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Association between plasma selenium level and NRF2 target genes expression in humans

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ABSTRACT

Animal studies in rodent and in vitro studies indicate compensatory role of nuclear factor (erythroid-derived 2)-like (Nrf2) and Nrf2-regulated antioxidant and phase II biotransformation enzymes for the dietary selenium (Se) deficiency or for the loss of selenoproteins.

To explore associations between plasma Se level and NRF2-regulated cytoprotective genes expression, an observational study was conducted in a population of 96 healthy non-smoking men living in Central Poland aged 18–83 years with relatively low plasma Se level. *NRF2*, *KEAP2*, *CAT*, *EPHX1*, *GCLC*, *GCLM*, *GPX2*, *GSR*, *GSTA1*, *GSTM1*, *GSTP1*, *GSTT1*, *HMOX1*, *NQO1*, *PRDX1*, *SOD1*, *SOD2*, *TXNRD1* transcript levels in peripheral blood leukocytes and polymorphism of *NRF2*-617C/A (rs6721961) in blood genomic DNA were determined by means of quantitative real-time PCR.

Mean plasma Se level was found to be 51.10 ± 15.25 μg/L (range 23.86–96.18 μg/L). *NRF2* mRNA level was positively correlated with expression of investigated NRF2-target genes. The multivariate linear regression adjusting for selenium status showed that plasma Se level was significantly inversely associated only with expression of *GSTP1* (β-coef. = −0.270, $p = 0.009$), *PRDXR1* (β-coef. = −0.245, $p = 0.017$) and *SOD2* with an inverse trend toward significance (β-coef. = −0.186, $p = 0.074$), but without an effect of *NRF2* gene variants. *NRF2* expression was inversely associated with age ($r = -0.23$, $p = 0.03$) and body mass index ($r = -0.29$, $p < 0.001$). The findings may suggest a possible link between plasma Se level and cytoprotective response at gene level in humans.

Introduction

Selenium (Se) is an essential trace element, responsible for various physiological and cellular processes in humans. The major role of Se is incorporation in catalytic sites of selenoproteins as selenocysteine – 21st proteinogenic aminoacid. Selenoproteins are further involved in maintaining redox balance which, when disturbed, can lead to various adverse health outcomes, including cancer and type 2 diabetes [1]. The (erythroid-derived 2)-like 2 (NFE2L2, Nrf2) nuclear factor is involved in redox functions via a signaling pathway, which controls the expression of various antioxidant and metabolic genes possessing antioxidant response element (ARE) sequence in their promoters [2,3]. Similarly to selenoproteins, up-regulation of various Nrf2-target cytoprotective genes in response to dietary compounds, xenobiotics and selected physical agents is involved in the mechanisms that enable adaptation to the redox state alterations.

Recently, various studies have indicated direct link between dietary Se supply and Nrf2-regulated cytoprotective enzymes. The relationship between Nrf2-modulated genes and Se status was observed for over-optimal/toxic and suboptimal levels. It was found that the administration of supraphysiological level of metabolites of Se resulted in increased activity of the enzymes of phase II biotransformation in rodents under chemically induced carcinogenesis [4] and Se toxicity in rats resulted in differential expression of Nrf2 targets [5]. Interestingly, during late 70s of XX century it was also observed that induction of phase II enzymes was related with Se deficiency in rat [6,7]. Over the next decades, several studies have indicated induction of Nrf2-target enzymes under low dietary Se supply, Se deficiency or loss of selenoproteins [5,8–18]. These results clearly indicate that decreased selenoprotein activity due to Se deficiency and also impaired

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selenoprotein expression is counterbalanced by increased activity of Nrf2-regulated cytoprotective system to prevent oxidative stress and maintain redox homeostasis [17,18].

Unfortunately, there is no accessible data on Se level and NRF2-target gene expression in humans with different dietary Se supply or plasma/serum Se concentration. It is generally considered that plasma glutathione peroxidase (GPX3) and plasma selenoprotein P1 (SEPP1) as the unique secreted selenoproteins can reflect Se level. The activity of GPX3 reaches a steady state with levels of Se intake that are lower than those required for the saturation of SEPP1. Se intake responsible for maximizing the activity of GPX3 occurs at a plasma Se concentration around 95 μg/L (range 89–114 μg/L) [19]. In a review of supplementation studies, the maximization of SEPP1 concentration was found to be associated with plasma Se concentration between 90 and 140 μg/L [20]. Therefore, the aim of our observational study was to test the hypothesis whether there is any association between plasma Se level in the population with relatively low Se supply (with respect to intake required to maximize GPX3 activity and SEPP1 concentration) and *NRF2*, *KEAP1*, *CAT*, *EPHX1*, *GCLC*, *GCLM*, *GPX2*, *GSR*, *GSTA1*, *GSTM1*, *GSTP1*, *GSTT1*, *HMOX1*, *NQO1*, *PRDX1*, *SOD1*, *SOD2*, *TXNRD1* gene expression in peripheral blood leukocytes.

Materials and methods

Study population

Healthy controls ($n = 96$) non-smoking males with mean age 60.3 ± 16.1 years (range 18–83 years) were recruited from Military Teaching Hospital in Lodz and Nofer Institute of Occupational Medicine in Lodz. The mean body mass index (BMI) was $27.16 \pm 3.82\ kg/m^3$ (range $19.69–38.51\ kg/m^3$). The Institutional Ethics Committee for Scientific Research approved the study protocol and a written informed consent was obtained from each participant of the study.

