Effect of astaxanthin on human sperm capacitation.


Abstract

In order to be able to fertilize oocytes, human sperm must undergo a series of morphological and structural alterations, known as capacitation. It has been shown that the production of endogenous sperm reactive oxygen species (ROS) plays a key role in causing cells to undergo a massive acrosome reaction (AR). Astaxanthin (Asta), a photo-protective red pigment belonging to the carotenoid family, is recognized as having anti-oxidant, anti-cancer, anti-diabetic and anti-inflammatory properties and is present in many dietary supplements. This study evaluates the effect of Asta in a capacitating buffer which induces low ROS production and low percentages of acrosome-reacted cells (ARC). Sperm cells were incubated in the presence or absence of increasing concentrations of Asta or diamide (Diam) and analyzed for their ROS production, Tyr-phosphorylation (Tyr-P) pattern and percentages of ARC and non-viable cells (NVC). Results show that Asta ameliorated both sperm head Tyr-P and ARC values without affecting the ROS generation curve, whereas Diam succeeded in enhancing the Tyr-P level but only of the flagellum without increasing ARC values. It is suggested that Asta can be inserted in the membrane and therefore create capacitation-like membrane alteration which allow Tyr-P of the head. Once this has occurred, AR can take place and involves a higher numbers of cells.
Effect of astaxanthin on human sperm capacitation: in vivo and in vitro studies

Gabriella Donà 1, Chiara Sabbadin 1, Claudia Carraro 2, Eugenio Ragazzi 3, Decio Armanini 1, Guido Ambrosini 2, Alessandra Andrisani 2 and Luciana Bordin 4

1 Department of Medical – Endocrinology, University of Padova.
2 Department of Pharmacological and Pharmacological Sciences, University of Padova.
3 Department of Women’s and Child’s Health, University of Padova.
4 Department of Molecular Medicine-Biological Chemistry, University of Padova.

As far as sperm quality is concerned, many efforts in the last two decades have been devoted to improve human healthy lifestyle, regarding food, sport and any other habits just to maintain the best conditions for human life and reproduction. The growing interest towards male infertility mirrors a serious concern since it is becoming a global public health issue affecting the 15% of all reproductive age couples, with the decreased semen quality responsible for 25% of cases of infertility (1).

Not yet established the etiology, it has been proposed that the main spermatogenic damage can be ascribed to physiological, infectious (2), environmental and genetic factors, including oxidative stress (3,4). OS is considered as one of the main responsible of the drop of sperm capacitating ability, due to the formation of exogenous as well as endogenous reactive oxygen species (ROS), which induce DNA fragmentation and cause male sub-fertility, together with protein degradation and membrane denaturation by lipid peroxidation (5, 6). In addition to OS, reduced spermogramm, which lowers the number of sperm produced in the testis, low motility and membrane weakening, in absence of any other patho-physiological disorders, co-operate to lower sperm quality and, in turn, improve male infertility. In the attempt to address the wide range of possible alteration leading to the poor sperm quality, we chose a commercially available formulation (Fertylor15 - FERpharma s.r.l. Milan, Italy) including many factors known for potential specific beneficial effects on human semen parameters. In this formulation (7) should be considered for strengthening cell membrane and reducing the DNA breaks (10,11); arginine and folic acid for helping spermatogenesis (12); and CoQ and selenium for improving vitality (11) (9). In addition, to counteract oxidative stress injuries, conditions lessens the number of sperm produced in the testis, low motility and membrane weakening, in absence of any other patho-physiological disorders, co-operate to lower sperm quality and, in turn, improve male infertility. In the attempt to address the wide range of possible alteration leading to the poor sperm quality, we chose a commercially available formulation (Fertylor15 - FERpharma s.r.l. Milan, Italy) including many factors known for potential specific beneficial effects on human semen parameters. In this formulation (7) should be considered for strengthening cell membrane and reducing the DNA breaks (10,11); arginine and folic acid for helping spermatogenesis (12); and CoQ and selenium for improving vitality (11). In addition, to counteract oxidative stress injuries, conditions lessens the number of sperm produced in the testis, low motility and membrane weakening, in absence of any other patho-physiological disorders, co-operate to lower sperm quality and, in turn, improve male infertility.

Methods

Study population: We recruited 51 male patients with couples’ infertility problems treated at the Centre of Assisted Reproduction - U.O.C. Obstetrics and Gynecology Clinic - Padua. The subjects, presenting the inclusion/exclusion characteristics and who refused their written consent to participation at the study, were sequentially recruited. The study was approved by Padua Ethics Committee for Research and Clinical Trials.

