Int Urol Nephrol (2008) 40:679–683
DOI 10.1007/s11255-008-9339-6

ORIGINAL ARTICLE

Comparison of antioxidant enzyme activity in the internal spermatic vein and brachial veins of patients with infertile varicocele

Emin Ozbek · Mustafa Cekmen · Abdulmuttalip Simsek · Yusuf Turkoz · Ahmet Soylu · Y. Ozlem Ilbey · M. Derya Balbay

Received: 9 October 2007 / Accepted: 8 January 2008 / Published online: 9 February 2008

Abstract
Aim Recent studies have shown that both oxidative and reductive stresses are present within the internal spermatic vein of patients with varicocele. The aim of this study was to compare the activities of antioxidant enzymes in the internal spermatic vein and brachial vein of patients with varicocele.
Methods Fifteen primary infertile varicocele patients and ten normal-nonvaricocele-fertile control subjects participated in this study. The patients and subjects were first given a physical and color doppler examination, and then whole blood samples were drawn from the brachial vein and a dilated internal spermatic vein during surgery. Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) enzyme activities were assessed by enzymatic methods, and the results were compared using the Mann–Whitney *U* test.
Results The activity of SOD in the internal spermatic veins and brachial veins of patients with varicocele was 60.17 ± 2.15 and 42.10 ± 1.60 U/g protein, respectively; that of GSH-Px was 5.44 ± 0.14 and 3.92 ± 0.14 U/g protein, respectively. The results were statistically significant ($P < 0.05$). In the control group, the activity of SOD in the internal spermatic veins and brachial veins was 43.12 ± 1.80 and 40.01 ± 2.10 U/g protein, respectively; that of GSH-Px was 3.35 ± 0.20 and 3.7 ± 0.10 U/g protein, respectively ($P > 0.05$).
Conclusions Increased antioxidant enzyme activity in the internal spermatic vein may be due to increased oxidative stress in the internal spermatic vein: the increase in antioxidant enzyme activity may be a response to offset the toxic actions of reactive oxygen species. Further studies are needed to confirm this suggestion.

Keywords Antioxidant enzyme activity · Glutathione peroxidase · Infertility · Superoxide dismutase · Varicocele

E. Ozbek (✉) · A. Simsek · Y. O. Ilbey
Department of Urology, Bezm-I Alem Valide Sultan Vakif Gureba Research and Education Hospital, Istanbul, Turkey
e-mail: ozbekemin@hotmail.com

M. Cekmen
Department of Biochemistry, Kocaeli University, Kocaeli, Turkey

Y. Turkoz · A. Soylu
Department of Biochemistry and Urology, Kocaeli and Inonu University, Malatya, Turkey

M. D. Balbay
Department of Urology, Atatürk Research and Education Hospital, Ankara, Turkey

Introduction

Varicocele is known to be associated with male infertility, and approximately 19–41% of male

patients attending infertility clinics have varicocele [1]. The pathophysiology of testicular dysfunction in patients with varicocele has been studied, and although the exact mechanism is poorly understood, the most commonly accepted explanation is that testicular hypoxia by venous stasis and small vessel occlusion leads to Leydig cell and germinal cell dysfunction [2]. Alternative explanations include the retrograde flow of adrenal and renal metabolites from the renal vein down the left internal spermatic vein [3], elevation in scrotal temperature and a depression of gonadotropin or androgen secretion [4].

Since Aitken and Clarkson noted the role of reactive oxygen species (ROS) in male infertility, attention has been focused on the possible relationship between male infertility and oxidative stress [5]. Sperm dysfunction is associated with an excessive generation of ROS in 53% of varicocele patients [5]. The most important ROS produced by human sperm are hydrogen peroxide, superoxide anion and hydroxyl radicals [6, 7]. Human seminal plasma and sperm possess antioxidant systems to scavenge ROS and prevent ROS-related cellular damage. Extensive investigations have been conducted on superoxidase dimutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities in the human sperm and seminal plasma [6, 8, 9]. It has been reported that ROS is very important in the pathophysiology of varicocele in experimental.

The aim of the study reported here was to evaluate the antioxidant enzyme activities of SOD and GSH-Px in the internal spermatic vein and brachial vein of patients with infertile varicocele patients. To the best of our knowledge, this is the first published study on this subject.

