Urol Res (2000) 28:357–359

ORIGINAL PAPER

H. Aksoy · Y. Aksoy · I. Özbey · I. Altuntas · F. Akçay

The relationship between varicocele and semen nitric oxide concentrations

Received: 21 February 2000 / Accepted: 20 June 2000

Abstract We investigated the relationship between seminal plasma nitric oxide (NO) concentrations and conventional semen parameters in patients with varicocele. Semen samples were obtained from infertile patients with varicocele ($n = 55$) and from normal controls ($n = 48$). The mean NO concentration in the seminal plasma of patients with varicocele was significantly higher than that of the controls ($P < 0.01$). A significant negative correlation was noted between NO and sperm motility ($r = -0.29$, $P = 0.003$), NO and sperm concentration ($r = -0.26$, $P = 0.008$) and NO and normal morphology (normal %) ($r = -0.25$, $P = 0.01$). It was concluded that increased NO production may influence sperm production, motility and morphology in patients with varicocele.

Key words Nitric oxide · Seminal plasma · Varicocele

Introduction

Nitric oxide (NO) is synthesized from L-arginine by nitric oxide synthase (NOS) in various cells. It is increasingly being recognized as having some biological effects on mammalian cells, such as vasodilation, neurotransmission, immune targeting, hormone production and gene expression [13]. Both male and female genital tracts contain several types of cells (phagocytes, endothelial cells, smooth muscle cells and fibroblasts) that are capable of generating NO [12]. Many investigators have studied the relationship between varicocele and infertility. They have reported that varicocele occurs more frequently in populations of infertile subjects and that varicocele treatment improves spermiogram parameters, subsequently increasing pregnancy rates [3, 5]. However, the pathophysiological mechanism of testicular dysfunction in patients with varicocele is not clearly understood. In relation to this, some mechanisms have been proposed, such as induction of testicular hypoxia by venous stasis [2], elevation in scrotal temperature [15] and decreased secretion of gonadotropin and androgen [17]. Sperm motility, which is maintained by high levels of adenosine triphosphate (ATP), is required for fertilization in humans. It is also known that NO can reduce ATP levels in cells by inhibiting glycolysis and the electron-transport chain [4]. Recent studies have reported that there is an excessive release of NO within the dilated spermatic vein in sub-fertile patients with varicocele [10, 14] and that NO is capable of inhibiting human sperm motility in vitro [19].

To date, we could not find any study that determined the seminal plasma NO concentration in patients with varicocele during literature review. In the present study, we aimed to measure and compare NO concentration in the seminal plasma of both healthy subjects and patients with varicocele.

Materials and methods

A total of 103 semen samples were analysed in this study. Of these, 48 samples were provided by normal subjects with a proven fertility without varicocele and 55 samples were provided by men with normal gonadotropic parameters who were admitted to the Department of Urology because of prolonged (more than 2 years) infertility. At physical examination, all patients ($n = 55$) had a unilateral varicocele that was detectable by palpation. Testicular size was estimated using an orchidometer and no testicular atrophy was found. After a 48–72 h period of sexual abstinence, semen samples were produced by masturbation and collected in sterile containers. Specimens were allowed to liquefy for 30 min at room temperature and a conventional semen analysis was performed [20] within 1 h of collection. Liquefied semen was then centrifuged at 1000 *g* for 15 min; the supernatant was aspirated and stored at −80 °C until assay. Men with leucocytospermia (more than 10^6 white blood cells/ml) were excluded from the study.

H. Aksoy (✉) · I. Altuntas · F. Akçay
Department of Biochemistry, Medical School,
Atatürk University, 25240 Erzurum, Turkey
e-mail: hulyaaksoy@superonline.com
Tel.: +90-442-2331122/2329; Fax: +90-442-2345028

Y. Aksoy · I. Özbey
Department of Urology,
Medical School, Atatürk University, Erzurum, Turkey

NO analysis

Determination of the NO radical itself is difficult because it decomposes rapidly in biological solutions into nitrite (NO_2^-) and nitrate (NO_3^-). Therefore, determination of NO_2^- and NO_3^-, is often used as a measure of NO radical production. Seminal plasma total nitrite + nitrate levels were measured using the Griess reagent, as previously described [11]. Griess reagent consists of sulphanilamide and *N*-1-naphtyl ethylenediamine. The method is based on a two-step process. The first step is the conversion of nitrate to nitrite using nitrate reductase. The second step is the addition of Griess reagent, which converts nitrite into a deep purple azo compound; photometric measurement of the absorbance at 540 nm due to this azo chromophore accurately determines the nitrite concentration (sodium nitrate is used as a standard). Protein interference is eliminated by treating the reacted samples with zinc sulphate and centrifuging them for 10 min at 2000 *g*.

