Mar Drugs. 2013 Jun 3;11(6):1909-19. doi: 10.3390/md11061909.

Effect of astaxanthin on human sperm capacitation.

Donà G[1], Kožuh I, Brunati AM, Andrisani A, Ambrosini G, Bonanni G, Ragazzi E, Armanini D, Clari G, Bordin L.

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 Medicine - Endocrinology, University of Padova.
[2] Department of Women's and Chilren's Health, University of Padova.
[3] Department of Pharmaceutical and Pharmacological Sciences, University of Padova.
[4] Department of Molecular Medicine-Biological Chemistry, University of Padova.

ISGE International Society of Gynecological Endocrinology
Gynecological Endocrinology
The 18th World Congress
7-10 MARCH 2018
FIRENZE, ITALY

Context

As far as sperm quality is concerned, many efforts in these two decades have been spent 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 almost 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 suboptimal semen quality can be ascribed to physiological, infectious (2), environmental and genetic factors, including oxidative stress (OS) (3, 4). OS is considered as one of the main responsible of the drop of sperm fecundating ability, due to the formation of exogenous as well as endogenous reactive oxygen species (ROS), which induce DNA fragmentation and cause male in/sub-fertility, together with protein degradation and membrane denaturation by lipid peroxidation (5, 6). In addition to OS, reduced spermatogenesis, which lessens the number of sperm produced in the ejaculate, low motility and membrane weakness, in absence of any other patho-physiological disorders, co-operate to lower semen quality (7).
Hence a number of nutraceutical/vitamin supplements have been developed to lower the oxidative stress in semen (7, 8) and, alone or in association with many various cofactors, upgrade sperm parameters (7, 9), including sperm counts, morphology and motility, and improve male fertility. In the attempt to address the widest 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 zinc (Zn) should be considered for streghtening cell membrane and reducing the DNA breaks (10, 11); arginine and folic acid for helping spermatogenesis (12), and CoQ and selenium for improving motility (13). In addition, to counteract oxidative stress conditions, vitamins C and E should be added for having antioxidant synergic action, with vitamin C locating the cytoplasm and vitamin E inserting in the lipid bilayer, together with astaxanthin (Asta), which has been described to be an antioxidant 65 times more powerful than vitamin C, 54 times stronger than β-carotene, 100 times more effective than α-tocopherol (14).
Several studies are emerging about the use of combinations of various micronutrients (15-17), but, besides spermiogram-based common evaluation, a biochemical quantification of the effects has not yet been assessed in the framework of a research study. Hence, the aim of the present investigation was to evaluate in vivo the short term effect of a combination of eight micronutrients (including Asta) and to compare the results with in vitro effects of Asta incubation on sperm capacitation.

Methods

Study population: We recruited 51 male patients with couple's 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 released 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.

Seminal parameters analysis: After 3 days of abstinence, semen samples from all recruited patients, were collected by masturbation and assessed for sperm parameters (T_0). During the baseline visit were also collected data on the pathophysiology of the couple's infertility and on the physio/pathological anamnesis. Recruited patients (n= 24) were asked to take Fertylor15 with dosage 1 capsule/day for 30 days. At the end (T_1), seminal fluid was subjected to the same analysis carried out at baseline. The sample obtained from 27 patients was incubated in the presence/absence 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 (PSW), incubated for up to 180 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 monitored with acrosome-specific FITC-labeled peanut (Arachis hypogaea) agglutinin (FITC-PNA) in conjunction with DNA-specific fluorochrome propidium iodide (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, $GM1$, which can be stained by the cholera toxin subunit B (CTB)-FITC (18, 19). Cells were analyzed by immunofluorescence cytochemistry.

Results

In vivo study

Figure 1. Seminal parameters (sperm concentrations, progressive motility and normal morphology) of 24 recruited patients, before (T0) and after 30 days of Fertylor15 intake (T1).

Great individual variability of the seminal parameters.

There are no statistically significant changes in mean values after 30 days of therapy.

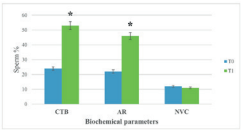

Figure 2. Biochemical parameters of sperm before (T0) and after Fertylor15 intake (T1). Semen samples from recruited subjects (n=24) were centrifuged, washed, incubated for 180 min at 37°C in capacitating conditions and then analyzed for cholera toxin subunit B-FITC labelling (**CTB**) for evaluation of membrane rafts. Number of cells showing CTB labelling in the head, expressed as % of total number of cells, were detected and reported.
In order to induce AR, aliquots of each sample were incubated for 30 min at 37°C, in the presence of 10 μM Ca^{2+} ionophore A23187, dissolved in dimethyl sulphoxide (DMSO), or only DMSO in order to test spontaneous AR (data not shown). After incubation sperm were treated with propidium iodide, to assess not viable cells (**NVC**) and then stained with FITC-PNA for evaluation of acrosome reaction (AR) by immunofluorescence cytochemistry. Percentage of cells undergoing Ca^{2+} Ionophore-induced AR and NVC were also detected and reported.
Values are means±SD. * p<0.01 comparison Asta *vs* C. Student's t-test for paired data.

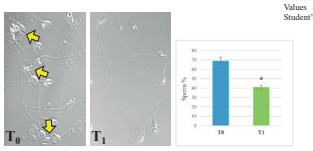

Figure 3. Microscopy images of sperm before (T0) and after Fertylor15 intake (T1). Semen samples from recruited subjects (n=24) were analyzed by contrast fase microscopy. Percentage of aggregate cells were counted and reported. Values are means±SD. * p<0.01 comparison Asta vs C. Student's t-test for paired data.

