Reproductive Toxicology 53 (2015) 33–38

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Cadmium may impair prostate function as measured by prostate specific antigen in semen: A cross-sectional study among European and Inuit men

Alessandro Andreucci[a,*], Emina Mocevic[b], Bo AG Jönsson[c], Aleksander Giwercman[d], Yvonne Lundberg Giwercman[e], Gunnar Toft[f], Thomas Lundh[c], Davide Bizzaro[a], Ina Olmer Specht[b], Jens Peter Bonde[b]

[a] Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy
[b] Department of Occupational and Environmental Medicine, Copenhagen University Hospital, 2400 København NV, Denmark
[c] Department of Laboratory Medicine, Lund University Hospital, SE-221 85 Lund, Sweden
[d] Reproductive Medicine Centre, Malmö University Hospital, SE 205 02 Malmö, Sweden
[e] Lund University, Department of Translational Medicine, SE 205 02 Malmö, Sweden
[f] Department of Occupational Medicine, Aarhus University, Aarhus C, 8000 Aarhus, Denmark

ARTICLE INFO

Article history:
Received 26 February 2014
Received in revised form
25 December 2014
Accepted 26 January 2015
Available online 3 February 2015

Keywords:
Prostatic specific antigen
Cadmium
Zinc
Androgen receptors
CAG repeats
Prostate

ABSTRACT

We investigated the association between cadmium in blood and the concentration of the prostate specific antigen (PSA) in semen, including the modifying effects of zinc or the CAG polymorphism in the androgen receptor (AR). Blood and semen samples were collected from 504 partners of pregnant women in Greenland, Poland and Ukraine. We found an inverse trend between cadmium and PSA ($\log(\beta) = -0.121$, 95% confidence interval (CI): −0.213; −0.029, $P = 0.0103$) in Greenlandic men. Similar results were observed in men with a high number of CAG repeats (CAG 24) ($\log(\beta) = -0.231$, 95% CI: −0.363; −0.098, $P = 0.0009$). Inverse trends between cadmium and PSA were found when semen zinc concentrations were below the median value for men from Ukraine and Greenland. These outcomes suggest that cadmium may impair prostate function, as measured by PSA in semen, while high zinc levels and a low number of CAG repeats protects against this action.

1. Introduction

Cadmium is a silver-white metal found in the earth's crust at low concentrations, most often together with zinc in mineral deposits. The general population is primarily exposed through food and tobacco smoking [1], and occupational exposure occurs in manufacture of batteries, pigments, plastic stabilizers and by electroplating [2]. People who base their diet on seafood, are more exposed to cadmium, due to the bioaccumulation of cadmium in marine animals [3,4], even if the bioavailability of cadmium from marine diet may be low [4].

In vivo and in vitro experimental studies suggest that cadmium exerts toxic actions to the prostate, which are antagonized by zinc [5–7]. Prostate specific antigen (PSA) is a protein produced by the prostate necessary for the liquefaction of the semen [8], used as a biomarker of prostate function. The PSA release is androgen induced through the interaction with the androgen responsive elements (AREs) in the PSA promoter [9].

The effect of the number of CAG repeats present in the AR gene is still uncertain on determining the AR activity. An inverse association between the number of CAG repeats and AR activity is generally assumed, while in vitro experiments showed that subjects with 22 CAG repeats had higher AR activity, compared to individuals with 16 and 28 CAG repeats [10–12].

Human studies focusing on relations between cadmium and PSA concentrations in body fluids are few and conflicting. Positive associations between cadmium concentration in blood and serum PSA have been shown [5,6,13,14]. In one of these studies, in which 437 men over the age of 40 were investigated, a clear, positive association was found when zinc intake was low [5]. However, a reversed association between serum PSA levels and cadmium concentration in urine and blood was reported [15]. The goal of the present study was to investigate if higher blood concentrations of cadmium were

Abbreviations: PSA, prostatic specific antigen; AR, androgen receptor.

* Corresponding author. Tel.: +39 07135494/+39 3338478228.
E-mail address: ale.andreucci86@gmail.com (A. Andreucci).

http://dx.doi.org/10.1016/j.reprotox.2015.01.010
0890-6238/

associated with concentrations of PSA in semen, and to examine the possible modifying effect of zinc and AR CAG repeats.

2. Methods

2.1. Study design

This cross-sectional study was based on data from the INUENDO project[1] which addressed links between several environmental contaminants and a range of biological measures of human reproductive health. Men with measurements of blood cadmium, AR status and semen concentration of PSA and zinc were included in this study.

2.2. The study population

The study population consisted of spouses of pregnant women from Greenland, Poland and Ukraine. To be eligible, the participants had to be born in the country where the study was conducted and they had to be at least 18 years at the time of enrolment [16]. The data collection started in May 2002 and lasted to February 2004 when semen samples from approximately 200 subjects were collected at each research site [17]. The participation rates were 32% (206/638 men) in Ukraine, 28% (198/690 men) in Poland and 77% (199/256 men) in Greenland. The final dataset included 504 individuals since 99 men were excluded because of absence of cadmium values (30 individuals from Greenland, 20 from Poland and 49 from Ukraine). All individuals in the present study were considered to be healthy and did not have any reproductive problems, even those who underwent urogenital surgery.