Material and methods

Patients

Fifteen normal gonadotropic patients, aged between 24–33 years, attending at male infertility unit because of prolonged (more than 3 years) subfertility were included in the study. The patients were evaluated by physical examination and scrotal color doppler ultrasonography. No testicular atrophy was diagnosed, and all had unilateral G-II or G-III primary varicocele. On the right, scrotal doppler and physical examination were normal in all cases. Those with abnormal hormone analysis, testicular atrophy, genito-urinary tract infection, azoospermia were excluded. Previous repeated semen analysis showed persistent oligoasthenozoospermia (less than 20×10 spermatozoa per milliliter, more than 50% immotile spermatozoa after 1 h). The mean sperm concentration ($\pm$ SD) was $10 \times 10^6 \pm 2.6 \times 10^6$/ml spermatozoa, while the mean percentage of motile spermatozoa after 1 h was 32 ± 1.6. The mean percentage of morphologically normal spermatozoa was $29.3 + 0.8$. Serum specimens obtained from ten fertile-nonvaricocele-patients, either during inguinal hernia repair (four patients, age range 22–33 years) or open lower ureteral stone surgery (six patients; age range 23–30 years) 23–30, were used as a control. Whole blood samples (1.5 ml) were drawn using a 25-gauge-needle during surgery from a brachial vein and the internal spermatic vein. The dilated spermatic vein was punctured immediately after exposing the spermatic cord and before any further manipulations. Following centrifugation at 800*g* for 10 min, the serum was separated and kept at -20°C until analyzed.

Biochemical determinations

Superoxide dismutase activity was measured by determining the reduction of nitroblue tetrazolium by the superoxide anion produced with xanthine and xanthine oxidase [12]. One unit of SOD was defined as the amount of protein that inhibits the rate of NBT reduction by 50% and the results reported as units per milligram protein. The specific activity of the enzymes was expressed in units per milligram protein. Proteins in the seminal fluid were determined by the method of Lowry et al. [13]. Glutathione peroxidase activity was measured by monitoring the oxidation of reduced nicotinamide–adenine dinucleotide phosphate at 340 nm, as described by Paglia and Valentine, [14], and the results are reported as units per gram protein using an extinction coefficient of 6.22 $\times$ 10–6/*M*/cm.

Statistical analysis

The Mann–Whitney *U* test was used for statistical analysis. A *P* value of <0.05 was considered to be

statistically significant. Data were expressed as mean ± standard error (SEM) with 95% confidence intervals (95% CI).

Results

The mean age of the patients was 27 ± 2.2 years (range 24–33 years). Superoxidase dimutase and GSH-Px enzyme activity in the internal spermatic veins were 60.17 ± 2.15 and 5.44 ± 0.14 U/g protein; in the brachial veins, these values were 42.10 ± 1.60 and 3.92 ± 0.14 U/g protein, respectively (Table 1). Consequently, the activities of the SOD and GSH-Px enzymes were significantly higher in the internal spermatic vein than in the brachial vein ($P < 0.05$). In the control group, the enzyme activities of SOD and GSH-Px in the internal spermatic veins were 43.12 ± 1.80 and 3.35 ± 0.20 U/g protein; in the brachial veins, these values were 40.01 ± 2.10 and 3.7 ± 0.10 U/g protein, respectively ($P > 0.05$) (data not shown).

Discussion

The association between male infertility and varicocele have been well known for several years, and approximately 19–41% of male patients attending infertility clinics have varicocele [1]. However, the mechanism causing infertility in those patients is still completely unknown. Recent studies have shown that increased ROS and decreased antioxidant enzyme activity in the seminal plasma of patients with varicocele is associated with sperm dysfunction and subsequent male infertility [15]. Human spermatozoa can produce ROS, and the incidence and level of ROS production are greater in sperm from infertile patients than in that from fertile controls [16, 17]. Despite the damaging effects of ROS on human spermatozoa, ROS also play a physiological role in sperm activation, capacitation and the acrosome reaction [18, 19].

We found that the activities of antioxidant enzymes (AOEs) (SOD, GSH-Px) in the internal spermatic vein of patients with varicocele are higher than those found in the brachial vein of the same patients. There was no statistically significant difference between the activities of AOEs in the brachial and internal spermatic veins of the non-varicocele-normal-fertile controls ($P > 0.05$). To the best of our knowledge, this is the first published study on this topic. In a previous study we showed increased nitric oxide production in the internal spermatic vein of patients with varicocele [20].