Inclusion criteria: Age from 25 to 50 years. Seminal parameters: Seminal volume >1.5 ml; Total sperm number >39 mil; Concentration >15 mil/ml; Total motility >30%; Vitality >58%; Normal morphology >4%; pH 7.2; Leucocytes <1.0/ml.

Exclusion criteria: Subjects presenting genitourinary pathologies, cancer, infectious, autoimmune or endocrinological diseases were excluded from the study. Subjects, before and during therapy, should not take drugs that affect sperm function.

In order to induce AR, aliquots of each sample were incubated for 30 min at 37°C. In the presence of 1 μM of calcium ionophore, a calcium-dependent step was observed. Following this, spermatozoa were considered as cells showing AR. DNA breaks (10, 11) were evaluated by quantifying the percentage of cells showing CTB-FITC labelling in the head. Apoptotic cells were detected by cytochemistry for evaluation of acrosome-intact sperm using the animal model for the study of human sperm capacitation. Immunocytochemistry images of sperm from the control and Asta samples were taken to evaluate the changes in membrane rafts formation.

In order to assess the effect of treatment on sperm capacitation, semen analysis was performed. The samples were obtained from 27 patients included in the present study of Asta for the in vitro study.

Biochemical investigations: seminal fluid remaining after the execution of the basal and follow-up semen analysis was transferred to Department of Molecular Medicine - Biological Chemistry. Sperm were separated by centrifugation or discontinuous gradient, washed with Pure Sperm Wash, incubated for up to 100 min in capacitating conditions and then analyzed for the evaluation of two fundamental processes for the physiology of the sperm cell: capacitation and acrosome reaction.

• Acrosome reactions and viability: was evaluated with acrosome-specific FITC-labeled penaeid (Arachosia hyas) antibody (FITC-PNA) in conjunction with DNA-specific fluorescein peroxidase isoperoxidase (PI) as a viability test by immunofluorescence cytochemistry. Only sperm cells showing evenly distributed fluorescence over the acrosomal region will be considered acrosome-intact.

• Evaluation of capacitation by membrane rafts localization: We have previously showed that when correct capacitation occurs membrane micro-domains, called rafts, have to shift from the sperm tail to the head. This relocation was monitored by a membrane raft marker, OME, which can be stained by the cholera toxin B subunit (CTB)-FITC (18, 19). Cells were analyzed by immunofluorescence cytochemistry. In vivo study

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Conclusions

Asta is configured as a molecule functional to the improvement of the fertilizing capacity both in couples with idiopathic infertility, that in infertile couples in which the male factor is not causal. As suggested by our in vitro findings, the addition of Asta in capacitation buffers could increase the possibility of success in Medically Assisted Procreation treatment.

References

Astaxanthin Prevents Human Papillomavirus L1 Protein Binding in Human Sperm Membranes

Gabriella Donà 1, Alessandra Andrisani 2, Elena Tibaldi 1, Anna Maria Brunati 1, Chiara Sabbadin 3, Decio Armanini 3, Guido Ambrosini 2, Eugenio Ragazzi 4 and Luciana Bordin 1,*

1 Department of Molecular Medicine-Biological Chemistry, University of Padova, 35131 Padova, Italy; gabriella.dona@unipd.it (G.D.); elena.tibaldi@unipd.it (E.T.); annamaria.brunati@unipd.it (A.M.B.)
2 Department of Women’s and Children’s Health, University of Padova, 35131 Padova, Italy; alessandra.andrisani@unipd.it (A.A.); guido.ambrosini@unipd.it (G.A.)
3 Department of Medicine-Endocrinology, University of Padova, 35131 Padova, Italy; ChiaraSabbadin@libero.it (C.S.); decio.armanini@unipd.it (D.A.)
4 Department of Pharmaceutical and Pharmacological Sciences, University of Padova, 35131 Padova, Italy; eugenio.ragazzi@unipd.it

* Correspondence: luciana.bordin@unipd.it; Tel.: +39-049-827-6113; Fax: +39-049-807-3310

