Seminal plasma can be a predictive factor for male infertility

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Seminal plasma from ejaculates of 10 healthy, fertile volunteers and 63 infertile males was analysed for superoxide dismutase (SOD) and xanthine oxidase (XO) activities using a chemiluminometer. There was no statistically significant difference in the activity of either enzyme between control and infertile populations (113 ± 74 IU/ml for SOD and 1.17 ± 0.52 IU/ml for XO) in samples from normozoospermic ejaculates. Sperm progressive motility was positively correlated with SOD activity in seminal plasma of corresponding ejaculates (P < 0.05) and negatively with XO activity (P < 0.001). An `oxido-sensitive' index was defined as the SOD/XO ratio and was found to be inversely related to sperm progressive motility samples (P < 0.01). Analysing this index among all tested samples of semen including those with pathological spermograms, as well as normospermic (N) samples we found statistically significant (elevated) differences in oligoaesthenoteratospermia (OAT) samples in comparison with N (P < 0.05); OAT samples were also significantly different from oligospermic (O) and oligoteratospermic (OT) samples (P < 0.05). This suggests that the `oxido-sensitive' index of seminal plasma may be a simple diagnostic factor, useful in the determination of male infertility.

Key words: antioxidants/infertility/oxidative stress

Introduction

The influence of toxic oxygen metabolites on the quality of spermatozoa has long been recognized (McLeod, 1943; Todic, 1947). Methodological progress has led to the classification of these metabolites as those of low (superoxide anion) or high oxidizing potentials (hydroxyl radical, singlet oxygen). There is extensive literature concerning the active secretion of oxygen free radicals by spermatozoa (Holland et al., 1982; Alvarez and Storey, 1984). More recently, more precise measurements have been used to determine superoxide release by spermatozoa (Alvarez et al., 1987; Miesel et al., 1993), and its role in sperm/oocyte interaction and fusion has been intensively discussed (Aitken et al., 1989a; Miesel et al., 1993).

Further attempts have been made to correlate the excessive production of reactive oxygen species (ROS) with infertility (Aitken et al., 1989a,b; Lamirande and Gagnon, 1992). However, the identification of other related factors suggests that the mechanism of oxidative stress is rather complex. Firstly, the antioxidant systems (which in semen act as ROS-counteracting substances) were examined including superoxide dismutase (SOD) (Mennen and Jones, 1980), the glutathione peroxidase/glutathione reductase system (Alvarez and Storey, 1989), and the catalase system (Jeulin et al., 1989). The dominating views stressed the importance of intracellular antioxidant systems (Kobayashi et al., 1991; Lamirande and Gagnon, 1993) rather than those present in seminal plasma, and related low sperm motility to the deficient intracellular activity of SOD. Secondly, other reports documented additional sources of ROS production within semen e.g. leukocytes or atypical sperm (Kessopoulou et al., 1992; Mazzilli et al., 1994); those cells can be stimulated to enhance ROS generation by cytokines or growth factors (Weese et al., 1993; Buch et al., 1994). However, seminal plasma seems to have a stronger capacity to maintain a relatively neutral and protective environment for sperm function in comparison with the intracellular compartments, since as well as glutathione and SOD, seminal plasma contains vitamins E and C, and taurine/hypotaurine (Alvarez and Storey, 1983; Halliwell and Gutteridge, 1989). Recently, low molecular weight antioxidants, ascorbic acid and urate were found to be present in semen (Thiele et al., 1995). Out of the growing numbers of substances which appear to participate in the `oxidative stress phenomenon', we have selected only two substances for this study in an attempt to determine the `oxidative sensitivity' of semen in a simplified form.

Thus we have determined the activity of SOD (antioxidant) and xanthine oxidase (XO; a potential source of ROS generation) which, in our view, may critically influence the oxidative status. Seminal plasma samples of healthy and infertile males were examined and the values obtained were correlated with sperm motility; an `oxido-sensitive' index was then created.

Materials and methods

Chemicals

Unless otherwise stated, all the reagents were purchased from Sigma (Munich, Germany).