The most common ROS that are potentially implicated in reproductive biology are superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), peroxyl radicals (ROO^-) and very reactive hydroxyl radicals (OH^-) [21]. Under normal conditions, superoxide anions are detoxified by SOD and peroxyl radicals from various peroxides, including hydrogen peroxides removed by GSH-Px, a selenium-containing antioxidant enzyme with glutathione as the electron donor [22]. In addition, GSH-Px and GSH-reductase may directly act as antioxidant enzymes involved in the inhibition of sperm lipid peroxidation (LPO) [23].

Varicocele is a chronic disease; in chronic diseases, there is mild to moderate free radical production because a compensatory mechanism reacts against oxidative stress. For this reason H_2O_2 production is very low. glutathione peroxidase plays a major role in removing the cytotoxic H_2O_2 formed in chronic pathologies. Catalase also detoxifies high amounts of H_2O_2, but GSH-Px possesses a higher affinity for H_2O_2 than CAT (Km for H_2O_2

Table 1 Superoxidase dimutase (SOD) and glutathione peroxidase (GSH-Px) activity in the internal spermatic and brachial veins

	Patient group		Control group	
	Spermatic vein	Brachial vein	Spermatic vein	Brachial vein
SOD	60.17 ± 2.15*	42.10 ± 1.60*	43.12 ± 1.80**	40.01 ± 2.10**
GSH-Px	5.44 ± 0.14*	3.92 ± 0.14*	3.35 ± 0.20**	3.7 ± 0.10**

Values are given as means ± standard error of the mean. The units are units per gram protein

SOD and GSH-Px activities in the internal spermatic veins and brachial veins of patients group were statistically significant (*$P < 0.05$); in the control group, antioxidant enzyme activities were not significant (**$P > 0.005$)

degradation is about 40 μM) [24]. It has bee suggested that GSH-Px plays a major role in removing the cytotoxic H_2O_2 formed in chronic pathologies, and a number of reports support the suggestion that GSH-Px makes the cells more resistant to oxidative stress and that GSH-Px is more important than CAT in this respect [25–27]. Due to the abundance of data on CAT, we did not measure CAT activity.

The internal spermatic venous blood of patients with varicocele is characterized by venous stasis and a hypoxic condition, suggesting that increased ROS via neutrophil activation may be found in this venous blood. Mitropoulos et al. reported high oxidative stress due to the release of nitric oxide synthase and xanthine oxidase within the dilated spermatic vein [28]. These authors suggested that peroxynitrite, which is formed from nitric oxide and superoxide, could be a causative factor for sperm dysfunction in patients with varicocele. Under normal conditions there is a positive balance between antioxidant and prooxidants in various biological systems [29]. In women with preeclampsia, which is associated with oxidative stress, the levels of erythrocyte GSH-Px and erythrocyte GSH, both major LPO scavenger systems, are increased in comparison to those women having a normal pregnancy; this increase is probably compensatory [30].

We propose that in the acute cases when the levels of ROS increase, the levels of antioxidant enzymes decrease because of high consumption. However, in chronic cases, as in preeclampsia, the increase in the levels of ROS is accompanied by an increase in AOE activity to offset the toxic actions. More studies are needed to determine the role of increased AOE activity in the pathophysiology of varicocele and male infertility.