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Abstract: Astaxanthin (Asta), red pigment of the carotenoid family, is known for its anti-oxidant, anti-cancer, anti-diabetic, and anti-inflammatory properties. In this study, we evaluated the effects of Asta on isolated human sperm in the presence of human papillomavirus (HPV) 16 capsid protein, L1. Sperm, purified by gradient separation, were treated with HPV16-L1 in both a dose and time-dependent manner in the absence or presence of 30 min-Asta pre-incubation. Effects of HPV16-L1 alone after Asta pre-incubation were evaluated by rafts (CTB) and Lyn dislocation, Tyr-phosphorylation (Tyr-P) of the head, percentages of acrosome-reacted cells (ARC) and endogenous reactive oxygen species (ROS) generation. Sperm membranes were also analyzed for the HPV16-L1 content. Results show that HPV16-L1 drastically reduced membrane rearrangement with percentage of sperm showing head CTB and Lyn displacement decreasing from 72% to 15.8%, and from 65.1% to 13.9%, respectively. Accordingly, both Tyr-P of the head and ARC decreased from 68.4% to 10.2%, and from 65.7% to 14.6%, respectively. Asta pre-incubation prevented this drop and restored values of the percentage of ARC up to 40.8%. No alteration was found in either the ROS generation curve or sperm motility. In conclusion, Asta is able to preserve sperm by reducing the amount of HPV16-L1 bound onto membranes.

Keywords: human papillomavirus 16 (HPV16); astaxanthin (Asta); acrosome reaction; cholera toxin subunit B (CTB); L1 protein

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Article

Astaxanthin Improves Human Sperm Capacitation by Inducing Lyn Displacement and Activation

Alessandra Andrisani 1, Gabriella Donà 2, Elena Tibaldi 2, Anna Maria Brunati 2, Chiara Sabbadin 3, Decio Armanini 3, Gualtiero Alvisi 4, Salvatore Gizzo 1, Guido Ambrosini 1, Eugenio Ragazzi 5 and Luciana Bordin 2,*

1 Department of Women’s and Chilren’s Health, University of Padova, Padova 35100, Italy; E-Mails: alessandra.andrisani@unipd.it (A.A.); salvatore.gizzo@gmail.com (S.G.); guido.ambrosini@unipd.it (G.A.)

2 Department of Molecular Medicine-Biological Chemistry, University of Padova, Padova 35129, Italy; E-Mails: gabriella.dona@unipd.it (G.D); elena.tibaldi@unipd.it (E.T); annamaria.brunati@unipd.it (A.M.B.)

3 Department of Medicine-Endocrinology, University of Padova, Padova 35100, Italy; E-Mails: ChiaraSabbadin@libero.it (C.S.); decio.armanini@unipd.it (D.A.)

4 Department of Molecular Medicine-Microbiology Section, University of Padova, Padova 35129, Italy; E-Mail: gualtiero.alvisi@unipd.it

5 Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Padova 35129, Italy; E-Mail: eugenio.ragazzi@unipd.it

* Author to whom correspondence should be addressed; E-Mail: luciana.bordin@unipd.it; Tel.: +39-049-827-6113; Fax: +39-049-807-3310.

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Abstract: Astaxanthin (Asta), a photo-protective red pigment of the carotenoid family, is known for its multiple beneficial properties. In this study, the effects of Asta on isolated human sperm were evaluated. Capacitation involves a series of transformations to let sperm acquire the correct features for potential oocyte fertilization, including the generation of a controlled amount of reactive oxygen species (ROS), cholesterol depletion of the sperm outer membrane, and protein tyrosine phosphorylation (Tyr-P) process in the head region. Volunteers, with normal spermogram values, were divided in two separate groups on the basis of their ability to generate the correct content of endogenous ROS. Both patient group (PG) and control group (CG) were analysed for Tyr-phosphorylation (Tyr-P) pattern and percentages of acrosome-reacted cells (ARC) and non-viable cells (NVC), in the presence
or absence of Asta. In addition, the involvement of ROS on membrane reorganization and the presence of Lyn, a Src family kinase associated with lipid rafts, were investigated. Results show that Lyn is present in the membranes of human sperm, mainly confined in midpiece in resting conditions. Following capacitation, Lyn translocated to the head concomitantly with raft relocation, thus allowing the Tyr-P of head proteins. Asta succeeded to trigger Lyn translocation in PG sperm thus bypassing the impaired ROS-related mechanism for rafts and Lyn translocation. In this study, we showed an interdependence between ROS generation and lipid rafts and Lyn relocation leading the cells to undergo the successive acrosome reaction (AR). Asta, by ameliorating PG sperm functioning, may be utilised to decrease male idiopathic infertility.

**Keywords:** astaxanthin; tyrosine kinase Lyn; human sperm capacitation; acrosome reaction; cholera toxin subunit B (CTB)