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·*Original Article*·

Effects of epidermal growth factor on sperm content and motility of rats with surgically induced varicoceles

Dong Cheng, Xin-Min Zheng, Shi-Wen Li, Zhi-Wei Yang, Li-Quan Hu

Research Center of Urology and Andrology, Zhongnan Hospital, Wuhan University, Wuhan 430071, China

Abstract

Aim: To investigate the effect of epidermal growth factor (EGF) on the sperm content and motility of the varicocelized rats. **Methods:** Forty-eight male Wistar rats were randomly divided into five groups. Experimental varicocele was induced by partial ligation of the left renal vein in the varicocele, the varicocele repair, the varicocele with EGF and the varicocele repair with EGF groups, whereas the control group only received a sham induction of varicocele. Surgical repair of varicocele was performed 4 months later in the varicocele repair and varicocele repair with EGF groups. EGF administration was performed daily by s.c. injection in the varicocele with EGF and varicocele repair with EGF groups at the dose of 10 μg/(kg·day) from the next day of the second surgery. One month later, all animals were killed and bilateral cauda epididymal sperm counts and motility were evaluated. **Results:** The mean sperm count and percentage of motile spermatozoa were significantly higher bilaterally in the varicocele with EGF group than in the varicocele group ($P < 0.05$). They were also significantly higher bilaterally in the varicocele repair with EGF group than in the varicocele repair and the varicocele with EGF groups ($P < 0.05$). **Conclusion:** EGF can improve bilateral epididymal sperm content and motility of the rat with surgically induced varicocele. The administration of EGF in combination with surgical repair is more effective than surgical repair or EGF administration alone. EGF might be useful for the treatment of infertility induced by varicocele. *(Asian J Androl 2006 Nov; 8: 713–717)*

Keywords: epidermal growth factor; varicocele; infertility; rats; therapy

1 Introduction

Varicocele is the most common cause of male infertility. The incidence of varicocele in the general population is approximately 15%, whereas 19–41% of men presenting for infertility investigation demonstrate varicocele [1]. Varicocelectomy is currently the most popular treatment for varicocele. Surgical correction of varicocele in infertile men has demonstrated improved semen parameters in 50–80% of patients and pregnancy rates of 31–71% [1], which indicates that there are still many patients who fail to restore their fertility after surgery. Some investigators consider that varicocelectomy is not an effective treatment for infertility induced by varicocele, because they did not find higher pregnancy rates when patients operated on were compared with controls not operated on [2]. Therefore, it is still a worthwhile subject to investigate pharmacological treatment for varicocele-related infertility.

Epidermal growth factor (EGF) is a cytokine that

Correspondence to: Prof. Xin-Min Zheng, Research Center of Urology and Andrology, Zhongnan Hospital, Wuhan University, Wuhan 430071, China
Tel: +86-27-6781-3104, Fax: +86-27-6781-3090
E-mail: zhengxinmin008@hotmail.com
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promotes cell proliferation, regulates tissue differentiation and modulates organogenesis [3]. It is also one of the important cytokines that have significant effects on male fertility. The elimination of circulating plasma EGF by sialoadenectomy significantly suppresses spermatogenesis of the adult male mouse and results in a marked decrease in sperm content and motility [4]. This suggests that EGF is important for sperm production and motility acquisition. Administration of exogenous EGF is found to markedly improve the testicular injuries caused by testicular torsion [5] and cryptorchidism in rats [6] and streptozotocin-induced diabetes in mice [7]. However, the influence of EGF on varicocele has not been reported before. In the present study, we investigate the possible effect of exogenous EGF on sperm content and motility of rats with surgically induced varicocele when combined with or without surgical repair of varicocele.

2 Materials and methods

2.1 Animals

Forty-eight healthy male Wistar rats (6–7 weeks old) were randomly divided into five groups: the control group (n = 8), the varicocele group (n = 10), the varicocele repair group (n = 10), the varicocele with EGF group (n = 10) and the varicocele repair with EGF group (n = 10). Animals were kept in individual cages at a constant temperature (22 ± 2ºC), with a 12 h : 12 h Light: Dark cycle and fed standard rat chow with free access to tap water. Before surgical procedures, animals were fasted for 12 h, but allowed free access to water. All rats were obtained from the Experimental Animal Center of Tongji Medical College (Wuhan, China) and treated in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). The study was approved by the Ethical Committee, Wuhan University School of Medicine. The procedures performed in each group are summarized in Table 1.

