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Selenium speciation in paired serum and cerebrospinal fluid samples of sheep

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ABSTRACT

This study was performed to characterise selenium (Se) and Se species in cerebrospinal fluid (CSF) of sheep and its relation to the respective Se concentrations in serum. Paired samples from 10 adult sheep were used for the study. Five sheep were fed a diet with a marginal Se concentration of <0.05 mg Se/kg diet dry weight (dw, Se^-), and five animals were fed the same diet supplemented with sodium selenite revealing a concentration of 0.2 mg Se/kg diet dw (Se^+). The feeding strategy was conducted for two years; The results on metabolic effects were published previously. At the end of the feeding period, paired samples of serum and CSF were collected and analysed using ion exchange chromatography inductively coupled plasma–dynamic reaction cell–mass spectrometry (IEC-ICP-DRC-MS) technique for total Se concentration and concentrations of Se species. Albumin concentrations were analysed additionally.

The feeding strategy caused significant differences ($p < 0.01$) in serum Se concentrations with 33.1 ± 5.11 μg Se/l in the Se^- group and 96.5 ± 18.3 μg Se/l in the Se^+ group, respectively. The corresponding total Se concentrations in CSF were 4.38 ± 1.02 μg Se/l and 6.13 ± 1.64 μg Se/l in the Se^- and the Se^+ group, respectively, missing statistical significance ($p = 0.077$). IEC-ICP-DRC-MS technique was able to differentiate the Se species selenoprotein P-bound Se (SePP), selenomethionine, glutathione peroxidase-bound Se (Se-GPx), selenocystine, thioredoxin reductase-bound Se, ovine serum albumin-bound Se (Se-OSA), SeIV and SeVI in ovine serum and CSF. Quantitatively, SePP is the main selenoprotein in ovine serum followed by Se-GPx. The CSF/blood ratio of albumin ($Q_{Albumin}$) reflected a physiological function of the blood-CSF barrier in all sheep. $Q_{Se\text{-}species}$ were higher than $Q_{Albumin}$ both feeding groups, supporting the hypothesis of local production of Se species in the brain. Significant positive regression lines for CSF vs. serum were found for albumin and Se-OSA only, suggesting a role of albumin to convey Se across the blood-CSF barrier. The ovine model, together with the IEC-ICP-DRC-MS technique to characterise the Se species, might be a worthwhile model for further studies as repeated sample collection as well as modification of the nutritional status is feasible and effective.

1. Introduction

Selenium (Se) is an essential trace element for mammals. Several regions of northern Europe as well as some parts of the US have low soil Se concentrations, and forage grown in these areas provide a marginal or even deficient Se supply for farm animals [1]. Therefore, undernutrition with Se was found frequently also in sheep [2]. The main biological function of Se is believed to exist through its incorporation into selenoproteins, which happens mainly in the form of selenocystine residues as an integral constituent of reactive oxygen species detoxifying selenoenzymes such as glutathione peroxidases (GPx), thioredoxin reductases (TrxR) and selenoprotein P (SePP) [3]. Marginal Se supplementation leads to a decrease of the antioxydative enzyme activity, in the first instance obvious in cytosolic GPx activity [4], probably due to the low rank of GPx in the hierarchic regulation of selenoprotein expression [5].

Se and selenoproteins are also known to play an important role in brain function [6], however, little is known about the mode

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of exchange of Se or selenoproteins between the extracellular compartments blood and cerebrospinal fluid (CSF). CSF fills the larger spaces within and around the central nervous system (CNS) and regulation of the composition and the volume of the CSF is indispensable for proper brain functions [7]. At brain cell level the interstitial fluid (ISF) provides the environment for water and solutes to enter and to leave cells. ISF and CSF are very similar in composition, but differ significantly from that of blood plasma [7]. The responsible barriers are the blood-brain barrier separating blood and ISF within the parenchyma as well as the blood-CSF barrier. However, only the CSF is attainable for clinical laboratory diagnostics by puncture of the central spinal canal. The anatomical basis of the blood-CSF barrier is the arachnoid membrane, the pia mater, the ependyma lining the ventricles and the choroid plexus. The choroid plexus seems to be predominantly responsible for the CSF formation [7]. CSF is removed mainly by the arachnoid villus system, the lymphatic outflow system as well as by the perineural pathway [8,9].

Se concentration in the brain seems to be provided with high priority. Depletion studies in mice showed a strong decrease of Se in body tissues, but an only slight decrease in the brain [10]. Radiolabelling studies documented that the uptaken amount of ^{75}Se into the brain of rats depends on the Se status and shows a significant higher quantity in Se-deficient animals. The authors assumed that the Se uptake is regulated by a brain region-specific expressed receptor and that the expression of the receptor is nutritionally regulated by the Se status. Within the CNS, CSF showed the highest uptake of the applied ^{75}Se (intraperitoneal injection) and the authors suggested a high turnover rate of this Se in the ventricular system [11].