Fertylor15 intake for 30 days improves the percentage of capacitated and acrosome reacted cells. Asta enhances *in vivo* fertilizing capacity of sperm and sensitively reduces sperm aggregation.

In vitro study

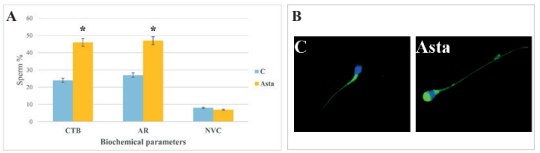

Figure 4. Biochemical parameters of human sperm after incubation in absence (C) or presence of Asta 2 μM (Asta). Semen samples from recruited subjects (n=27), were subjected to gradient separation. Sperm samples were collected three by three in a single pool to obtain a sufficient number of cells to perform all tests, and incubated for 180 min at 37°C in capacitating conditions in absence (C) or presence of Asta 2 μM (Asta).
Panel A: Sperm from each samples were then analyzed with cholera toxin subunit B-FITC labelling (**CTB**) for evaluation of membrane rafts. Number of cells showing CTB labelling in the head, expressed as % of total number of cells, were detected and reported.
In order to induce AR, aliquots of each sample were incubated for 30 min at 37°C, in the presence of 10 μM Ca^{2+} ionophore A23187, dissolved in dimethyl sulphoxide (DMSO), or only DMSO in order to test spontaneous AR (data not shown). After incubation sperm were treated with propidium iodide, to assess not viable cells (**NVC**) and then stained with FITC-PNA for evaluation of acrosome reaction (AR) by immunofluorescence cytochemistry. Percentage of cells undergoing Ca^{2+} Ionophore-induced AR and NVC were also detected and reported.
Panel B: Immunocytochemistry images of sperm from C and Asta samples, staining CTB-FITC for evaluation of membrane rafts.
Values are means±SD. The figure is representative of 9 separate experiments conducted in triplicate.
* p<0.01 comparison Asta *vs* C. Student's t-test for paired data.

The incubation with Asta in *in vitro* experiments increases the percentages of capacitated sperm (CTB) and acrosome reacted cells (AR) by 22% and 20%, respectively, confirming *in vivo* finding.

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

1. Boivin J et al. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. Hum Reprod 2007; 22:1506–1512.
2. Garolla A et al. Association, prevalence, and clearance of human papillomavirus and antisperm antibodies in infected semen samples from infertile patients. Fertil Steril 2013; 99: 125-131.
3. World Health Organization. Laboratory manual for the examination and processing of human semen. 5th ed. New York: Cambridge University Press; 2010.
4. Jungwirth A et al. European association of urology guidelines on male infertility: the 2012 update. Eur Urol 2012; 62: 324–332.
5. Agarwal A et al. Clinical utility of sperm DNA fragmentation testing: practice recommendations based on clinical scenarios. Transl. Androl Urol 2016; 5: 935-50.
6. Gharagozloo P, Aitken RJ. The role of sperm oxidative stress in male infertility and the significance of oral antioxidant therapy. Hum Reprod 2011; 26: 1628-40.
7. Salas-Huetos A et al. Dietary patterns, foods and nutrients in male fertility arameters and fecundability: a systematic review of observational studies. Hum Reprod Update 2017; 23: 371-389.
8. Buhling KJ, Laakmann E. The effect of micronutrient supplements on male fertility. Curr Opin Obstet Gynecol 2014; 26: 199–209.
9. Collins GG, Rossi BV. The impact of lifestyle modifications, diet, and vitamin supplementation on natural fertility. Fertil Res Pract 2015; 1: 11.
10. Yuyan L et al. Are serum zinc and copper levels related to semen quality? Fertil Steril 2008; 89: 1008-1011.
11. Ames BN. A role for supplements in optimizing health: the metabolic tune-up Arch Biochem Biophys 2004; 423: 227-34.
12. Wu G et al. Arginine metabolism and nutrition in growth, health and disease. Amino Acids 2009; 37: 153-68.
13. Buhling KJ, Laakmann E. The effect of micronutrient supplements on male fertility. Curr Opin Obstet Gynecol 2014; 26: 199-209.
14. Ambati RR et al. Astaxanthin: sources, extraction, stability, biological activities and its commercial applications--a review. Mar Drugs 2014;12: 128-52.
15. Stanislavov R, Rohdewald P. Sperm quality in men is improved by supplementation with a combination of L-arginine, L-citrullin, roburins and Pycnogenol®. Minerva Urol Nefrol 2014; 66: 217-23.
16. Wong WY et al. Effects of folic acid and zinc sulfate on male factor subfertility: a double-blind, randomized, placebo-controlled trial. Fertil Steril 2002; 77:491–8.
17. Lipovac M et al. Comparison of the effect of a combination of eight micronutrients versus a standard mono preparation on sperm parameters. Reprod Biol Endocrinol 2016; 14: 84.
18. Donà G et al. Effect of astaxanthin on human sperm capacitation. Mar Drugs 2013; 11: 1909-19.
19. Andrisani A et al. Astaxanthin Improves Human Sperm Capacitation by Inducing Lyn Displacement and Activation. Mar Drugs 2015; 13: 5533-51.

marine drugs

MDPI

Article

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 Chilren'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

Received: 15 September 2018; Accepted: 1 November 2018; Published: 2 November 2018

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 63.1% 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

Mar. Drugs **2015**, *13*, 5533-5551; doi:10.3390/md13095533

OPEN ACCESS

marine drugs

ISSN 1660-3397

www.mdpi.com/journal/marinedrugs

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.

Academic Editor: Keith B. Glaser

Received: 24 June 2015 / Accepted: 12 August 2015 / Published: 25 August 2015

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 spermiogram 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)