Testicular Oxidative Stress
Effects of Experimental Varicocele in Adolescent Rats

A.S. Özdamara A.G. Soylua M. Çulhaa M. Özdenb A. Gökalpa
Departments of aUrology and bBiochemistry, Kocaeli University Faculty of Medicine, Kocaeli, Turkey

Key Words
Oxidative stress • Varicocele

Abstract
Objective: This present study was undertaken to determine the levels of malondialdehyde (MDA), nitric oxide (NO) and total antioxidant status (TAS) in testes of adolescent rats with experimental bilateral varicocele and to determine the effects of oxidative stress on testis produced by varicocele. Methods: 6-week-old, male Wistar rats, weighing 146–334 g (228.37 ± 41.34 g), were randomly allocated into two groups. The first group underwent selective and bilateral partial ligation of the spermatic vein (n = 28), and the second group underwent sham operation and served as the controls (n = 15). Animals were sacrificed 12 weeks after surgery and dilatation of the spermatic veins was observed in the first study group. Bilateral orchietomy was performed in all rats, and MDA, NO and TAS levels were measured. Results: In the study group, the mean MDA (SEM) level was 15.58 ± 6.07 μmol/g protein, and in the control group, it was 11.59 ± 3.86 μmol/g protein, respectively; this difference was statistically significant (p < 0.05). The mean NO level was 82.73 ± 77.84 nmol/g protein in the study group, whereas 28.65 ± 20.18 nmol/g protein in the control group, this difference was also statistically significant (p < 0.005). The mean TAS levels of the study and control groups were 0.91 ± 0.32 and 1.78 ± 0.46 nmol/g tissue, respectively, and this difference was also statistically significant (p < 0.001). But there was no correlation between these three parameters (MDA→TAS: r = −0.103, p > 0.05; MDA→NO: r = −0.104, p > 0.05; NO→TAS: r = −0.123, p > 0.05). Conclusion: These findings suggest that varicocele may change the testicular oxidative status and may play a role in testicular dysfunction that causes infertility.

Introduction

The association of testicular dysfunction with varicocele has been known for many decades. Varicocele is found in approximately 10% of young men and consists of dilatation of the pampiniform plexus above the testis, with the left side being most commonly affected. Sperm concentration and motility are significantly decreased in 65–75% of subjects. Infertility is often observed and can be reversed in a high percentage of patients by correction of the varicocele [1]. Although the varicocele is often referred to as ‘the most common cause of correctable infertility’, the exact pathophysiological mechanism of varicocele is not clearly understood [2]. Several theories have been postulated, such as hyperthermia, testicular blood flow and venous pressure changes, reflux of renal/
adrenal products, hormonal dysfunction, autoimmunity, defects in acrosome reaction, and oxidative stress [3].

Oxidative stress results from an imbalance between production and removal of reactive oxygen species (ROS), leading to both a steady-state concentration of reactive intermediates higher than normal and to increased cellular damage. The production of ROS, antioxidants and nitric oxide (NO) is a normal physiological process. However, abnormal production (over or down) of these molecules can be detrimental to sperm and recently has been association with male infertility [4]. Several authors postulated similar correlation between varicocele and oxidative stress in tests [5, 6]. In most of these studies, oxidative stress parameters were investigated in seminal plasma or in the cellular level of ejaculated sperm.

Methods

Ethics

This study was approved by the ‘Local Animal Ethical Committee’ of Kocaeli University, Faculty of Medicine (AEK-248/3).

Animals

Forty-three adolescent male Wistar rats, 6 weeks old, and weighing between 146 and 334 g (228.37 ± 41.34 g), were kept in a controlled environment with food and water ad libitum.

Rats were randomly allocated into two groups (study group n = 28; control group n = 15).

Group 1 (study group): Under sterile conditions, anesthesia was induced by ether inhalation and modified bilateral varicoceles were created as previously described [7]. We performed bilateral selective spermatic vein ligation, renal vein ligation. We used an ‘L’-shaped 20-gauge needle. The needle was positioned parallel to the spermatic vein (approximately 1 cm distally from the renal vein) and a 5-zero silk suture was tied around the spermatic vein and needle. After placing the ligature, the needle was removed; by this method the lumen of the vein was reduced to 20-gauge effectively. The wound was closed with a 4-zero silk suture.

Group 2 (control group): These rats underwent a similar procedure without ligation of the spermatic vein.

All rats were sacrificed and dilatation of the spermatic veins was observed by comparing with the ureteral veins at the 12th week post-operatively [8]. After that bilateral orchiectomy was performed on all rats. The animals were fasted without ligation of the spermatic vein, adrenal products, hormonal dysfunction, autoimmunity, defects in acrosome reaction, and oxidative stress [3].

