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Cerebrospinal fluid of newly diagnosed amyotrophic lateral sclerosis patients exhibits abnormal levels of selenium species including elevated selenite☆

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ABSTRACT

Exposure to selenium, and particularly to its inorganic forms, has been hypothesized as a risk factor for amyotrophic lateral sclerosis (ALS), a fast progressing motor neuron disease with poorly understood etiology. However, no information is known about levels of inorganic and some organic selenium species in the central nervous system of ALS patients, and recent observations suggest that peripheral biomarkers of exposure are unable to predict these levels for several Se species including the inorganic forms. Using a hospital-referred case–control series and advanced selenium speciation methods, we compared the chemical species of selenium in cerebrospinal fluid from 38 ALS patients to those of 38 reference neurological patients matched on age and gender. We found that higher concentrations of inorganic selenium in the form of selenite and of human serum albumin-bound selenium were associated with increased ALS risk (relative risks 3.9 (95% confidence interval 1.2–11.0) and 1.7 (1.0–2.9) for 0.1 μg/L increase). Conversely, lower concentrations of selenoprotein P-bound selenium were associated with increased risk (relative risk 0.2 for 1 μg/L increase, 95% confidence interval 0.04–0.8). The associations were stronger among cases age 50 years or older, who are postulated to have lower rates of genetic disease origin. These results suggest that excess selenite and human serum albumin bound-selenium and low levels of selenoprotein P-bound selenium in the central nervous system, which may be related, may play a role in ALS etiology.

1. Introduction

Selenium (Se), an essential trace mineral, has both nutritional and toxicological properties, with a broad range of biological activities which strongly depend on its chemical form. Inorganic species of Se are generally more toxic and of lower nutritional value than the organic species (Vinceti et al., 2009; Valdiglesias et al., 2010; Nogueira and Rocha, 2011), in some cases exhibiting entirely different effects (Borella et al., 1996; Hoefig et al., 2011; Nogueira and Rocha, 2011; Bodnar et al., 2012; Bitencourt et al., in press), and more generally, each Se compound has specific and distinctive biological properties. Unfortunately, most human studies have investigated the overall Se content of body tissues without assessing the different chemical species of the metalloid. Moreover, the levels of Se and of specific Se compounds in the different body tissues may considerably vary and are frequently uncorrelated, hampering or even precluding assessment of their concentrations in target organs and compartments of relevance for specific diseases on the basis of peripheral indicators of exposure (such as blood or toenails Se) or dietary intake of the metalloid (Behne et al., 2010; Dennert et al., 2011; Vinceti et al., 2012b). This may be particularly true when attempting to assess involvement of Se species in the etiology of human neurological diseases: a recent Se speciation studies has found that while peripheral (blood) levels of some organic Se compounds tend to reflect central nervous

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system (CNS) levels, no such correlation exists for other organic species such as selenoprotein P, overall Se content and the inorganic forms selenate and selenite (Solovyev et al., 2013), confirming previous observations on overall Se (Michalke et al., 2009). Other observations appear to confirm the independence of brain selenoprotein P compared with its plasma circulating levels (Scharpf et al., 2007).

Amyotrophic lateral sclerosis (ALS), a severe degenerative motor neuron disease of largely unknown etiology apart from the few cases of genetic origin, is among the diseases whose etiology is hypothesized to be related to overexposure to selenium (Vinceti et al., 2012a). This hypothesis was first proposed based on observation of a cluster of ALS cases in a seleniferous area of South Dakota, and an excess incidence of ALS in an Italian population consuming drinking water with high concentrations of the inorganic Se species selenate (Kilness and Hochberg, 1977; Vinceti et al., 1996, 2010b). Consistent with the hypothesis is the demonstration of Se-induced neurotoxicity in laboratory studies (Ammar and Couri, 1981; Nogueira et al., 2003; Maraldi et al., 2011; Estevez et al., 2012) and its selective toxicity on motor neurons in species such as swine (Casteignau et al., 2006; Raber et al., 2010; Vinceti et al., 2012a), generally showing a much higher or even an exclusive toxicity of inorganic species compared with the organic ones. Moreover, recent epidemiologic observations suggest deleterious effect of Se on neurological end-points such as visual evoked potentials (Saint-Amour et al., 2006) and, even at low doses of exposure, neonatal neurological and behavioral development (Yang et al., 2013).

