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Selenium Supplementation Prevents Metabolic and Transcriptomic Responses to Cadmium in Mouse Lung

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Abstract

Background—The protective effect of selenium (Se) on cadmium (Cd) toxicity is well documented, but underlying mechanisms are unclear.

Methods—Male mice fed standard diet were given Cd (CdCl_2 , 18 $\mu\text{mol/L}$) in drinking water with or without Se (Na_2SeO_4 , 20 $\mu\text{mol/L}$) for 16 weeks. Lungs were analyzed for Cd concentration, transcriptomics and metabolomics. Data were analyzed with biostatistics, bioinformatics, pathway enrichment analysis, and combined transcriptome-metabolome-wide association study.

Results—Mice treated with Cd had higher lung Cd content (1.7 ± 0.4 pmol/mg protein) than control mice (0.8 ± 0.3 pmol/mg protein) or mice treated with Cd and Se (0.4 ± 0.1 pmol/mg protein). Gene set enrichment analysis of transcriptomics data showed that Se prevented Cd effects on inflammatory and myogenesis genes and diminished Cd effects on several other pathways. Similarly, Se prevented Cd-disrupted metabolic pathways in amino acid metabolism and urea cycle. Integrated transcriptome and metabolome network analysis showed that Cd treatment had a network structure with fewer gene-metabolite clusters compared to control. Centrality measurements showed that Se counteracted changes in a group of Cd-responsive genes including *Zdhhc11*, (protein-cysteine S-palmitoyltransferase), *Ighg1* (immunoglobulin heavy constant gamma-1) and associated changes in metabolite concentrations.

Conclusion—Co-administration of Se with Cd prevented Cd increase in lung and prevented Cd-associated pathway and network responses of the transcriptome and metabolome. Se protection against Cd toxicity in lung involves complex systems responses.

General Significance—Environmental Cd stimulates proinflammatory and profibrotic signaling. The present results indicate that dietary or supplemental Se could be useful to mitigate Cd toxicity.

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Keywords

Cadmium burden; Dietary supplement; integrative omics; nutritional requirements; selenium metabolism

1. Introduction

Selenium (Se) is an essential nutrient for humans and animals. It is incorporated into selenoproteins which function in a wide range of oxidation-reduction reactions, redox signaling, antioxidant defense, thyroid hormone metabolism, and immune responses [1–3]. An important role of Se in antioxidant defense has provided a basis to test Se supplementation for prevention of cancers, type 2 diabetes and cardiovascular disease [2,4]. Se supplementation has also been used as a strategy to mitigate toxicity of heavy metals, such as cadmium (Cd), thallium, inorganic mercury, and methylmercury, by blocking oxidative stress and/or modulating their interaction with biomolecules [3,5,6].

Cd is a ubiquitous environmental pollutant that causes multiple adverse health effects in lung, including cancer, chronic obstructive pulmonary disease and emphysema [7,8]. Increased lung cancer risks and comprised pulmonary function linked to occupational exposure and cigarette smoking are well established [9–11] [8,12]. More recent research in human lung cells and mouse models shows that Cd concentrations comparable to the lung Cd burden of nonsmoking Americans activates profibrotic [13–15] and proinflammatory signaling [16] and contributes to lung inflammation [17] and fibrosis [18]. The major exposure to Cd for nonsmoking humans is from diet, with an average intake estimated by the European Food Safety Authority to be approximately 19–21 µg per day [19]. Humans have no effective way to excrete Cd, and consequently, Cd has a long biological half-life of 10 – 30 years [8].

Interaction of Se with Cd is long known, and multiple approaches have been attempted to use Se as antidote for Cd toxicity [20]. Following the initial observation that selenite prevents Cd toxicity in the testes, experiments showed that Se and Cd can form a complex in approximately 1:1 atomic ratio in rat plasma after simultaneous injection with selenite. Biotransformation of selenite to selenide is required [6,20] and the complex further binds to selenoprotein P to form a ternary complex in the blood stream [21]. Cd interacts with other selenoproteins in tissues [22], and research shows that Se [4] protects against Cd toxicity [23] by restoring antioxidant enzyme activities [2], reducing lipid peroxidation [24–28], and increasing Cd interaction with zinc-coordinated thiolate (e.g. metallothionein) [29]. Much of the research focused on Cd levels from occupational exposures and cigarette smoking, however, and little information is available on the protective effect of Se on Cd toxicity at dietary intake levels.

