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Selenium speciation in human serum and its implications for epidemiologic research: a cross-sectional study

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ABSTRACT

Observational studies addressing the relation between selenium and human health, particularly cancer risk, yielded inconsistent results, while most recent randomized trials showed a fairly consistent pattern suggesting null or adverse effects of the metalloid. One of the most plausible explanations for such inconsistencies is inadequate exposure assessment in observational studies, commonly carried out by measuring total Se content without taking into account the specific exposure to the individual chemical forms of the metalloid, whose toxic and nutritional properties may vary greatly. Data on the distribution of these species in human blood and their correlation with overall selenium levels are very limited.

The concentrations of organic and inorganic selenium species were analyzed in serum of fifty subjects sampled from the general population of the municipality of Modena, northern Italy, aged from 35 to 70 years. Samples were collected during a 30-month period, and determinations of selenium species were carried out using high pressure liquid chromatography coupled with inductively coupled plasma dynamic reaction cell mass spectrometry.

The majority of selenium was found to be present as organic species, but the inorganic forms showed higher levels than expected. These species showed limited correlations with age, sex and body mass index, while the organic forms increased in subjects consuming selenium-containing dietary supplements and decreased in smokers. The length of the sample storage period strongly influenced the distribution of selenium compounds, with a clear tendency towards higher inorganic and lower organic selenium levels over time. In multivariate analysis adjusting for potential confounders, total serum selenium correlated with human serum albumin-bound selenium and, in males, with two organic species of the metalloid (selenocysteine and glutathione peroxidase-bound selenium), while little association existed with the other organic forms and the inorganic ones.

These findings highlight the potential for exposure misclassification of observational epidemiologic investigations based on overall selenium content in blood and possibly other tissues, and the critical role of the storage conditions for speciation analysis.

Introduction

The relation of metalloid selenium (Se) with human health, and particularly with cancer, is puzzling and still not entirely defined. It encompasses both the possibility of beneficial and adverse effects, the latter being supported by the most recent results of the Selenium and Vitamin E Cancer Prevention Trial [1–3]. There is a clear consensus about its safe range of exposure being very and unusually narrow, but opinions considerably diverge about the upper and lower safe Se limits as well as the specific outcomes linked to altered Se status, particularly for chronic exposure [4–7]. Currently, a marked reassessment of the relation of Se with cancer risk is in progress, since the expectation of a beneficial effect of selenium supplements has vanished [7,8] and indications of an excess risk for skin and prostate cancer [9–12] have emerged. Such a trend is also occurring for cardiovascular and metabolic diseases [13,14] and neurological and cognitive disorders [15–17].

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In particular, improvements in the assessment of Se exposure retains a critical position in observational epidemiologic research in order to correctly define its effects on human health. This issue has long attracted the attention of investigators, in order to identify indicators of long-term selenium exposure, such as its concentration in blood, urine, hair and toenails, as well as its average dietary intake through foods and rarely through drinking water [4,6,7,18]. Inadequate exposure assessment, particularly long-term, in addition to unmeasured confounding due to lifestyle and environmental factors and to genetic factors including selenoprotein polymorphisms, has been suggested to explain the conflicting results yielded by observational studies, and may have severely hampered the possibility to reliably identify the health effects of the metalloid. Among the potential pitfalls of the exposure assessment methods, a key role is played by the inability of overall tissue Se levels to adequately reflect exposure to specific selenium compounds [8,19]. In fact, this element is usually present in environmental sources and in living organisms in various inorganic and organic forms, having considerable and even extreme variations in both toxicological and physiological properties [4,15,20–28], and little is known in the human about the relation of these Se species to each other and more generally with the overall Se level.

In the present study, we aimed to investigate the relation between blood levels of overall Se and individual Se species in a population-based sample from an Italian community, in order to test the adequacy of the exposure assessment based on the former biological indicator.

Methods

Study participants

We selected the study participants from the population of the municipality of Modena, Northern Italy (around 180,000 inhabitants), aiming to recruit a representative sample of 50 individuals. To do so, we randomly sampled eligible subjects from each sex- and age-specific subgroup of Modena residents aged between 35 and 70 years. Fifty-one out of the 150 contacted individuals agreed to participate in this investigation (34% response rate, substantially similar in all subgroups except for a lower one in males <50 years). After we had obtained their written informed consent, the participants were invited to a Modena National Health Unit Center in the morning, to give a fasting venous blood sample. The sample was collected in a plastic tube, immediately centrifuged for 10 min at 3000 rpm and serum aliquots of 1 ml were stored at −15 °C until use.