Sperm preparations

Ejaculates were obtained through masturbation from 10 healthy volunteers (control individuals with proven fertility) and 63 infertile patients who were recruited from an andrology out-patient clinic in Poznan, Poland. Semen was allowed to liquefy for 30 min at room
temperatures were observed in asthenozoospermic (A; 271 ± 172 IU/ml) samples although not statistically different from normospermia. Leukocyte numbers in ejaculated samples were determined by May—Grunwald—Giemsa staining although the concentration of these cells was never >2.5x10^6 cells/ml of seminal plasma.

Progressive motility of each sample was assessed in a fresh drop of semen by two independent observers and the mean of these observations (percentage of motile spermatozoa after scoring at least 200 spermatozoa) was calculated.

Among infertile samples, 23 were classified as oligozoospermic, three as asthenozoospermic, six as oligoteratozoospermic, five as asthenoteratozoospermic, and 26 as oligoasthenoteratozoospermic. Cell-free seminal plasma fractions were obtained by semen centrifugation at 600 g for 15 min.

**Superoxide dismutase**

SOD activity was quantified by chemiluminescence using the xanthine/xanthine oxidase lucigenin assay. A final volume of 1 ml contained the following components: 100 μl seminal plasma, 10 mM diethylenthinitrimatepenta-acetic acid (DTPA), 100 mM lucigenin, 0.009 IU/ml XO and 50 μM xanthine in 50 mM HEPES, pH 7.4.

The reaction was started by the addition of xanthine and the resulting photon emission was recorded in a Berthold LB 9505 C luminometer at 25°C. Bovine Cu-Zn SOD was used for calibration. One unit represents the concentration of SOD required to inhibit the release of superoxide by 50% and equals 5 mM Cu. SOD specific activity was confirmed using an enzyme inhibitor (diethylthiocarbamate), as described by Miesel et al. (1993).

**Xanthine oxidase**

XO activity was determined by chemiluminescence. A final volume of 1 ml contained the following: 100 μl seminal plasma, 100 mM lucigenin, 10 μM DTPA in 100 mM HEPES, pH 7.4. The ability to generate superoxide was monitored in a Berthold LB 9505 C luminometer at 25°C over 40 min and integrated. Chromatographically-purified XO from buttermilk was used for calibration. One unit of XO converts 1 μM of xanthine to uric acid per 1 min. XO specific activity was confirmed by using the enzyme inhibitor, allopurinol, as previously described by Miesel et al. (1994).

**Statistical analysis**

Statistical evaluation was performed using linear regression analysis (correlations were calculated by the Spearman rank test). Values were given as mean ± SD and were analysed by one-way analysis of variance (ANOVA) adjusting by least significant determination (LSD). A probability value of P < 0.05 was considered to be significant. The 'oxido-sensitive' index was calculated by computing the ratio of superoxide dismutase (SOD) and xanthine oxidase (XO) activity values, obtained in seminal plasma samples from males with different spermograms. Statistically significant differences (P < 0.05) were observed in values obtained from oligoasthenoteratozoospermia (OAT) versus normozoospermia (N), OAT versus oligozoospermia (O) and OAT versus oligoteratozoospermia (OT). A = asthenozoospermia; AT = asthenoteratozoospermia.

**Results**

Seminal plasma from fertile, normozoospermic patients (n = 10) gave a mean SOD value (113 ± 74 IU/ml) which was not significantly different from the value obtained in a group of infertile males (183 ± 123 IU/ml). However, markedly higher (although not statistically different from normospermia) SOD values were observed in asthenozoospermic (A; 271 ± 172 IU/ml), oligoteratozoospermic (OT; 229 ± 124 IU/ml) and asthenoteratozoospermic (AT; 172 ± 144 IU/ml) subgroups.

In samples of infertile individuals, seminal plasma SOD activity showed a statistically significant correlation (P < 0.05) with the sperm progressive motility (r = 0.392).

Similarly, XO activity from seminal plasma of infertile males (1.13 ± 0.50 IU/I) did not differ significantly from the values observed in the control, fertile population (1.17 ± 0.52 IU/I) although XO reached its highest value (1.43 ± 0.38 IU/I) in the OT group.

As a consequence of the observed reciprocal correlations between these two semen components and sperm progressive motility, we created an 'oxido-sensitive' index which is defined as the ratio of SOD/XO activity values, obtained in seminal plasma samples from males with different spermograms. The only positive correlation (P < 0.01) was obtained for normozoospermic, fertile individuals (r = 0.569).