2.3. Data collection

All interviews, sampling, processing, storage and shipment of semen samples and blood were performed according to uniform study protocols, questionnaires and forms [17].

2.4. Interviews

Men were interviewed regarding lifestyle factors, occupational factors, urogenital disorders, periods of abstinence and other issues relating the delivery of a semen sample. Questionnaires used were translated to native languages in the participating countries and back translated to English [17].

2.5. Blood sampling and analysis

Venous blood samples were collected within one week from the semen collection, except for 116 Greenlandic samples that were collected up to 1 year in advance [18]. Cadmium exposure was measured in blood by inductively coupled plasma-mass spectrometry (ICP-MS; Thermo X7, Thermo Elemental, Winsford, UK) at the Department of Occupational and Environmental Medicine, Lund University. The detection limit of 0.01 μg/L was calculated as three times the standard deviation of the blank. The coefficient of variation for duplicate preparations measurements was 2.9%. Seronorm Trace elements whole blood (Lot.: MR4206, 503109 and MI1256, SERO AS, Billingstad, Norway) was used for analytical accuracy checking. The results were 0.62 ± 0.04 (mean ± SD), $n = 55$ vs. recommended 0.68–0.80 μg/L, 6.2 ± 0.37, $n = 36$ vs. 5.6–6.4 μg/L and 11.5 ± 0.80, $n = 34$ vs. 10.6–11.5 μg/L. In addition cadmium was quantified on certified human blood samples from Centre de Toxicologie du Quebec, International Comparison Program, Canada (Lot C0515, obtained value was 0.80 ± 0.07, $n = 52$ vs. certified 0.79 ± 0.23 μg/L) [19]. Cotinine, the major metabolite of nicotine, was measured in blood. The detection limit for cotinine was 0.7 ng/mL and values under this limit were considered equal to 0.35 ng/mL.

2.6. Semen sampling and analysis

The analyses of the semen samples from Greenland were carried out at the local hospital or nursing station in 9 municipalities and one settlement. Analyses of samples from Ukraine and Poland were performed at the central hospital in each region [16]. The participants were requested to abstain from sexual activities for at least 2 days before they delivered the sample and to declare the precise actual abstinence time. If the sample was collected at home, it was kept close to the body to maintain a temperature of approximately 37 °C during the transportation to the local hospital [16]. Zinc and PSA concentrations were analyzed from the semen samples. The Prostatus™ kit (Wallac Oy, Finland) was used to determine the concentration of PSA in seminal plasma at Skane University Hospital Malmö. The control samples were collected from healthy men in general population, and stored in multiple aliquots. Then, they were used for determining the performance of the PSA and zinc assays. The reference value for zinc in semen is 1.6 mmol/L. Control samples had a coefficient of variation of 12% and a mean PSA concentration of 660 mg/L [20]. A colorimetric method was used to determine the concentration of zinc in seminal plasma [21]. Trichloroacetic acid was used for the precipitation of the proteins in the sample. The supernatant was mixed with a water-soluble pyridylazo dye and the absorbance measured at 560 nm. Control samples had a coefficient of variation of 7% and a mean zinc concentration of 2.0 mmol/L [20].

2.7. Assessment of CAG repeats

Genomic DNA was extracted from peripheral leucocytes. The length of CAG repeats in the AR gene was quantified by PCR amplification in a 50 μL PCR reaction containing approximately 100 ng DNA, 0.3 μM of each of the primers: forward 5′-TTAGGGCTGGGAAGGGTCTA-3′ and reverse 5′-TGGGGCCTCTACGATGGGCT-3′ (Invitrogen, Stockholm, Sweden), 1.5 mM $MgCl_2$, 200 μM of dATP, dCTP, dTTP and dCTP each (Roche, Bromma, Sweden), 45 mM KCl, 10 mM Tris and 1 unit of DNA polymerase (Finnzymes Oy, Espoo, Finland). Amplification was performed for 40 cycles in an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany) with denaturation at 96 °C for 1 min, annealing at 61 °C for 45 s, extension step at 72 °C for 2 min, with an initial denaturation step at 96 °C for 3 min and a final extension step at 72 °C for 7 min. The PCR products were purified, run with the reverse primer in a sequencing PCR, precipitated, dissolved in highly deionized formamide (Applied Biosystems, Stockholm, Sweden) and directly sequenced on an eight-capillary AB3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

2.8. Statistical methods

The participants were grouped by tertiles of blood cadmium and semen zinc concentrations, respectively, and according to the number of CAG repeats: <20, 20/21, 22/23, 24 and >24.

The concentration of PSA in semen as a function of cadmium in blood was examined and subsequent analyses were performed stratifying by level of zinc in semen and number of CAG repeats, respectively. Results are presented as geometric means, medians

[1] INUENDO (INUit-ENDOcrine) is acronym for “Biopersistent organochlorines in diet and human fertility. Epidemiologic studies of time to pregnancy, semen quality and reproductive hormones in Inuit and European populations.”