Asian Journal of Andrology, ISSN 1008-682X
by Asian Society of Andrology and
Shanghai Institute of Materia Medica, Chinese Academy of Sciences
294 Taiyuan Road, Shanghai 200031, China
Fax: +86-21-6471 9309 Tel: +86-21-6431 1833-207
Email: aja@mail.shcnc.ac.cn

Effect of vitamin E on human sperm motility and lipid peroxidation *in vitro*

Anil Verma[1], K.C. Kanwar

Department of Biophysics, Panjab University, Chandigarh 160014, India

Asian J Androl 1999 Sep; 1: 151-154

Keywords: reactive oxygen species; vitamin E; sperm motility; lipid peroxidation

Abstract

Aim: To assess the protective efficacy of vitamin E to counteract the reactive oxygen species (ROS) mediated damage on sperm motility, viability and lipid peroxidation. **Methods:** Human semen samples were obtained from the local hospital. The split seminal fractions freed of seminal plasma were reconstituted in Ringer-Tyrode and subjected to varied vitamin E concentrations (0.1-2 mmol/L). **Results:** Dose-dependent improvement in both motility and viability accompanied by concomitant decrease in malondialdehyde (MDA—an end product of lipid peroxidation) following vitamin E supplementation was noticed. **Conclusion:** Vitamin E protects against the ROS mediated damage on spermatozoa. Vitamin E supplementation could be of clinical importance for prolonged spermatozoal storage whenever needed.

1 Introduction

Reactive oxygen species (ROS) such as the superoxide anion ($O_2^{\cdot -}$), the hydroxyl radical (·OH) and hypochlorite radical (·OHCl) produced by the spermatozoa and the contaminating leucocytes in the seminal fluid adversely affect sperm motility[1,2] and also impair their fertilizability[3].

The damage by ROS gets exacerbated when sperm are washed and freed of seminal plasma[3] since ROS constantly produced by the spermatozoa and the contaminating leucocytes are not neutralized by the antioxidants present in the seminal plasma[4,5].

In assisted reproduction, poor sperm motility rather than a low sperm count in the semen is most often the cause of male infertility[6]. An antioxidant that reduces oxidative stress and improves sperm motility could be of clinical significance in the management of male infertility[7]. The present in vitro studies are aimed at finding out the efficacy of vitamin E—a biological antioxidant[8]—in reversing the free radical mediated oxidative damage on sperm motility, viability and lipid peroxidation.

2 Materials and methods

Human sperm samples (n=8) from healthy donors were obtained from the local hospital in accordance with the Helsinki Declaration and only those having motility higher than 60% and count over 20 millions/mL were used for the present studies[9]. Each human ejaculate was split into four equal fractions. The split seminal fractions were centrifuged at 1000×g and the supernatent (plasma) discarded. Thereafter, these were reconstituted in Ringer-Tyrode (NaCl 0.8 g, KCl 0.02 g, $CaCl_2$ 0.02 g, $NaHCO_3$ 0.1 g, NaH_2PO_4 0.005 g, $MgCl_2$ 0.01 g, glucose 0.1 g, Hepes 0.5 g, and 100 mL twice-distilled water). Ringer-Tyrode (RT fraction without vitamin E) served as control. The experimental fractions comprised RT supplemented with three different concentrations of vitamin E (alpha-tocopherol acetate, E. Merck, India, 0.1, 1.0 and 2.0

mmol/L).Vitamin E was dissolved in ethanol and an emulsion was formed by vortex mixing in RT medium before addition to the spermatozoa. The final ethanol concentration was <2% by volume[10].

The number of motile and immotile spermatozoa from control as well as experimental fractions were counted at room temperature (30℃±2℃) at different time intervals ranging from 0.5 h to 6 h. The viability of spermatozoa was also evaluated using eosin[11].

MDA formation was estimated by the reaction of thiobarbituric acid (TBA) with lipid peroxides according to the method of Beuge & Aust[12]. The data were evaluated statistically by unpaired Student's *t* test. A probability value of $P<0.05$ was considered significant.