We conducted a case–control study to examine the hypothesis that Se species and particularly the inorganic ones are associated with ALS risk by using a CNS biomarker of exposure, cerebrospinal fluid (CSF), which appears to play a key role in assessing exposure to etiopathogenetic and therapeutic factors in this disease (Tarasiuk et al., 2012; von Neuhoff et al., 2012; Wilson et al., 2013; Winer et al., 2013).

2. Methods

2.1. Study participants

ALS patients were recruited from a case series of residents of the Emilia-Romagna region, northern Italy, who were diagnosed with clinically definite or clinically probable ALS using the revised El Escorial Criteria (Georgoulopoulou et al., 2011) at the ALS Center of the Modena University Neurological Department from May 1998 to April 2011, and who underwent lumbar puncture during diagnostic procedures. This group comprised 72 consecutive patients with sporadic ALS, some of whom were enrolled in a previous study (Mandrioli et al., 2006). Thirty-eight patients had at least 1 mL CSF available when the present study was designed and composed the enrolled cases. We recently performed genetic screening including all the major genes that are implicated in ALS (SOD1, C9ORF72, FUS, TDP43) in 12 of these patients, generally the younger ones, with none testing positive. We were not allowed to perform genetic analysis in the remaining patients because of lack of informed consent and/or available blood samples.

The control population consisted of patients residing in the Emilia-Romagna region who were admitted to the same department between 1999 and 2010, inclusive, and underwent lumbar puncture because of suspected but later unconfirmed neurological disease, and had a sample of at least 1 mL of CSF still available in September 2011. Among these individuals, we randomly selected 38 subjects matched 1:1 to ALS cases on age (±10 years, in most cases ±5 years) and gender. Signs or symptoms that led to neurological examination and lumbar puncture were: headache ($n = 17$), paresthesias ($n = 5$), diplopia ($n = 6$), vertigo ($n = 3$) and other ($n = 7$). All control patients were subsequently discharged from hospital without a diagnosis of a major disease; final diagnosis was primary headache in 13 individuals, and other diagnoses with negative instrumental tests in the remaining 25 individuals.

Informed consent for diagnostic lumbar puncture was obtained from all patients, and utilization of the CSF specimens for the present study was approved by the Modena Ethical Committee.

2.2. Sample collection

Approximately 6 mL of CSF were collected by lumbar puncture from each patient and immediately stored at −80 °C in polypropylene tubes. A 1 mL aliquot was transported by air courier deep frozen in dry ice to the Munich laboratory, and kept continuously frozen until use. For analysis, samples were slowly thawed in a refrigerator at 4 °C, vortexed and subsequently analyzed.

2.3. Chemicals

Suprapure grade chemicals were used throughout. Selenite, selenate, selenomethionine (Se-Met), selenocystine (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 ordered from Sigma–Aldrich, Deisenhofen, Germany. Certified Se and Rh stock standards (1000 mg/L) were purchased from CPI International, Santa Rosa, CA, USA. Ammonium acetate (NH_4Ac) and acetic acid (HAc) were obtained from Merck, Darmstadt, Germany. Ar_{liq} and methane (99.999% purity) were purchased from Air Liquide, Gröbenzell, Germany. Selenite and selenate stock solutions were prepared 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 Se-HSA 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-bound Se (SePP) is not commercially available as a standard compound, but it can be prepared from serum using affinity chromatography (AFC): The AFC-SePP fraction was purified by a mass-calibrated size exclusion chromatography (SEC) column, where the SePP fraction eluted at a RT calculated for 61.8 kDa.

2.4. Selenium speciation

We determined total Se and the Se species selenite, selenate, Se-Met, Se-Cys, Se-TrxR, Se-GPx, SePP and Se-HSA in the CSF samples using high pressure liquid chromatography (HPLC) coupled with inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS) according to methodologies previously established for biological matrices, specifically for CSF (Michalke and Berthele, 2011; Solovyev et al., 2013). Se speciation was conducted by strong anion exchange (SAX)-ICP-DRC-MS (Michalke and Berthele, 2011). The SAX separation followed Xu et al. (2008) but was slightly modified for baseline separation of close eluting peaks.

A Knauer 1100 Smartline inert Series gradient HPLC system was connected to an anion exchange column ProPac SAX-10 (250 × 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 0.20 mL min^{-1}. For internal standardization the column effluent was mixed with 1 μg/L Rh (final