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Analysis of all calculated 'oxido-sensitive' indices (Figure 1) from seminal plasma samples (with pathological spermograms), we found that the OAT samples had significantly higher values than normospermia controls (P < 0.05); these values were also significantly different from oligozoospermic and oligoteratozoospermic samples (P < 0.05).

**Discussion**

We investigated SOD and XO activity in seminal plasma from fertile, healthy volunteers and in samples from an infertile male population. As previously observed by Mennella and Jones (1980) and Nissen and Kreysel (1983), there was no
correlation between SOD values in semen and the presence of a particular pathological spermogram. Moreover, fertile and infertile populations did not differ significantly in the activity of either SOD or XO in seminal plasma, although XO has been suggested as a potentially important substance participating in 'oxidative stress' by generating a cytotoxic effect (Aiikten et al., 1993).

Contrary to other reports, where the only intracellular SOD activity correlated positively with sperm progressive motility (Kobayashi et al., 1991) we have found a positive correlation between SOD seminal plasma activity and sperm motility in an infertile group ($P < 0.05$). However, there was a much stronger negative correlation between XO activity in seminal plasma and sperm progressive motility from the same ejaculates ($P < 0.001$). This has led us to consider that perhaps not just SOD and XO activities (alone) in seminal plasma are important. However, since their activities were opposingly correlated with sperm progressive motility, their ratio could be a more valuable indicator than either compound independently.

Indeed, the relationship between the SOD/XO 'oxido-sensitive' index and progressive motility revealed a significant positive correlation with sperm progressive motility from normozoospermic, fertile individuals ($P < 0.01$; $r = 0.569$). Analysing such indices separately for particular pathological spermograms, we found significantly elevated values for OAT seminal plasma samples ($P < 0.05$), which were not only higher in comparison to normospermia but also to oligospermic and oligoteratospermic patients. This created the interesting situation where the 'oxido-sensitive' factor increased in parallel with the more complex sperm pathology from oligospermia through oligoteratospermia, reaching its maximum with the most defective spermogram which marks OAT. This may mean that in the absence of appropriate compensation by the antioxidant, excessive ROS production is reflected in the deteriorating condition of the semen sample. A group of oligospermic patients is particularly interesting when we recall an earlier report describing the intensive ROS production by defective spermatozoa in this pathological (O) specimen (Aiikten et al., 1989b). Defective semen samples of O, OT and OAT subgroups can be clearly distinguished from A + AT subgroups in which the 'oxido-sensitive' index did not significantly depart from normospermic values. This phenomenon may support different aetiologies based upon the asthenospermic versus oligo- (oligoterato) basis of pathological spermograms.

Until now, insufficient SOD protection was thought to be a major factor which could lead to immotility or structural changes in motility apparatus (Lamirande and Gagnon, 1992). Subsequently, catalase-deficient seminal plasma samples were also postulated to be associated with asthenozoospermic, infertile men (Jeulin et al., 1989). Now, it appears that XO is a potential source which may augment the ROS production can be at least equally important for extending the 'oxidative stress' situation.

Another key question that has to be addressed is the presence of leukocyte subsets in pathological ejaculates (specifically in oligozoospermia where non-spermatozoal cells are often encountered). No one can deny that leukocytes have a stronger capacity for ROS production than spermatozoa (Tomlinson and Barratt, 1993; Aiikten et al., 1995; Rajasekaran et al., 1995; Zalata et al., 1995) and we may expect that such non-spermatozoal contamination might be extremely deleterious to sperm physiology (Aiikten et al., 1995; Zalata et al., 1995). That such situation does not take place (Tomlinson and Barratt, 1993) would be proof of the strong antioxidant properties of seminal plasma and the importance of its capacity for biological protection (Kovalski et al., 1992) up to a certain threshold (Aiikten et al., 1995). As suggested in this study, the SOD/XO 'oxido-sensitive' index may be a simple but helpful factor to indicate the relationship between the source of ROS and scavenging ability of semen, regardless of whether leukocytes or defective spermatozoa are the predominant oxygen species producers (in oligozoospermia). Further studies are required to verify the potential of this factor in evaluation of male infertility.

References


Received on November 9, 1995; accepted on April 24, 1996