3 Results

As compared with the control, a significant dose-dependent improvement in sperm motility was noticed from 1 h onwards in the experimental fractions supplemented with 1.0 mmol/L and 2.0 mmol/L concentrations of vitamin E; sperm motility was maximum after a 6-h incubation in 2.0 mmol/L vitamin E where it approximated 52.9% ($P<0.01$) higher in comparison with the control (Figure 1A).

Likewise percent spermatozoal viability also improved significantly from 2 h onwards in 1.0 and 2.0 mmol/L vitamin E. Increase in viability vs the control was the highest at 2.0 mmol/L concentration where it approximated 62.6% ($P<0.01$) following a 6-h incubation (Figure 1B).

As compared with the control, statistically significant decrease in MDA formation was noticeable only after 4 h and 6 h of incubation of the experimental sperm fraction supplemented with 2.0 mmol/L vitamin E. MDA formation was maximally reduced (30%; $P<0.01$) following 4 h of incubation in 2.0 mmol/L vitamin E sperm fraction (Figure 1C).

Figure 1. Percentage motility (A), percent viability of human spermatozoa (B) and MDA formation (C, nmol/10^8 sperm in human spermatozoa suspended in Ringer-Tyrode (RT) and in different vitamin E concentrations at various time intervals. n=8. mean±s. $^bP<0.05$; $^cP<0.01$ vs the spermatozoa suspended in RT only.

4 Discussion

A gradual decrease in spermatozoal motility and viability with concomitant increase in MDA in RT from 0.5 h to 6 h observed during the course of present study have been attributed to oxidative stress to which spermatozoa are subjected to during storage[13,14]. Human spermatozoa contain little antioxidant enzymes (viz. catalase, glutathione peroxidase, and superoxide dismutase) to counteract fully the oxidative stress[15]. Oxygen free radicals generated by the spermatozoa and the contaminating leucocytes produce a fall in intracellular ATP levels which adversely affect the sperm motility and also initiate lipid peroxidation in the polyunsaturated fatty acid rich sperm plasma membrane[13,16,17] culminating in increased cell permeability, enzyme inactivation and production of spermicidal end products[14,18,19]. Lipid peroxidation also impairs fertilizing potential of sperm owing to loss of membrane fluidity[20] or to selective inactivation of some of the biochemical pathways leading to acrosomal reaction[21]. The present observations record a significant improvement in sperm motility and viability following vitamin E treatment in vitro. Higher MDA levels (representative of lipid peroxidation) are recorded in spermatozoal fractions suspended in RT only. Following vitamin E treatment, MDA levels in the experimental fractions are lowered. These findings are supported by earlier workers who have reported improved testicular histoarchitecture and sperm quality following vitamin E dietary supplementation in the different animal species[8,22,23]. Vitamin E, a chain breaking antioxidant, not only scavenges oxygen radicals from within the membrane but also intercepts peroxyl and alkoxyl radicals which are generated during the conversion of lipid hydroperoxides that fuel the peroxidative chain reaction thereby preventing this damaging process from propagating through plasma membrane[3].

5 Acknowledgment

One of the authors (A. Verma) is thankful to the Indian Council of Medical Research, New Delhi, India, for the financial assistance.