References

1. Pryor JL, Howards SS (1987) Varıcocele. Urol Clın North Am 14:499
2. Cohen MS, Plaine L, Brown JS (1975) The role of internal spermatic vein plasma catecholamine determination in subfertile men with varicocele. Fertil Steril 26:1243
3. Goldstein M, Eid JF (1989) Elevation of intratesticular and scrotal temperature in men with varicocele. J Urol 142:743
4. Ross JA, Watson NE Jr, Jarrow JP (1994) The effect of varicoceles on testicular blood flow in man. Urology 44:535
5. Aitken RJ, Clarkson JS (1987) Cellular basis of defective sperm function and its association with the genesis of reactive oxygen species by human spermatozoa. J Reprod Fertil 81:459–469
6. Alvarez J.G, Storey BT (1989) Role of glutathione peroxidase in protecting mammalian spermatozoa from loss of motility caused by spontaneous lipid peroxidation. Gamete Res 23:77–90
7. Aitken RJ, Clarkson JS, Fishel S (1989) Generation of reactive oxygen species, lipid peroxidation and human sperm function. Biol Reprod 40:183–197
8. Alvarez JG, Touchstone CJ, Blasco L, Storey BT (1987) Spontaneous lipid peroxidation and production of hydrogen peroxide and superoxide in human spermatozoa. Superoxide dismutase as major enzyme protectant against oxygen toxicity. J Androl 8:338–348
9. Jeulin C, Soufir JC, Weber P, Laval-Martin D, Calvayrac R (1989) Catalase activity in human spermatozoa and seminal plasma. Gamete Res 24:185–196
10. Koksal IT, Usta M, Orhan I, Abbasoglu S, Kadioglu A (2003) Potential role of reactive oxygen species on testicular pathology associated with infertility. Asian J Androl 5:95–99
11. Koksal T, Erdogru T, Toptas B, Gulkesen KH, Usta M, Baykal A, Baykara M (2002) Effect of experimental varicocele in rats on testicular oxidative stress status. Andrologia 34:242–247
12. Sun Y, Oberly LW, Li Y (1988) A simple method for clinical assay of SOD. Clin Chem 34:479–500
13. Lowry O, Rosenbraugh N, Farr L, Rondell R (1951) Protein measurement with the folin-phenol reagent. J Biol Chem 183:265–275
14. Paglia DE, Valentine WN (1967) Studies on the qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 70:158–163
15. Hendin EN, Kolettis PN, Sharma RK, Thgomas AJ Jr, Agarwal A (1999) Varicocele is associated with elevated spermatozoal reactive oxygen species production and diminished seminal plasma antioxidant activity. J Urol 161:1831–1834
16. Simchowitz L, Spilberg I (1979) Chemotactic factor-induced generation of superoxide radicals by human neutrophils: Evidence for the role of sodium. J Immunol 123:2428
17. Metcalf JA, Gallin JI, Nauseef WM, Root RK (1986) Superoxide production. In: Laboratory manual of neutrophil function. Raven Press, New York, pp 109–118
18. Sigman M, Lipshultz LI, Howards SS (1991): Evaluation of the subfertile male. In: Lipshultz LI, Howards SS (eds) Infertility in the male, 2nd edn. Mosby Year Book, St. Louis, pp 179–210
19. Allen RC (1982) Biochemiexcitation: chemiluminescence and the study of biological oxygenation reactions. In: Adam W, Cilento G (eds) Chemical and biological generation of excited states. Academic Press, New York, pp 310–341
20. Ozbek E, Turkoz Y, Gokdeniz R, Davarci M (2000) Increased nitric oxide production in the internal spermatic vein of patients with varicocele. Eur Urol 37:172–175
21. Koppenol W, Moreno J, Pryor W, Ischiropoulus H, Beckman JS (1992) Peroxinitrite, a cloaked oxidant formed

by nitric oxide and superoxide. Chem Res Toxicol 5: 834–842
22. Calvin HI, Cooper GW, Wallace EW (1981) Evidence that selenium in rat sperm is associated with cysteine-rich structural proteins of the mitochondrial capsule. Gamete Res 4:139–145
23. Alkan I, Simsek F, Haklar G, Kervancıoglu E, Ozveri H, Yalcin S, Aktas A (1997) Reactive oxygen species production by the spermatozoa of patients with idiopathic infertility: Relationship to seminal plasma antioxidants. J Urol 157:140–143
24. Spector A, Wang GM, Wang RR (1993) Photochemically induced cataracts in rat lenses can be prevented by AL-3823A, a glutathione peroxidase mimic. Proc Natl Acad Sci USA 90:7845–7849
25. Taylor SD, Davenport LD, Speranza MJ, Mullenbach GT, Lynch RE (1993) Glutathione peroxidase protects cultured mammalian cells from the toxicity of adriamycin and paraquat. Arch Biochem Biophys 305:600–605
26. Cohen G, Hochstein P (1963) Glutathione peroxidase: The primary agent for the elimination of hydrogen peroxide in erythrocytes. Biochemistry 2:1420–1428
27. Maulik N, Yoshida T, Das DK (1999) Regulation of cardiomyocyte apoptosis in ischemic reperfused mouse heart by glutathione peroxidase. Mol Cell Biochem 196:13–21 Jun
28. Mitropoulos D, Deliconstantinos G, Zervas A, Villiotou V, Dimopoulos C, Stavrides J (1996) Nitric oxide synthase and xanthine oxidase activities in the spermatic vein of patients with varicocele: a potential role for nitric oxide and peroxynitrite in sperm dysfunction. J Urol 156:1952–1958
29. Dekkar GA, van Geijn HP (1996) Endothelial dysfunction in preeclampsia part 1: Primary prevention. Therapeutic perspectives. J Perinat Med 24:99–117
30. Uotila J, Tuimala R, Pyykko K (1990) Erythrocyte glutathione peroxidase activity in hypertensive complications of pregnancy. Gynecol Obstet Invest 29:559–564