In addition, each participant completed a questionnaire collecting detailed information on education, occupational history, height and current weight, consumption of dietary supplements and smoking habits. In particular, we asked them to detail specific intake (product, quantity/day, duration) of any dietary product, supplement or drug containing selenium taken at the blood sampling time for at least six months continuously. Assessment of commercial supplements containing Se and their metalloid content was done on the basis of a systematic search on all over-the-counter products marketed in Italy, with the help of the Reggio Emilia Drug Documentation Service. The recruitment of subjects included in the study, which was approved by the Modena province Ethical Committee, lasted 30 months.

Laboratory analysis

A 1 ml serum aliquot for each study subject was transported by air courier deep frozen in dry ice to the Munich laboratory, and kept continuously frozen until use. We slowly thawed samples in a refrigerator at 4 °C, vortexed and subsequently analyzed them. Suprapure grade chemicals were used throughout. Selenite (Se(IV)), selenate (Se(VI)), selenomethionine (Se-Met), selenocysteine (Se-Cys), thioredoxin reductase (EC 1.8.1.9.)-bound selenium (Se-TrxR), glutathione peroxidase (EC 232-749-6)-bound selenium (Se-GpX), human serum albumin (HSA) and Tris buffer were from Sigma–Aldrich, Deisenhofen, Germany. We purchased certified Se and Rh stock standards (1000 mg/l) from CPI International, Santa Rosa, CA, USA, and we obtained ammonium acetate (NH_4Ac) and acetic acid (HAc) from Merck, Darmstadt, Germany. $Argon_{liq}$ and methane (99.999% purity) were purchased from Air Liquide, Kleve Germany. We prepared stock solutions of Se(IV) and Se(VI) at a concentration of 1000 mg Se/l by dissolving in Milli-Q water (18.2 MΩ cm, Milli-Q system, Millipore, Bedford, MA, USA). HSA was prepared at a concentration of 1000 mg/l. Preparation of HSA-Se was performed by mixing 10 mg Se/l selenite with this stock solution and incubating for at least 14 days. Working standards of Se species were prepared daily from their stock standard solutions by appropriate dilution with Milli-Q H_2O. Selenoprotein P (SePP) is not commercially available as a standard compound, but it can be prepared from serum using affinity chromatography (AFC). We purified the AFC-SePP fraction by a mass-calibrated size exclusion chromatography (SEC) column, where the SePP fraction eluted at an RT calculated for 62 kDa. The complete preparation process of SePP by AFC + SEC and purity checking by anion exchange chromatography (IEC) coupled with inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS) is detailed in Solovyev et al. [20].

We determined total Se and the Se species Se(IV), Se(VI), Se-Met, Se-Cys, Se-TrxR, Se-GpX, SePP and HSA-Se in serum samples using anion exchange chromatography (IEC) coupled with inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS) according to methodologies previously established for biological matrices [20,22]. In general the IEC separation followed Xu et al. [29] but was slightly modified by flattening the elution gradient (see below) for complete peak resolution.

We connected a Knauer 1100 Smartline inert Series gradient HPLC system to an anion exchange column AG 11 (precolumn 50 mm × 4 mm) + AS 11 (analytical column 250 mm × 2 mm I.D.) from Thermo (Dionex Idstein, Germany) for species separation. The sample volume was 100 μl. The mobile phases were: eluent A: 10 mM Tris–HAc, pH 8.0; and eluent B: A + 500 mM NH_4Ac, pH 8.0. Gradient elution expressed as %-eluent A: 0–3 min 100%; 3–10 min 100–60%; 10–23 min 60–45%; 23–26 min 45–43%; 26–28 min 43–0%; 28–52 min 0%; 52–60 min 100%. The flow rate was 0.70 ml/min. For internal standardization we mixed the column effluent with 1 μg/l Rh (final concentration, Rh flow rate: 0.1 ml/min) and directed to ICP-MS.

The experimental settings chosen for ICP-DRC-MS (Perkin Elmer NexIon) after optimization were: radio frequency power: 1250 W, plasma gas flow: 15 L Ar/min auxiliary gas flow: 1.05 L Ar/min, nebulizer gas flow: 0.94 L Ar/min, daily optimized, dwell time 300 ms, ions monitored: ^{78}Se, ^{80}Se, ^{103}Rh, DRC reaction gas: CH_4 reaction at 0.58 ml/min, DRC rejection parameter q: 0.6.

We analyzed total serum Se by graphite furnace atomic absorption spectrometry based on the method of the MAK collection-biomonitoring methods [30].

We performed peak quantification from chromatograms by comparing peak areas with peak area calibration curves. We used the standard addition method for standard-retention-time matched identification of Se species and as QC means in quantification. Species identity was further confirmed using a 2-D approach of IEC-capillary electrophoresis (CE)-ICP-DRC-MS as described earlier [20]. Species identification was regarded as acceptable when the species matched the standard compounds with both chromatography/electrophoretic techniques (match in first and second