Table 1. Procedures performed in each group. VC, varicocele; NS, normal saline; EGF, epidermal growth factor.

Group	Procedure
Control	Sham varicocele induction
VC	Varicocele + sham repair + NS
VC repair	Varicocele + repair + NS
VC with EGF	Varicocele + sham repair + EGF (10 μg/[kg·day])
VC repair with EGF	Varicocele + repair + EGF (10 μg/[kg·day])

2.2 Surgical procedures

Rats were weighed and anaesthetized with an i.p. injection of pentobarbital (40 mg/kg). The experimental varicocele was induced according to the method of Turner [8] in all groups, except the control group. In brief, through a midline laparotomy incision, the upper-left abdominal quadrant was approached and the left renal, adrenal and internal spermatic veins were identified. Using careful blunt dissection, the left renal vein was cleared of adhering tissue in a position medial to the insertion of the internal spermatic and adrenal veins. For the evaluation of varicocele development, the external diameter of the left internal spermatic vein at the level of the iliolumbar vein was measured using a metal micrometer. Then the left renal vein was ligated using a 4-0 silk suture together with a metal probe in the position that had been cleared of adhering tissue. The probe was then carefully removed and approximately a 50% reduction in the diameter of the left renal vein was achieved. The anastomotic branch between the left internal spermatic vein and the left common iliac vein and all the small anastomotic branches of the left internal spermatic vein were also carefully ligated. The control group underwent similar procedures, but without the partial ligation of the left renal vein. After the incisions of the abdominal muscle and the abdominal wall were repaired, the rat was taken into the cage and fed the same way as before. Four months later, the rat was weighed and anaesthetized in the same way as previously. The presence of dilatation of the left internal spermatic vein was checked by measuring its external diameter at the same previous level. Dilatation of the vein more than double of its original external diameter was considered a varicocele. The rats that did not have a varicocele, except those in the control group, were excluded from the present study. After varicocele evaluation, surgical repair of varicocele was performed in the varicocele repair and the varicocele repair with EGF groups by ligation of the left internal spermatic vein at its junction with the left renal vein using the method of Sofikitis *et al*. [9], whereas the varicocele and varicocele with EGF groups only underwent sham repair operations. Neither a repair nor a sham repair operation was performed in the control group.

2.3 Epidermal growth factor treatment

From the day following the second surgery, s.c. injections of recombinant EGF (PeproTech, Rocky Hill, NJ, USA) were performed daily in the varicocele with EGF and varicocele repair with EGF groups at a dose of 10 μg/(kg·day) (diluted in 0.2 mL normal saline [NS]); and the varicocele and varicocele repair groups were injected with an equal volume of NS. One month later, all animals were anaesthetized and the venous system was evaluated again. The animals were then killed and bila-teral testes and cauda epididymides were collected and weighed.

2.4 Cauda epididymal spermatozoa evaluation

The cauda epididymis was minced in 5 mL of the media (Hank's solution containing 0.5% bovine serum albumin) at 37ºC and filtrated for debris removal. The sperm suspension was placed on a slide glass that was warmed to 37ºC for observation of sperm motility. The percentage of motile spermatozoa was determined by counting more than 200 spermatozoa in randomly selected fields under a light microscope. The sperm count was calculated using a haemocytometer and expressed as the number of spermatozoa per gram of cauda epididymis. Each sample was evaluated by two investigators simultaneously and the average value was adopted.

2.5 Statistic analysis

SPSS version 11.5 for Windows (SPSS, Chicago, IL, USA) was used for the statistical analysis. All values were expressed as the mean ± SD. The intergroup differences were evaluated using analysis of variance followed by Duncan's multiple range test. The difference between the left and right sides within a group was evaluated using the paired *t*-test. $P < 0.05$ was considered significant.