Recent applications of omics methods to evaluate effects of Se supplementation on mouse liver showed that Se has very broad impacts on transcription and metabolism [30]. Transcriptomics measures changes in abundance of most transcripts, and high-resolution metabolomics (HRM) measures changes in metabolites from most metabolic pathways [31–34]. Integrated omics methods [35–37], such as transcriptome-metabolome wide association

study (TMWAS) [35], further allow integration of these data to define central hubs in complex systems [38–40]. The available data indicate that TMWAS could be sufficiently sensitive to reveal biologic responses to Se in alleviating adverse effects of low-dose Cd in lung.

The present study was to determine whether Se supplementation within an adequate intake range prevents transcriptome and metabolic effects of low-dose Cd in lungs of mice. The design included four groups of mice, a control group, a group receiving 2 mg/L Cd in drinking water (Cd group), a group receiving Cd with supplemental Se at 1.6 mg/L in drinking water (Cd+Se group), and a group receiving only Se in drinking water (Se group). The results showed that supplemental Se prevented many of the Cd-induced changes in transcripts, metabolites and associated functional pathways in lung. The results provide a strategy to evaluate potential beneficial effects of dietary or supplemental Se on adverse effects of toxic metals.

2. Materials and methods

2.1. Animals

Animal protocols were approved by the Institutional Animal Care and Use Committee at Emory University. Eight-week-old male C57BL/6J mice were purchased from Jackson Laboratory and housed under conventional conditions of 21–24 °C and 12 h light/dark cycle with commercial standard mouse diet (Laboratory Rodent Diet 5001, LabDiet, St. Louis, MO) containing 0.41 ppm Se [41] and 0.062 ppm Cd [42]. Forty mice were randomly divided (n=10/group) and assigned to vehicle control [purified water by reverse osmosis system, no detectable range of Cd (<0.0008 ppb) or Se (< 0.25 ppb)], Cd treatment [2.0 ppm Cd (18 µM CdCl₂) in purified water], Se treatment [1.6 ppm Se (20 µM Na₂SeO₄) in purified water] groups and Cd+Se treatment (2.0 ppm Cd and 1.6 ppm Se in purified water). All mice were provided with mouse diet and water *ad libitum* and maintained for 16 weeks; subsets were used for sample collections for different assays as described below. For assays not conducted on the day of tissue collection, samples were immediately frozen and maintained at –80 °C until assay. Mice were group housed, and individual food or water consumption was not determined. Urine samples were collected from the bladder at the time of tissue collection.

2.2. Cd measurement in human lung tissue

Cd levels were measured in 19 adult human lung tissues obtained from the Emory Transplant Center. Sample collection was approved by the Emory IRB protocol (CR10_IRB00006248). Excised tissues were stored at –80 °C until Cd measurement by ICP-MS as described below.

2.3. Quantification of Cd and Se

¹¹⁴Cd and ⁷⁷Se were measured in mouse lung and urine with inductively-coupled plasma mass spectrometry (ICP-MS, iCap Q, ThermoFisher Scientific) following procedures to measure trace metals that conformed to accuracy (100 ± 10%) and precision standards (RSD < 12%). Briefly, 50 mg lung samples were homogenized in 500 µL purified water and

digested in 2% nitric acid to a final volume of 10 mL. Se standard (1000 mg/L, 2% HNO₃) was purchased from Ricca Chemical (Arlington, TX) and diluted in series for standard curve. For urine analysis, samples were injected along with internal standards, nitric acid and hydrogen peroxide without digestion. Se concentration in urine was normalized to creatinine level (Creatinine urinary Colorimetric Assay, Cayman Chemical, Ann Arbor, MI).

Protein concentration was measured in 5 μ L of tissue homogenates using Bio-Rad DC kit. Indicators of oxidative stress, glutathione (GSH), glutathione disulfide (GSSG), cysteine (Cys), cystine (CySS) and calculated Nernst potentials (E_h) for the GSH/GSSG and Cys/CySS couples, were measured [43,44] but are not included because no significant effects were observed.

2.4. Transcriptomics

Total RNA was isolated from mouse lung ($n = 5/\text{group}$) with the mirVana RNA isolation kit (Life Technologies, Grand Island, NY) and stabilized in RNA_{later} (QIAGEN, Valencia, CA). The preserved RNA was hybridized on Affymetrix Mouse Gene ST 2.0 exon chips following NuGEN Ovation RNA Amplification. Robust multi-array average (RMA) was used to pre-process and summarize the chip data as an Affymetrix Expression set resulting in normalized expression levels of 33,793 transcripts. Among them, 10654 transcripts have been annotated with an official gene symbol, and these were used in the current study. To investigate the effect of Cd treatment and compare to that of Cd+Se treatment, Gene Set Enrichment Analysis (GSEA) was used to determine enriched gene sets and genes that differed between the biological states: Cd vs control and Cd+Se vs control [45]. Gene set enrichment statistics were obtained with the GSEA applet (v. 2.2; Broad Institute) with $P < 0.01$.