Oxidative stress results from an imbalance between production and removal of reactive oxygen species (ROS), leading to both a steady-state concentration of reactive intermediates higher than normal and to increased cellular damage. The production of ROS, antioxidants and nitric oxide (NO) is a normal physiological process. However, abnormal production (over or down) of these molecules can be detrimental to sperm and recently has been association with male infertility [4]. Several authors postulated similar correlation between varicocele and oxidative stress in tests [5, 6]. In most of these studies, oxidative stress parameters were investigated in seminal plasma or in the cellular level of ejaculated sperm.

Results

In the study group (group 2) mean MDA level was 15.58 ± 6.07 µmol/g protein (7.54–29.44) where in controls, this was 11.59 ± 3.86 µmol/g protein (5.38–16.71). In the study group the result was significantly higher than in the control group (p < 0.05) (fig. 1).

Mean NO level was 82.73 ± 77.94 nmol/g protein (10.77–254.50) in the study group, whereas 28.65 ± 20.18 nmol/g protein (10.37–67.85) in the control group. Difference between the two groups was statistically significant (p < 0.005) (fig. 1).

Mean TAS level was 0.91 ± 0.32 nmol/g tissue (0.30–1.58) in the study group and 1.78 ± 0.46 nmol/g tissue (0.93–2.42) in the control group. In the study group, the result was significantly lower than for the control group (p < 0.001) (fig. 1).

There was no correlation between the parameters that we used (MDA+TAS: r = −0.103, p > 0.05), (MDA+NO: r = −0.104, p > 0.05), (TAS+NO: r = −0.123, p > 0.05).
**Discussion**

Varicocele is found in 19–41% of infertile men, and is one treatable form of male infertility. Although a lot of studies can be found in the literature, the mechanism by which varicocele causes the variable effects on male infertility and spermatogenesis is still controversial [3]. Various mechanisms have been suggested to account for the testicular dysfunction associated with varicocele, e.g. retrograde flow of toxic metabolites from the adrenal or kidney, venous stasis with germinal epithelial hypoxia, alterations in the hypothalamic-pituitary-gonadal axis, and increased testicular temperature. Although clinically evident varicocele has been reported in 8–23% of men. Not all men with varicocele are infertile but varicocelectomy may resolve the infertility problem in these patients [13].

Naughton et al. [3] concluded that experimental animal models play a useful (but limited) role due to the sudden and variable iatrogenic nature of the varicoceles and the duration of the studies. In these studies, acute varicocele formation may not reflect the chronic effects of varicocele on the testis, but these studies may show the relationship between cause and results by eliminating other etiologic factors. In our study, we performed a selective spermatic vein ligation instead of renal vein ligation. By this modification, the possibility of reflux of toxic metabolites from kidney and adrenal gland was minimized. In addition to this surgical modification, we performed orchiectomies in the 12th postoperative week for elimination of the acute effects of ligation.

The biochemical mechanisms by which varicocele induce spermatogenic and spermatozoal dysfunction have not been completely elucidated. Researches during the last 10–15 years have implicated oxidative stress as a mediator of sperm dysfunction and may play a role in male infertility [10, 14]. It was concluded that seminal oxidative stress is strongly associated with varicocele and sperm dysfunction [15]. Irrespective of the clinical diagnosis and semen characteristics, the presence of seminal oxidative stress in infertile men suggests its role in the pathophysiology of infertility, and it was concluded that medical and surgical treatments for infertility in these men should include strategies to reduce oxidative stress [16].

Oxidant agents, NO, and antioxidants take place in the ‘oxidative stress’ concept [10, 14, 17, 18]. Oxidative stress results from an imbalance between production and removal of ROS, leading to both a steady state concentration of reactive intermediates higher than normal and to increased cellular damage. The production of ROS, NO and antioxidants is a normal physiological process. However, abnormal production (over or down) of these molecules can be detrimental to sperm and has recently been associated with male infertility [4].

Studies have shown that 40–88% of nonselected infertile patients have high levels of seminal ROS [18]. In non-oligospermic men with varicocele, spermatozoal ROS was significantly elevated and the concentration of seminal plasma antioxidant was lower in men with varicocele irrespective of fertility status, and it was concluded that seminal oxidative stress was strongly associated with varicocele and sperm dysfunction [15]. Uncontrolled and excessive production of ROS seems to have a significant role as one of the major factors leading to an infertile status and causes oxidative stress resulting in decreased sperm motility, viability, increased sperm capacitation and acrosome reaction defects [10, 14, 19]. Superoxide anion, hydrogen peroxide and hypochlorite have been proposed as major
components of ROS [10]. In these studies, the ROS levels were determined in seminal plasma, and to date, few experimental studies have examined ROS levels in testicular tissue [20–24]. It was postulated that increasing levels of MDA in testicular biopsies of infertile men are associated with higher grades of varicocele [22]. In another study by the same author, they could not find a relationship between oxidative status and testicular dysfunction in adolescent rats with varicocele [23].