3 Results

3.1 Varicocele evaluation

Of the 40 rats undergoing partial ligation of the left renal vein, 4 did not show the objective dilatation of the left internal spermatic vein at 4 months after the model creation and another 2 died. Finally, 9, 8, 8 and 9 rats in the varicocele, the varicocele repair, the varicocele with EGF and the varicocele repair with EGF groups were included in the study, respectively. No animals in the control group showed a similar dilatation of the left internal spermatic vein. Before the animals were killed, the venous system was also evaluated. Although the external diameters of the left internal spermatic veins of the repair groups did not completely return to the normal level (< 0.3 mm), they were all markedly reduced by more than 50% when compared with their original values shown at the time the repair operation was performed. The incomplete recovery of the spermatic vein might be a result of the damage of the venous wall that had been formed in the process of surgical induction of the varicocele and subsequent repair of the varicocele.

3.2 Body weight

When the rats were killed, the mean body weights were 459.1 ± 42.7, 445.5 ± 36.9, 438.3 ± 47.4, 469.5 ± 29.4 and 464.7 ± 40.6 g in the control, the varicocele, the varicocele repair, the varicocele with EGF and the varicocele repair with EGF groups, respectively. There were no significant differences ($P > 0.05$) among various groups.

3.3 Testicular weight

The mean weight of the testis is shown in Table 2. The mean testicular weight was significantly lower ($P < 0.05$) bilaterally in the varicocele group than that in all the other groups. The mean testicular weight was significantly higher ($P < 0.05$) bilaterally in the varicocele repair with EGF group and the control group than in the varicocele repair and the varicocele with EGF groups. The mean testicular weight was significantly lower ($P < 0.05$) on the left side than on the right side in all groups, except the control group.

3.4 Sperm count and motility

The mean sperm count and percentage of motile spermatozoa are shown in Table 3. The mean sperm count and percentage of motile spermatozoa were significantly lower ($P < 0.05$) bilaterally in the varicocele group than

Table 2. Testicular weight. VC, varicocele; EGF, epidermal growth factor. $^{b}P < 0.05$, compared with the VC group; $^{e}P < 0.05$, compared with the VC repair and the VC with EGF groups.

Group	Number	Testicular weight (g)	
		Left	Right
Control	8	$1.85 \pm 0.07^{b,e}$	$1.94 \pm 0.06^{b,e}$
VC	9	0.92 ± 0.10	1.20 ± 0.08
VC repair	8	1.34 ± 0.11^{b}	1.56 ± 0.09^{b}
VC with EGF	8	1.19 ± 0.07^{b}	1.45 ± 0.05^{b}
VC repair with EGF	9	$1.68 \pm 0.11^{b,e}$	$1.81 \pm 0.06^{b,e}$

Table 3. Epididymal sperm count and motility. VC, varicocele; EGF, epidermal growth factor. $^{b}P < 0.05$, compared with the VC group; $^{e}P < 0.05$, compared with the VC repair and the VC with EGF groups.

Group	Number	Sperm count (10^6/g)		Sperm motility (%)	
		Left	Right	Left	Right
Control	8	$880.1 \pm 55.1^{b,e}$	$885.6 \pm 49.8^{b,e}$	$79.1 \pm 5.3^{b,e}$	$81.2 \pm 4.9^{b,e}$
VC	9	224.5 ± 37.9	407.6 ± 41.0	30.4 ± 5.8	35.7 ± 6.2
VC repair	8	531.7 ± 58.3^{b}	672.0 ± 62.5^{b}	53.5 ± 6.4^{b}	57.8 ± 4.8^{b}
VC with EGF	8	402.4 ± 46.9^{b}	578.3 ± 44.5^{b}	48.1 ± 4.4^{b}	57.0 ± 5.1^{b}
VC repair with EGF	9	$785.6 \pm 60.2^{b,e}$	$814.8 \pm 58.7^{b,e}$	$72.0 \pm 4.1^{b,e}$	$74.9 \pm 3.8^{b,e}$

in all the other groups. The mean sperm count and percentage of motile spermatozoa were significantly higher ($P < 0.05$) bilaterally in the varicocele repair with EGF group and the control group than in the varicocele repair and in the varicocele with EGF groups. The mean sperm count was significantly lower ($P < 0.05$) in the left epididymis than that on the right side in all groups, except in the control and the varicocele repair with EGF groups. The mean percentage of motile spermatozoa was significantly lower ($P < 0.05$) in the left epididymis than that on the right side in the varicocele with EGF group, whereas there was no significant difference ($P > 0.05$) between the left and right sides within any other groups.