2.5. High-resolution metabolomics (HRM)

Ultra-high resolution mass spectrometry with liquid chromatography was used for metabolic profiling [38] of lung extracts from control ($n = 10$) and Se ($n = 10$) mice as described previously [38]. Samples were extracted at 0 °C by homogenization in acetonitrile:water (2:1) containing a mixture of stable isotope-labeled internal standards [46]. The acetonitrile:water mixture was added to a weighed tissue sample (approximately 20 mg tissue) at a ratio of 15 μ L/mg tissue. Following homogenization and incubation on ice for 30 min, extracts were centrifuged to remove protein [38], randomized, and 10 μ L aliquots were analyzed with three technical replicates using C18 liquid chromatography and mass spectrometry (50 – 2000 m/z) with positive electrospray ionization (ESI) on LTQ-Velos Orbitrap mass spectrometer (Thermo Fisher) [31,47]. Mass spectrometry data were extracted using apLCMS [48] and xMSanalyzer [49]. Data were pre-filtered to retain only features with non-zero values in > 80% in all samples, retaining 10,427 features. Limma test was used to select features that differed between Cd and control group. Significant features (raw $P < 0.05$ by limma test) were further studied by pathway enrichment analyses using *mummichog* [50]. This approach protects against type 2 statistical error by including all features at $P < 0.05$ and protects against type 1 statistical error by permutation testing in pathway enrichment analysis [51]. Hierarchical clustering analysis (HCA) and principal

component analysis (PCA) were used for untargeted comparison of the significant features associated with Cd (raw $P < 0.05$ by limma test) across all groups.

2.6. Transcriptome-metabolome wide association study (TMWAS)

Transcriptome and metabolome data from the same set of samples ($n = 5/\text{group}$) were integrated using xMWAS [36]. We used sparse partial least squares (sPLS) regression [52–54], a variable selection and dimensionality reduction method, to conduct pairwise correlation analysis between transcriptome (5 samples $\times$ 10,654 transcripts with official gene symbols) and metabolome (5 samples $\times$ 10,427 metabolic features selected by relative standard deviation). Selected metabolic features were mapped to metabolic pathways using *mummichog* [50] and annotated with xMSannotator [33] using HMDB (Human Metabolome Database) [55]. These approaches provide level 5 identification by the criteria of Schymanski et al [56]; confidence scores for annotation by xMSannotator are derived from a multistage clustering algorithm. Confirmed identities were obtained by co-elution and ion dissociation mass spectrometry (MS/MS) relative to authentic standards (level 1 identification by criteria of Schymanski et al [56]) or by MS/MS relative to METLIN spectral database [57] (level 2 identification by criteria of Schymanski et al [56]).

2.7. Statistics

Data are presented as the mean and standard error of the mean. Limma t -test and student's t test (two-tailed with Welch's correction for unequal variance) was used to test significance. The significance level was $P < 0.05$ for all univariate tests; Benjamini and Hochberg false discovery rate was used for multiple comparisons [58]. The data integration and association analysis was performed using xMWAS [36], which uses multivariate statistical methods for obtaining correlation. More details are described in the Supplement Section 1 for xMWAS [36].

3. Results

3.1. Se supplementation prevented Cd accumulation in lung

Se effects on Cd levels in mouse lung tissue and urine were measured after 16 weeks of exposure. The results showed that lung Cd levels were significantly higher in mice treated with Cd (1.7 ± 0.4 pmol/mg protein, Fig. 1A) compared to control (0.8 ± 0.3 pmol/mg protein) or Se (0.4 ± 0.1 pmol/mg protein) groups. The lung Cd content in the Cd group was similar to the range of 19 human lungs (1.3 ± 0.4 pmol/mg protein) analyzed with the same methods. In the Cd+Se group, the lung Cd content was significantly lower (0.2 ± 0.03 pmol/mg protein) than the Cd group (Fig. 1A), showing that oral Se prevented Cd deposition in lung.