References

[1] Plante M, de Lamirande E, Gagnon C. Reactive oxygen species released by activated neutrophils, but not by deficient spermatozoa, are sufficient to affect sperm motility. Fertil Steril 1994 ; 62: 387-93.
[2] Aitken RJ. A free radical theory of male infertility. Reprod Fertil Dev 1994 ; 6: 19-24.
[3] Aitken RJ, Clarkson JS. Significance of reactive oxygen species and antioxidants in defining the efficacy of sperm preparation techniques. J Androl 1988; 9: 367-76.
[4] Jones R, Mann T, Sherins R. Peroxidative breakdown of phospholipids in human spermatozoa: spermicidal properties of fatty acid peroxides and protective action of seminal plasma. Fertil Steril 1979 ; 31: 531-7.
[5] Maruyama DK, Hale RW, Rogers BJ. Effects of white blood cells on the in vitro penetration of zona-free hamster eggs by human spermatozoa. J Androl 1985 ; 6: 127-35.
[6] Cai XQ, Marrik JJ. Improving penetrating capacity of spermatozoa with poor motility by addition of caffeine at co-incubation with zona-free hamster ova. Fertil Steril 1989 ; 51: 719-23.
[7] Aitken RJ. Free radicals, lipid peroxidation and sperm function. Reprod Fertil Dev 1995 ; 7: 659-68.
[8] Vezina D, Mauffette F, Roberts KP, Bleau G. Selenium- vitamin E supplementation in infertile men. Effects on semen parameters and micronutrient levels and distribution. Biol Trace Element Res 1996; 53: 665-83.
[9] World Health Organization. Laboratory manual for the examination of human semen and semen-cervical mucus interaction. 3rd ed. Cambridge: Cambridge University Press; 1992.
[10] Baker HW, Brindle J, Irvine DS, Aitken RJ. Protective effect of antioxidants on the impairment of sperm motility by activated polymorphonuclear leucocytes. Fertil Steril 1996; 65: 411-9.
[11] Eliasson R. Supravital staining of human spermatozoa. Fertil Steril 1977; 28: 1257.
[12] Beuge JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 1978; 52: 302-10.
[13] Aitken RJ, Buckingham D, Harkiss D. Use of xanthine oxidase system to investigate the cytotoxic effects of reactive oxygen species on human spermatozoa. J Reprod Fertil 1993; 97: 441-50.
[14] Halliwell B. Free radicals, antioxidants and human disease: curiosity, cause or consequence? Lancet 1994; 344: 721-4.
[15] Mann T, Mann C. Male reproductive function and semen. Berlin, Springer Verlag, 1981.
[16] de Lamirande E, Gagnon C. Reactive oxygen species and human spermatozoa. I. Effects on the motility of intact spermatozoa and on sperm axonemes. J Androl 1992; 13: 368-78.
[17] de Lamirande E, Gagnon C. Reactive oxygen species and human spermatozoa. II. Depletion of adenosine triphospate plays an important role in the inhibition of sperm motility. J Androl 1992; 13: 379-86.
[18] Selly ML, Lacey MJ, Bartlett MR, Copeland CM, Ardlie NG. Content of significant amounts of cytotoxic endproducts of lipid peroxidation in human semen. J Reprod Fertil 1991; 92: 291-8.
[19] Cummins JM, Jequier AM, Kan R. Molecular biology of human male infertility: links with ageing, mitochondrial genetics and oxidative stress. Mol Reprod Dev 1994; 37: 345-62.
[20] Ohyashiki T, Ohtasuka T, Mohri T. Increase of molecular rigidity of the protein conformation in the intestinal brush-border membranes by lipid peroxidation. Biochim Biophys Acta 1988; 939: 383-92.
[21] De Jonge CJ, Han HL, Lawrie HL, Mack SR, Zaneveld LJD. Modulation of the human sperm acrosome reaction by effectors of adenylate cyclase/cyclic AMP second-messenger system. J Exp Biol 1991; 258: 113-25.
[22] Haidl G, Schill WB. Guidelines for drug treatment of male infertility. Drugs 1991; 41: 60-8.
[23] Kitabchi AE. Hormonal status in vitamin E deficiency : A comprehensive treatise. New York: Marcel Dekker Inc; 1980. p 348-71.

home

Correspondence to Dr. K.C. Kanwar.
Tel: +91-172-54 1441, ext 1364 Fax: +91-172-77 8269
E-mail: staff%zoo@puniv.chd.nic.in
[1]Present address: Department of Biophysics, Postgraduate Institute of Medical Education and Research, Chandigarh 160012, India.
Received 1999-05-19 Accepted 1999-08-19