There is also the demand to characterise Se species crossing the blood-CSF barrier. This topic was firstly approached by measuring Se species in paired samples (serum and CSF) of healthy human patients using SAX–ICP–DRC–MS as published recently [12]. The study presented here conducts this topic by Se speciation in paired ovine samples having sufficient vs. marginal nutritional Se supplementation levels. The results are a part of a comprehensive study with a main focus on metabolic changes comparing marginal and sufficient Se supplementation published previously [4].

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and reagents used were of suprapure grade. The chemical list consists of the following: From CPI, Santa Rosa, USA: Certified selenium and rhodium (Rh) stock standards (1000 mg/l). From Sigma–Aldrich, Deisenhofen, Germany: Selenite, selenate, selenomethionine (Se-Met), selenocystine (Se-Cys), Se-TrxR (EC 1.8.1.9.), Se-GPx (EC 232-749-6), human serum albumin (HSA), TRIS buffer, protein standards for SEC mass calibration (α-2 macroglobuline, ferritin, γ-globuline, transferrin, glutamine synthetase, lysozyme, glutathione-disulfide, glutathione (reduced), citrate). From Merck, Darmstadt, Germany: Ammonium acetate and acetic acid. From Air Liquide, Gröbenzell, Germany: Ar_{liq} and methane (99.999% purity).

2.2. Preparation of SePP from human serum as a standard

SePP is not commercially available but its purification from human plasma using affinity chromatography is described in literature [13,14] and this method has been applied successfully for human samples in the past [12]. Briefly: 200 μl serum were separated at 1 ml/min with a Heparin-affinity column (Amersham, GE Healthcare Europe GmbH, Munich, Germany) using a linear 12 min gradient of A = 50 mM Tris, 10 mM NH_4-acetate/acetic acid, pH 6.0 to buffer B = A but 800 mM NH_4-acetate, pH 8.5, and remaining at 100% B for another 8 min. SePP elution was monitored at 280 nm and Se was subsequently determined in fractions with FI–ICP–DRC–MS. For verification an aliquot of SePP fraction was analysed by SEC–ICP–DRC–MS, where it eluted at RT calculated for 60 kDa, which fits to literature data [15].

2.3. Animals and feeding strategy

The study was part of a comprehensive project, the general study design was presented previously [4]. Ten sheep were used for the examination presented here, briefly the study design was as follows: The adult Cameroon crossbreed sheep were fed either a total ration providing <0.05 mg Se/ kg dry weight (dw, n = 5, Se^-) representing a marginal Se supplementation or a total ration providing 0.2 mg Se/kg dw (n = 5; Se^+) representing a sufficient Se supplementation for sheep. All other nutrients were adequately provided according to the dietary recommendations for sheep; the composition of the diets were analysed (including total Se concentrations) according to routine national standardised chemical analysis of animal feed. The feeding regime was applied for a biennial examination period. General inspection and recording of food intake was done daily. The sheep were weighed monthly.

2.4. Blood and CSF collections

Due to one main focus of the comprehensive study, all sheep underwent general anaesthesia to perform computed tomography (CT) of the lungs [16] at day 750 of the study. As it is impossible to collect CSF by puncture of the ependymal canal in sheep without general anaesthesia due to resistance of the animals and subsequent risk of cerebrospinal damage, the anaesthetic stage (for the CT) was used to collect the CSF samples. The animals laid down in a prone position with the hips flexed and bending the hind legs in a cranial direction alongside the body. The skin in the lumbosacral area was shared and defatted with alcohol. The lumbosacral space was identified being at the midline depression between the last palpable dorsal lumbar spine and the first palpable sacral dorsal spine as described by [17]. A sterile needle (1.2 × 40 mm, Nipro Europe N.V., Zaventem, Belgium) was used to penetrate through the skin, subcutaneous tissue, the interarcuate ligament and the ligamentum flavum into the dorsal subarachnoid space. CSF welled up in the needle hub and was collected into a plain, sterile tube for serum samples (Sarstedt, Nümbrecht, Germany). The CSF was centrifuged at 10.000 × g for 5 min, aliqoted in small tubes, shock frozen into liquid nitrogen and stored at −80 °C until further analysis.

Serum was collected immediately before the collection of CSF from the Vena jugularis using a plain serum collection system (S-Monovette®, Sarstedt, Nümbrecht, Germany). The serum was allowed to clot at room temperature for at least 30 min. Within one hour after collection the serum samples were centrifuged at 10.000xg for 10 min. The serum was obtained by careful aspiration using a standard 1000 μl pipette (Eppendorf AG, Hamburg, Germany). The serum was aliqoted and transferred to new tubes. Finally, the serum was shock frozen in liquid nitrogen and store at −80 °C until further analysis.

2.5. Analysis of albumin

Albumin concentration in serum was analysed using the bromocresol green method (L + T, Eberhard Lehmann GmbH, Berlin, Germany; inter-assay coefficient of variation 3.2%, detection limit 2 g/l).

The CSF was centrifuged for 10 min at 200 × g (Rotafix 32, Hettich GmbH und Co., KG, Tuttlingen, Germany) to eliminate cell