Although data suggested that Se increased urinary Cd, this effect was not significant (Fig. 1B). Analysis of Se contents showed that increased levels of Se were observed in urine from Cd+Se and Se groups, but no increase in lung Se occurred in either group (Fig. 1C and 1D). These results showed that lung Se content had achieved a plateau from mouse diet [59] and was not increased by supplemental Se even though supplemental Se caused increased urinary Se content.

3.2. Se supplementation prevented lung transcriptomic response to Cd exposure

Low dose Cd causes dysregulation in inflammatory response and cell proliferation at transcriptional and translational levels [15,16,31]. To determine whether supplemental Se can protect against Cd-induced perturbation of the lung transcriptome, we performed gene set enrichment analysis (GSEA) on lung transcriptome data, initially comparing the Cd group to the control group. The results showed that nine pathways were altered in the lung transcriptome of Cd treated mice compared to controls ($P < 0.01$, Supplemental Table 1). Eight pathways were positively enriched (i.e. upregulation of most genes in the pathway) compared to control. These included coagulation, mammalian target of rapamycin complex 1 (mTORC1) signaling, myc target genes, unfolded protein response, cholesterol homeostasis, complement activation, tumor necrosis factor- α (TNF α) signaling via nuclear factor kappa-light-chain-enhancer of activated B cells (NF κ B), and inflammatory response. Myogenesis was negatively enriched (i.e. downregulation of most genes) in Cd treatment (Fig. 2A, dark grey bars) compared to the controls.

To examine the effect of Se on Cd-altered genes, lung transcriptomics data of Cd+Se mice were compared to the Cd group using GSEA. The results showed that Se supplementation diminished Cd effects on genes enriched in seven of nine pathways and resulted in responses that were similar to the control group (Fig. 2A, white bars). These seven pathways are coagulation, mTORC1 signaling, myc target genes, unfolded protein response, cholesterol homeostasis, complement activation and TNF α signaling. In the other two pathways, the transcriptome responses of the Cd+Se group were exaggerated for the negatively enriched pathways in inflammatory responses and positively enriched pathways in myogenesis. Thus, the gene expression responses to Cd+Se were in opposite directions to the changes in Cd group, supporting the interpretation that Se counteracted the effects of Cd on gene expression.

To visualize gene expression in response to Cd, Se, and Cd plus Se, we performed two-way hierarchical clustering analysis (HCA) and presented results with a heatmap (Fig. 2B). The heatmap shows the Robust Multi-array Average (RMA)-normalized expression levels of the 481 genes from the nine GSEA significant pathways associated with Cd-induced response. Overall Cd treatment downregulated a cluster of genes representing myogenesis (Cluster 1; 107 genes) and upregulated the rest of genes from other pathways (Cluster 2; 374 genes). Se supplementation with Cd prevented the Cd effects and resulted in levels of most genes being similar to controls and clustering together with the controls (Fig. 2B). Of the total 481 genes, 28 were significantly altered by Cd treatment ($P < 0.05$), and only 5 of these differed between the Cd + Se group and controls (Supplemental Table 2).

To more specifically examine these effects, we plotted the expression levels of representative genes from four pathways in which Se antagonized the effects of Cd. These selections (complement, TNF- α /NF- κ B signaling, inflammatory response and myogenesis) were based upon effects of Se on Cd responses in the present study and previous results showing Cd effects on complement and proinflammatory signaling [16,60] (Chandler et al, unpublished). The genes included ubiquitin carboxyl-terminal hydrolase 16 (*Usp16*), a specific tag for epigenetic transcriptional repression in complement cascade (Fig. 3A); atypical chemokine receptor 3 (*Cxcr7*), a chemokine receptor that controls chemokine levels and localization in

TNF α signaling (Fig. 3B); cyclin-dependent kinase inhibitor 1 (*Cdkn1a*), an inflammation response gene (Fig. 3C); and adenylate cyclase type 9 (*Adcy9*) catalyzing the formation of cAMP in response to activation of G protein-coupled receptors (Fig. 3D). The results demonstrated responses consistent with the GSEA. Low dose Cd also stimulates profibrotic signaling, contributes to pathogenesis of lung fibrosis and increases expression of epidermal growth factor receptor (*Egfr*), a critical signaling controller in onset and progression of lung fibrosis [61,62]. Consistent with Se effects on the four genes described above, the results showed that Se supplementation partially blocked the Cd-dependent increase in *Egfr* (Fig. 3E).

