈曲張的疾病：而進一步改善睾丸的缺氧情形及使用缺氧誘發因子-1α 的抑制劑是否可以降低精索靜脈曲張病人手術後的復發率，則留待未來的探討。

關鍵字：缺氧誘發因子-1α，內精索靜脈，實驗誘導精索靜脈曲張，老鼠。