Plasma selenium determination

An inductively coupled plasma mass spectrometer (Elan DRC-e Perkin Elmer, SCIEX, USA) with dynamic reaction cell mode (DRC) was used for the determination of Se in plasma. Blood plasma was prepared by dilution in nitric acid (1%). A calibration curve was established for 1–200 μg/L range. Certificate reference material BCR-637 (IRMM, Geel, Belgium) was used in the analysis. Inter-assay precision of plasma Se determination expressed as coefficients of variability (CV) was 4.0% and intra-assay CV was 5.5%.

Gene expression analysis

NRF2 and 17 NRF2-targets selection: *KEAP1*, *CAT*, *EPHX1*, *GCLC*, *GCLM*, *GPX2*, *GSR*, *GSTA1*, *GSTM1*, *GSTP1*, *GSTT1*, *HMOX1*, *NQO1*, *PRDX1*, *SOD1*, *SOD2*, *TXNRD1* was made according to previous rodent studies data where associations between suboptimal Se supply and Nrf2-regulated genes were observed [5,14]. Total RNA was isolated from venous blood using QIAamp RNA Blood Mini Kit (Qiagen, Hilden, Germany). Transcript levels in peripheral blood leukocytes were determined by means of quantitative real-time PCR (qPCR). Primers for target genes were designed with Beacon Designer 7.0 (PREMIER Biosoft Int., Palo Alto, CA, USA) according to GenBank® genetic sequence database and amplicon sequence complied with exon-exon boundaries (Supplementary Table 1). The cDNA was synthesized on 200 ng RNA with Quantitect Kit (Qiagen, Hilden, Germany). PCR efficiencies were calculated using dilutions of five randomly selected pooled cDNA samples. All the samples were amplified in duplicate. Expression was quantified with FastStart SYBR Green Master (Roche, Basel, Switzerland) and using glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) as the endogenous control. To determine inter- and intra-assay variation, five cDNA samples (one sample per plate) in triplicate were run over three separate occasions by up to 30 days of the experiment. Inter-assay CV for 17 genes examined, calculated using relative expression values, were below 15% and intra-assay CV were below 10%. Gene expression data were evaluated by Pfaffl method [21] with reference gene-normalized relative quantification and efficiency correction using qbasePLUS software (Version: 2.3) (Biogazelle NV, Zwijnaarde, Belgium).

Table 1
Association between plasma selenium level, gene expression in circulating leukocytes and the *NRF2*-617 C/A (rs6721961) genetic polymorphism. Values of β (partial correlation coefficients) in the table were calculated using a linear regression model that incorporated both variables in the analysis.

	Selenium		*NRF2*-617CC vs. -617CA&AA	
	β-coef.	p	β-coef.	p
GSTP1	−0.270	0.009	0.096	0.367
PRDX1	−0.245	0.017	0.046	0.652
SOD2	−0.186	0.074	−0.012	0.915

Supplementary Table 1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jtemb.2014.11.008.

NRF2-617C/A (rs6721961) genotyping

Genomic DNA was isolated from buffy coats using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), in accordance with the manufacturer's instructions. Allelic discrimination of *NRF2* genotypes was performed with TaqMan® custom SNP assay using TaqMan® Genotyping Master Mix (Life Technologies, Carlsbad, CA, USA). Genotyping was performed using the control DNA samples with known genotypes and a negative control.

Statistical analysis

Univariate correlation analysis was performed using Spearman rank correlation test. All analyses of association between the *NRF2* polymorphic variants and gene expression were computed using multivariate linear regression adjusting for Se status. Log transformation of gene expression values was applied to normalize the distribution with STATISTICA 10 PL (StatSoft, Tulsa, OK, USA).

Results

Mean plasma Se level was 51.10 ± 15.25 μg/L (range 23.86–96.18 μg/L) and it was not associated with age ($r = -0.132$, $p = 0.198$), BMI ($r = -0.093$, $p = 0.369$). The highest level of gene transcripts in circulating leukocytes was observed for *SOD2*, while the lowest was attributable to *GSTA1*; *GPX2* transcripts were undetectable. Besides, we could assess *GSTA1* mRNA level in 92 samples, while *GSTM1* was found in 50 (52.1%) and *GSTT1* in 78 (81.3%) individuals, because of deletion polymorphism.

Inverse association was observed between *NRF2* expression and age ($r = -0.23$, $p = 0.03$), and BMI ($r = -0.29$, $p < 0.001$). Inverse associations between NRF2 targets and age were also found for *EPHX1* ($r = -0.47$, $p < 0.001$), *GSR* ($r = -0.27$, $p = 0.01$), *HMOX1* ($r = -0.24$, $p = 0.02$) and *TXNRD1* ($r = -0.22$, $p = 0.03$).

Multivariate linear regression adjusting for selenium status for *NRF2* and 16 NRF2-regulated gene expression indicated significant inverse relationships between plasma Se level and expression of *GSTP1*, *PRDXR1* and *SOD2* at the margin of statistical significance (Table 1, Fig. 1a–c). *NRF2*-617 C/A (rs6721961) genetic polymorphism, located in promoter region of *NRF2* gene had no impact on analyzed associations (Table 1).