3.3 Se supplementation prevented lung metabolic response to Cd exposure

To determine whether Se prevented Cd-induced effects on metabolism, samples from the same lungs analyzed for transcriptomic responses were examined for metabolic responses. Previously, we and others found that metabolic pathways in amino acid and lipid metabolism are disrupted by Cd exposure [31,38,63]. To understand the effect of supplemental Se on metabolic disruption of Cd, we selected metabolic features that were significantly changed by Cd compared to control groups (number of metabolic features; 301, $P < 0.05$, Supplemental Table 3 and Supplemental Fig. 1A). Pathway enrichment analysis using *mummichog* software [50] showed that the 301 features were enriched in amino acid metabolism (aspartate, asparagine, tyrosine and tryptophan), as well as the urea cycle (Fig. 4A, $P < 0.05$). The detailed information of metabolites associated with these pathways are provided in Supplemental Table 4. Several key metabolites were decreased in abundance by Cd treatment, including lysine (an essential amino acid with relatively high nitrogen content, $P < 0.05$), N-acetylglutamate (an enzyme cofactor of ureagenesis and activated by arginine, $P < 0.05$), dopa (metabolite of tyrosine, $P < 0.05$) and 3-hydroxyanthranilate (metabolite of tryptophan, $P < 0.1$), and these effects were reversed by supplemental Se (Fig. 4B – 4E).

To visualize effects of Se on Cd-dependent effects on metabolites, we performed 2-way HCA on the 301 metabolic features across all groups. Results showed that Cd+Se, Se and control groups clustered together while the Cd group formed a separate cluster (Supplemental Figure 1B). These results support the interpretation that Se supplementation prevented effects induced by Cd. Principal component analysis (PCA) also showed that the Cd+Se group clustered more closely to controls than the Cd group (see the overlap of 95% confident group area in Supplemental Fig. 1C). Together, these data show that co-exposure to Se prevented Cd-dependent impact on lung transcriptome and metabolome.

3.4. Se supplementation prevented Cd effects in the integrated network structure of transcriptome and metabolome

To visualize supplemental Se effects on Cd-altered transcriptome and metabolome, we used xMWAS [36] to integrate transcriptome and metabolome responses. Networks were obtained from correlations of the 10,428 metabolic features with the 10,654 transcripts identified in all four treatment groups (Fig. 5). With lower stringency criteria (e.g., $|\rho| > 0.5$), the network structures were complex (not shown), so higher correlation threshold ($|\rho| > 0.8$) was used. At high stringency, network substructures represent central hubs of strongly associated transcripts and metabolites (Fig. 5). The control condition showed multiple

communities, including a major hub (lower left corner of Fig. 5A) and other transcripts and metabolites with scattered connections, consistent with loose interconnected network structures that are relatively stable to perturbations. In contrast, the Cd group had a more condensed network structure (Fig. 5B) with strong correlations of transcripts and metabolites. Such tight communities tend to have less flexibility. In the Cd+Se group, the community network structure was similar to the more stabilized multi-community network found in the control, although fewer connections were present (Fig. 5C).

We used the eigenvector centrality measure in xMWAS to evaluate and compare the importance of genes between different conditions [36]. We found that 28 genes had the largest change between control and Cd groups (delta centrality ($|ECM_{\text{cadmium}} - ECM_{\text{control}}|$) > 0.8) (Supplemental Table 5). Twenty-one genes gained importance in Cd network indicated by increased centrality score, while seven lost importance compared to control indicated by decreased centrality score. Out of the total 28 genes, 19 showed similar centrality between Cd+Se and control, indicating that Se supplementation prevented the network change induced by Cd. Notably, zinc finger DHHC-type containing 11 (*Zdhhc11*), a gene encoding a protein-cysteine S-palmitoyltransferase, had a large increase in centrality in the Cd group. In the control network, *Zdhhc11* was associated with only a few metabolites (positive correlation: 0; negative correlation: 5; Fig. 5A). By contrast, the number of correlations in the Cd network at $|\rho| > 0.8$ increased to 24 positive and 127 negative correlations (Fig. 5B). In the Cd+Se network, the number of correlations decreased to 4 (negative) and 18 (positive). These changes indicate that Cd altered a few genes (e.g. *Zdhhc11*) that are closely related to the metabolic disruption, and supplemental Se inhibited this Cd effect to maintain the homeostasis of lung transcriptome and metabolome.