4 Discussion

Varicocele was traditionally described as being disadvantageous to spermatogenesis, characterized by a low sperm count, poor motility and abnormal morphology [10]. The present study confirms the previous findings that testicular weight, epididymal sperm content and motility were reduced bilaterally after the development of a left varicocele in the rat and that these decreases were more obvious on the left side than on the right side [9].

Varicocelectomy has been used for several decades as a therapeutic procedure for the treatment of infertility induced by varicocele. The effect of surgical repair of the experimental varicocele on the fertility of the animal has also been investigated. Sofikitis *et al.* [9] found a basically full recovery of bilateral testicular weight, epididymal sperm content and motility after surgical repair of the short-term (30-day) experimental varicocele. In the present study, however, we only found a partial recovery of those parameters 1 month after repair of the long-term (4-month) experimental varicocele in the rat model. The difference might be attributable to the different durations of varicocele before repair. Therefore, it seems that for those with a long duration of varicocele, surgical repair alone is not enough to restore those parameters completely, at least within a "short" time.

The exact mechanism by which varicocele causes infertility has not yet been fully elucidated. Some studies claim that it might be attributable to dysfunction of Leydig [1] and Sertoli cells [11], overproduction of reactive oxygen species (ROS) in the testis [12] and the epididymis [13], as well as dysfunction of the epididymis [14–16]. It is speculated that the pharmacological correction of these disturbances might help to improve the fertility of patients with varicocele. EGF is a cytokine that has attracted considerable attention in the field of fertility in recent years. In the present study, as an exploratory investigation, the effect of EGF on the testicular weight, epididymal sperm content and motility was observed in varicocele rat models. The results show that the EGF treatment significantly improved all these parameters, both for the rats that had undergone surgical repair of varicocele and those that had not received a varicocele correction. The results also show that the EGF administration in combination with surgical repair was more effective than surgical repair or EGF administration alone. No significant changes in the body weight nor obvious effects on the health of the animals was seen in EGF treated groups.

EGF is a polypeptide of 53 amino acids that was first isolated and purified from the submandibular glands of male mice [6]. EGF receptor (EGFR) has been shown in Leydig, Sertoli and peritubular cells of the testis and the epithelium of the epididymis [17], suggesting that EGF might affect both the function of the testis and the epididymis. The binding of EGF to its receptor initiates many biological effects on the testis, such as modulating Leydig cell proliferation, steroidogenesis, spermatogenesis and Sertoli cell activity [5]. EGF improves injuries of many organs, including the testis by decreasing ROS production [5]. EGFR is also present on sperma-

tozoa [18], suggesting that EGF might also influence fertility through its direct effects on spermatozoa. This was proved by Furuya *et al.* [19], who found that EGF could stimulate human sperm capacitation by activating the tyrosine kinase of EGFR on spermatozoa. Therefore, it seems possible that the beneficial effects of EGF in the present study might be a result of its stimulation of the testicular somatic cell functions and elimination of the increased ROS in the testis that was produced as a result of the surgical creation of the varicocele. It might also be related to its effects on the epididymis, because the epididymis plays a key role in sperm maturation, motility acquisition and antioxidant defense [20]. However, understanding the exact mechanism awaits further studies.

In summary, the present study shows that EGF administration improves bilateral epididymal sperm content and motility of a rat with a surgically created varicocele. It is also shown that the administration of EGF in combination with surgical repair is more effective than surgical repair or EGF administration alone. Therefore, for patients who fail to become fertile after varicocelectomy or those who are not suitable for surgery, EGF treatment might be a promising therapeutic option. However, this is only a preliminary investigation. Further studies are needed to address some correlative issues, such as the precise dose of EGF, the appropriate duration of treatment, the potential side effects and so on. A more effective method of EGF administration should also be investigated. For example, Kurokawa *et al.* [6] reported that the direct injection of EGF into the seminiferous tubules through the rete testis helps to improve spermatogenesis of the cryptorchid rat after orchiopexy. This might be a more economical method of treatment, which is worth of investigation.

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