The free radicals react with unsaturated fatty acids and cause lipid peroxidation. MDA is a stable product formed from the breakdown of polyunsaturated fatty acids and can therefore be used as a measure of lipid peroxidation [25].

We produced an experimental varicocele in rats, and the biochemical studies were done 12 weeks later to eliminate the acute effects of the procedure. In our study group, the mean MDA level was found to be statistically higher than in the control group (p < 0.05). This result suggests that varicocele itself may cause an elevation of lipid peroxidation in the testis.

Nitric oxide (NO) is a free radical formed by nitric oxide synthase and is an important regulator of the biology and physiology of the reproductive system [17]. It is produced by testicular tissues and acts as an autocrine/paracrine messenger in the local regulation of steroidogenesis [26]. Kostic et al. [27] reported that the stress-induced activation of NO synthases and elevation of intratesticular NO lead to inhibition of both steroidogenic and antioxidant enzymes. This in turn changes the contributions of these two enzymatic systems in the control of free radicals and their secondary actions and they concluded that interactions of antioxidant and steroidogenic enzyme systems with NO may provide a rationale for the occurrence of strong antagonal effects during acute stress. NO regulates sperm motility, with low concentrations of NO enhancing and medium/high concentrations of NO decreasing sperm motility. It is tempting to speculate that under physiological conditions, small amounts of NO are generated to neutralize free radicals which inhibit sperm motility. Thereby low concentrations of NO may protect against O2−-mediated reduction of sperm motility. In contrast, excessive generation of NO under pathological conditions such as infection or endometriosis can cause sperm toxicity as well as reduce sperm motility by contributing to the formation of peroxynitrite, a highly toxic anion of peroxidation [28]. There is a negative correlation between seminal plasma NO concentration and sperm motility, and it was concluded that increased NO concentration may be one of the causes of sperm damage in patients with varicocele [29, 30]. These negative effects of NO on sperm can be abrogated by inhibiting NO synthesis by L-NAME [31]. The specific localization of endothelial nitric oxide synthases to human spermatozoa suggests that nitric oxide may be involved in normal sperm physiology and it was concluded that aberrant patterns of sperm endothelial nitric oxide synthases expression are associated with decreased sperm motility, possibly through the generation of excessive cytotoxic oxidants [32].

We also found statistically higher levels of NO in the varicocele group than in the control group (p < 0.005). A variety of defense mechanisms comprising a number of anti-oxidants can be employed to reduce or overcome oxidative stress caused by excessive ROS. The first mechanism is the prevention of the formation or quick inactivation of radicals. It can be performed by enzymes like catalase or glutathione-peroxidase complex. The second mechanism is blocking of the lipid peroxidative propagation chain through a reaction with intermediate radicals, where the involved molecules are called ‘scavengers’. Some of these molecules are albumin, bilirubin, ascorbate and thiols, vitamin E and Coenzyme Q10. The third line includes mechanisms that can remove the molecules damaged by the ROS insult and therefore allow the healthy structures to be restored [33]. Oxidative injury to spermatozoa is a major cause of sperm dysfunction, and total nonenzymatic antioxidant defenses in human seminal plasma are inversely related to lipid peroxidation [34]. Antioxidant activity of seminal plasma was found to be significantly lower in idiopathic infertile patients than controls, and it was concluded that decreased seminal plasma antioxidant activity may be responsible for the observed ROS production and resulting defective sperm function [18, 19]. Zini et al. [35] could not find any deficiency of antioxidants but high levels of ROS in the seminal plasma of the infertile men, and they concluded that they could not exclude the possibility that a small subset of patients with high semen ROS levels had low levels of semen antioxidants. The antioxidant defenses both at the local (seminal plasma) and systemic (blood plasma) levels were found to be diminished in varicocele patients [36].

In our study, we measured TAS as a defense mechanism to oxidative stress produced by iatrogenic varicocele. In the study group, TAS levels were significantly lower than in the control group (p < 0.001). This result shows that varicocele may destroy the defense mechanisms of testis that can cause a relative elevation of oxidative status regarding the overproduction of oxidative agents and NO.
In conclusion, although our results confirm our hypothesis that varicocele produces ‘oxidative stress’ in testis and may destroy testicular function, we do not know whether this stress results in a decrease in fertility status. But our study shows that three components of oxidative stress (oxidants, NO and antioxidants) take place in the testis when varicocele is present. We believe that further studies are needed to determine the role and origin of oxidative stress in male infertility. Regarding our results, we suggest that varicocele may produce ‘oxidative stress’ on the testis, and we believe that oxidative stress begins in the testis and that this ‘stress’ may play a role in male fertility.

References