Statistical analysis

The values are expressed as the mean ± standard deviation. Statistical analyses were performed by Student's unpaired *t*-test and analysis of variance (ANOVA). The relationship between NO levels in seminal plasma and semen parameters was investigated by linear regression analysis. Probability values of <0.05 were considered to be significant.

Results

The mean NO concentration in the seminal plasma of the 55 men with varicocele was significantly higher than that of the 48 controls. Analysis of conventional sperm parameters showed reduced motility and concentration in the varicocele group when compared with the control group ($P < 0.001$ and $P < 0.01$, respectively; Table 1). Sperm morphology (normal %) was lower in the varicocele group than in the control group (Table 1). Of the 55 patients with varicocele, 21 were oligozoospermic, 32 were asthenozoospermic and the remaining two were normospermic. While the 21 oligozoospermic patients (32.00 ± 12.40 μmol/l) and the 32 asthenozoospermic patients (30.15 ± 11.71 μmol/l) had a significantly higher seminal plasma NO concentration than the control group ($P < 0.001$ for all), no difference was found between these two groups.

In the total (patient + control) group, a significant negative correlation was noted between NO concentration and motility ($r = -0.29$, $P = 0.003$). The correlation between NO and sperm concentration ($r = -0.26$, $P = 0.008$) and between NO and morphology (normal %) ($r = -0.25$, $P = 0.010$) was also significant. In addition, a significant negative correlation was noted between NO and motility ($r = -0.37$, $P = 0.005$) in the varicocele group. The correlation between NO and sperm concentration ($r = -0.30$, $P = 0.021$) and between NO and morphology (normal %) ($r = -0.33$, $P = 0.013$) was also significant (Table 2).

Discussion

Vascular endothelial cells, smooth muscle cells and phagocytes are included in a growing list of cell types

Table 1 Semen profiles and seminal plasma nitric oxide (NO) concentrations of normal controls and patients with varicocele

	Volume (ml)		Sperm concentration (10^6/ml)		Motility (%)		Morphology (normal %)		NO (μmol/l)	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Controls (n = 48)	3.58 ± 0.79	2–5	57.29 ± 19.97**	15–90	61.14 ± 14.41*	40–90	67.18 ± 10.86*	50–90	20.34 ± 9.23**	8.35–50.30
Patients with varicocele (n = 55)	3.30 ± 0.81	2–5	41.25 ± 30.51	2–90	46.81 ± 20.48	10–90	55.81 ± 16.40	10–80	26.66 ± 12.11	9.33–64.54

*$P < 0.001$, **$P < 0.01$

Table 2 Correlation coefficients of seminal plasma nitric oxide (NO) and the other parameters in groups

	Total group (patients + controls)		Patient group only	
	r	*P*	*r*	*P*
NO–motility	−0.29	0.003	−0.37	0.005
NO–morphology	−0.25	0.010	−0.33	0.013
NO–volume	−0.19	0.061	−0.18	0.190
NO–sperm concentration	−0.26	0.008	−0.30	0.021

that generate NO, which is derived from the oxidative deamination of L-arginine by the enzyme NOS [7]. Several isoforms of NOS exist: constitutively active forms have been described in neuronal and endothelial cells (Ca^{2+}-dependent), and inducible isoforms (Ca^{2+}-independent) are found mainly in phagocytes and smooth muscle cells [9]. Ca^{2+}-dependent NOS activity is reported to be localized to the vas deferens, prostate, seminal vesicles and corpus cavernosum [1]. NO has been implicated in protection against reactive oxygen species (ROS)-mediated damage; however, in situations of inappropriate NOS regulation, NO may exacerbate ROS-mediated pathology (8). In one study [10], NOS activity in the varicocele serum was found to be eightfold greater than the NOS contained in the corresponding peripheral serum. In addition, a 2- to 25-fold increase in the rate of NO production in the varicocele vein compared with the corresponding peripheral vein was also observed [10, 14]. These studies revealed that the seminal plasma NO concentration was higher in patients with varicocele than in the controls. NO, being a lipophilic molecule, may also diffuse out of the varicocele veins or may be produced by sex tissues (i.e. prostate) and exert cytotoxic effects on neighbouring sperm cells, resulting in their damage [10].

