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ORIGINAL ARTICLE

Angiogenesis inhibition impairs testicular morphology in experimental left varicocele rat model☆

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Angiogenesis;
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Vascular endothelial growth factor

Abstract
Introduction: It has been reported that varicocele might promote angiogenesis. However, it is not clearly identified how angiogenesis affect testicular morphology or spermatogenic activity. The objective of the study is to investigate the effect of spironolactone, as an angiogenesis inhibitor, on the ipsilateral testis morphology in left varicocele-induced rats.
Materials and methods: Twenty-four adult (12–14 mo), male Wistar albino rats were randomly assigned to four groups ($n = 6$, for each): 1. Control group, 2. Sham-operated group, 3. Experimental left varicocele group and, 4. Spironolactone (20 mg/kg/day)-treated experimental left varicocele group. Histopathological findings in rat testis were investigated.
Results: Microvessel density increased in varicocele group and spironolactone inhibited angiogenesis neither by antimineralocorticoid, nor by antiandrogenic effect. However, spermatogenesis impaired in spironolactone treated varicocele group.
Conclusion: Angiogenesis seems to be a protective process in varicocele. Spironolactone treatment, probably by inhibiting angiogenesis, impairs testicular morphology.

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PALABRAS CLAVE
Angiogénesis;
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Rata;
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La inhibición de la angiogénesis deteriora la morfología testicular en modelo de rata con varicocele izquierdo experimental

Resumen
Introducción: Se ha señalado que el varicocele podría favorecer la angiogénesis. Sin embargo, no se ha identificado claramente cómo la angiogénesis afecta a la morfología testicular o a la actividad espermatogénica. El objetivo de este estudio es investigar los efectos de la espironolactona, como inhibidor de la angiogénesis, en la morfología del testículo ipsilateral en ratas con varicocele inducido en el lado izquierdo.
Materiales y métodos: Veinticuatro ratas albinas Wistar, adultas (12–14 meses) y de sexo masculino, fueron asignadas aleatoriamente a 4 grupos (n = 6, para cada uno): 1) grupo de control; 2) grupo con operación simulada; 3) grupo experimental con varicocele izquierdo; y 4) grupo experimental con varicocele izquierdo tratado con espironolactona (20 mg/kg/día). Se investigaron los resultados histopatológicos en testículos de rata.
Resultados: La densidad microvascular aumentó en el grupo con varicocele y la espironolactona no inhibió la angiogénesis ni por efecto antimineralcorticoide ni por efecto antiandrogénico. No obstante, la espermatogénesis se vio afectada en el grupo con varicocele tratado con espironolactona.
Conclusión: La angiogénesis parece ser un proceso protector en el varicocele. El tratamiento con espironolactona, probablemente al inhibir la angiogénesis, afecta a la morfología testicular.

Introduction

Varicocele, one of the most common factors in male infertility, is defined as the dilatation and tourtousity of plexus pampiniformis leading to a retrograde reflux caused by various etiological factors. According to the results of epidemiological trials, varicocele is estimated in 19–41% infertile couples as a cause of primary infertility. However, the incidence of varicocele may be as high as 81% in patients with secondary infertility.[1,2]

The most accepted theories regarding the etiology of varicocle are anatomical differences between left and right testicular veins, the lack of venous valves and nutcracker phenomenon.[3–5] The pathogenesis of varicocele-related infertility is not completely identified. Although there have been some theories such as hyperthermia, alterations in the testicular blood flow, renal and/or adrenal reflux, hormonal disorders, autoimmunity, apoptosis, and oxidative stress, none of them can enlight the process.[6–12]

It has been reported that varicocele promotes angiogenesis; however, it is not exactly shown how spermatogenesis is affected by angiogenesis.[13] To our hypothesis, if angiogenesis increases the distribution of the toxic metabolites through the testis, inhibition of angiogenesis may improve spermatogenesis. However, if angiogenesis occurs as a protective process, inhibiting angiogenesis may impair spermatogenesis more.

Spironolactone (SPL), a competitive antagonist of aldosterone, has been widely used as a potassium-sparing diuretic drug. Recently, the antiangiogenic effect of SPL has been described, which is unrelated to the antimineralocorticoid effect.[14]

The objective of the present study is to investigate the effect of SPL on a rat model of experimental left varicocele. We hypothetize that SPL improves the detrimental effect of varicocele on spermatogenesis in experimentally-induced varicocele in rats by inhibiting the angiogenetic process.

Material and methods

After being approved by the Baskent University Local Ethical Comittee (DA 07/12), the study was performed in 24 adult (12–14 months) male Wistar albino rats (282, 75 ± 20.47 g). The animals were fed by standard rat chow and tap water *ad libitum* and maintained in the animal facility with constant environmental conditions (room temperature: 20 ± 2°C, relative humidity: 50 ± 10%, light-dark cycle: 12:12-h). The rats were randomized into 4 groups ($n = 6$, for each); 1. Control, 2.Sham operated 3.ELV 4. ELV + Spironolactone (20 mg/kg/d, *p.o.*, 45 days)-treated (V+S).

Each animal was anesthesized by 10% ketamine hydrocloride (60 mg/kg, *i.p.*) and xylazine (10 mg/kg, *i.p.*). After an abdominal midline incision; the left renal vein, inferior vena cava, and left spermatic vein were identified. A 4/0 silk ligature was loosely placed at the site of the left renal vein and left spermatic vein insertion over a rigid hydrophilic guide wire of 0.64 mm that was placed on the left renal vein. The ligature was loosely tied and the guide wire was removed leading to an immediate dilation of the left renal and left spermatic vein. The incision was sutured with 4/0 silk ligature. All of the surgical procedures in the sham-operated group were identical to that of the ELV group, except for the vein ligature step.

Animals in the V+S and ELV were weighed on the first day postoperatively, to adjust the dose of spironolactone or the volume of saline to be given, respectively. The rats in the V+S received spironolactone at the dose of 20 mg/kg/day through an orogastric catheter, for 45 days, beginning the first day postoperatively. Spironolactone was dissolved in saline (5 mg/ml). The ELV group received only the corresponding volume of saline for 45 days.