The mechanism(s) underlying the effect of NO on sperm motility is currently under investigation. $NO^{\bullet}$ and superoxide radical (O_2^-) rapidly react together to form peroxynitrite, which is an important mediator of free radical toxicity with strong oxidizing properties towards biological molecules, including protein and non-protein sulphydrates, DNA and membrane phospholipids [18]. Thus, peroxynitrite may be responsible for sperm dysfunction in varicocele patients, causing lipid peroxidation with subsequent changes of the physicochemical characteristics (e.g. membrane fluidity of plasma membranes in sperm cells). Weinberg et al. [19] found that NO decreased sperm motility in vitro, possibly by a mechanism involving the inhibition of cellular respiration, resulting in the depletion of sperm ATP.

The detrimental effect of varicocele on spermatogenesis in the sub-fertile male is most often reflected by abnormal seminal variables. Infertile men with varicocele may have abnormal semen quality, demonstrating a reduced sperm count, decreased motility and/or abnormal morphology [6, 16]. Furthermore, Yamamoto et al. [21] observed that varicocele repair led to a significant improvement in sperm and total motile counts.

The present study found a negative correlation between seminal plasma NO concentration and sperm motility in both the total (patient + control) and the patient groups, indicating the possible role of NO in sperm motility. Increased NO concentration may be one of the causes of damage to sperm in patients with varicocele. Detailed studies are required to examine this theory and to identify the condition for oligozoospermic patients without varicocele.

References

1. Burnett AL, Ricker D, Chamness SL (1995) Localization of nitric oxide synthase in the reproductive organs of the male rat. Biol Reprod 52: 1
2. Chakraborty J, Hikim AP, Jhunjhunwala JS (1985) Stagnation of blood in the microcirculatory vessels in the testes of men with varicocele. J Androl 6: 117
3. Cockett ATK, Urry RL, Dougherty KA (1979) The varicocele and semen characteristics. J Urol 121: 435
4. Dimmeler S, Lottspeich F, Brüne B (1992) Nitric oxide causes ADP-ribosylation and inhibition of glyceraldehyde-3-phosphate dehydrogenase. J Biol Chem 267: 16771
5. Greenberg SH (1977) Varicocele and male infertility. Fertil Steril 28: 669
6. Greenberg SH, Lipshultz LI, Wein AJ (1978) Experience with 425 subfertile male patients. J Urol 119: 507
7. Griffith OW, Stuerh DJ (1995) Nitric oxide synthase: properties and catalytic mechanism. Ann Rev Physiol 57: 707
8. Kanner J, Hare ST, Granit R (1991) Nitric oxide as an antioxidant. Arch Biochem Biophys 289: 130
9. Knowles RG, Moncada S (1994) Nitric oxide synthases in mammals (review). Biochem J 298: 249
10. Mitropolous D, Deliconstantinous G, Zervas A, Villitou V, Dimopoulos C, Stavrides J (1996) Nitric oxide synthase and xanthine oxidase activity in the spermatic vein of patients with varicocele: a potential role for nitric oxide and peroxynitrite in sperm dysfunction. J Urol 156: 1952
11. Moshage H, Kok B, Huizenga JR, Jansen PLM (1995) Nitrite and nitrate determinations in plasma: a critical evaluation. Clin Chem 41: 892
12. Nathan C (1992) Nitric oxide as a secretory product of mammalian cells. FASEB J 6: 3051
13. O'Bryan MK, Zini A, Yan Cheng C, Schlegel PN (1998) Human sperm endothelial nitric oxide synthase expression: correlation with sperm motility. Fertil Steril 70: 1143
14. Ozbek E, Turkoz Y, Gokdeniz R, Davarci M (2000) Increased nitric oxide production in the spermatic vein of patients with varicocele. Eur Urol 37: 172
15. Ross JA, Watson NE, Jarrow JP (1994) The effect of varicoceles on testicular blood flow in man. Urology 44: 535
16. Saypol DC (1981) Varicocele. J Androl 2: 61
17. Swerdloff RS, Walsh PC (1975) Pituitary and gonadal hormone in patients with varicocele. Fertil Steril 26: 1006
18. Van der Vliet A, Eiserich JP, O'Neil CA (1995) Tyrosine modification by reactive nitrogen species: a closer look. Arch Biochem Biophys 319: 341
19. Weinberg JB, Doty E, Bonaventure J, Haney AF (1995) Nitric oxide inhibition of human sperm motility. Fertil Steril 64: 408
20. World Health Organization (1992) Laboratory manual for the examination of human semen and sperm–cervical mucus interaction, 3rd edn. Cambridge University Press, Cambridge
21. Yamamoto M, Hibi H, Hirata Y, Miyake K, Ishigaki T (1996) Effect of varicocelectomy on sperm parameters and pregnancy rate in patients with subclinical varicocele: a randomized prospective controlled study. J Urol 155: 1636