Comparison of the Se group (Fig. 5D) to the Cd group (Fig. 5A) showed that Se and Cd appeared to cause opposite effects on the transcriptome-metabolome network structure. In the Cd network, correlations were mostly negative (blue edges) while in the Se network, a major hub consisted of only positive correlations (red edges). For example, the correlations of metabolites with the transcript for immunoglobulin heavy constant gamma 1 (*Ighg1*) were mostly negative (171 negative vs 27 positive correlations) in the Cd network, while all correlations in the Se network was positive (0 negative vs 49 positive correlations). The reverse effect of Se (compared to Cd) on *Ighg1* and its associated metabolites is shown in Fig. 6. The intensities of mass spectral features of lipophilic molecules with accurate mass matches (Schymanski Level 5 identification) to ganglioside GD3 (Fig. 6A) and cardiolipin (78:5) (Fig. 6B) were negatively correlated with *Ighg1* abundance in Cd treated animals, and positively correlated in control, Se and Cd + Se groups. Comparison of the Se group to controls further showed that fatty acid and lipid metabolism pathways were enriched in the lung metabolome of Se supplemented mice (*mummichog* results in Supplemental Table 6). Thus, the results show that Se prevention of Cd-induced effects on the metabolome are likely to occur both by preventing increased Cd burden in the lung and also by having opposing effects on lipid metabolism.

4. Discussion

The present study was to determine whether Se supplementation within an adequate intake range prevents transcriptome and metabolic effects of low-dose Cd in lungs of mice. Relatively high dose Cd from occupational exposures or cigarette smoking is a well-known cause of lung toxicities, and previous research in mouse models shows that Se supplementation protects against toxicity at high exposures [27,28]. More recent epidemiologic data indicate, however, that lower levels of Cd from environmental sources can also pose health risks [64]. Mouse models to study low-dose Cd are complicated because of the low absorption rates of dietary Cd and 10- to 30-year biological half-life of Cd in humans. In the present studies, the data show that the Cd exposure at 18 $\mu\text{mol/L}$ in drinking water resulted in a lung burden of 1.7 ± 0.4 pmol Cd/mg protein, which is lower than cumulative human lung content reported previously [42,65] and similar to the mean concentrations of 19 human lung samples (1.28 ± 0.41 pmol/mg protein) measured with the same instrumentation and analytic methods. Other studies with similar dosing in mice show that Cd exposures in drinking water at 54 $\mu\text{mol/L}$ caused fatty liver [66] and increased airway hyper-reactivity in lung [42] at 1.6 and 5.4 $\mu\text{mol/L}$, Cd caused lung fibrosis [14], and at 5.4 $\mu\text{mol/L}$ potentiated inflammation and lethality of H1N1 influenza virus (Chandler et al, unpublished observations). Thus, the results show that the Cd dose in the present study is suitable to test utility of Se in preventing Cd-induced responses at lung Cd burden similar to that found in non-smoking adults without occupational exposures.

To select an appropriate supplemental dose of Se in the mouse model, we examined conditions relevant to human Se exposures. The adequate intake range for humans is approximated by the span between the recommended dietary allowance (RDA) and the tolerable upper intake level (UL); this intake range has received growing attention for health benefits and risks from Se supplementation [4]. The human RDA for Se is 55 $\mu\text{g/day}$, and the UL is 7.3-fold higher (400 $\mu\text{g/day}$) [67]. Mouse diet contains 0.41 ppm Se (0.07 mg/kg body weight) and meets the minimal requirement for mice [68]. To test for Se protection against Cd, we noted that maximal scavenging of Cd would likely occur via a 1:1 ratio of Se:Cd to support formation of a Cd-Se complex [20]. We selected a slight excess of Se (20 $\mu\text{M Na}_2\text{SeO}_4$:18 $\mu\text{M CdCl}_2$) in drinking water) to provide this ratio. This dose (0.32 mg/kg body weight per day) included Se from diet (0.41 ppm Se) and drinking water (1.6 ppm) and was approximately 4-fold higher than controls receiving Se solely from diet (0.07 mg/kg body weight per day). Thus, the range between controls and Se supplemented mice is similar to the adequate intake range for humans. The Se supplementation dose of 0.32 mg/kg body weight per day for mice is under the no-observable adverse effect level (NOAEL) for the more toxic selenite [3], and represents a relatively high Se supplement level in animal nutritional research [59,69]. Inclusion of both Na_2SeO_4 and CdCl_2 in drinking water provided a convenient way to deliver a constant ratio of Se to Cd and was considered suitable because Cd is not redox-active and selenate must be reduced to form a complex with Cd. Thus, the conditions selected to test for Se effects on Cd responses in the mouse model appear relevant to both Cd and Se exposures in humans.

Cd and Se interact with many macromolecules and transporters, in addition to having direct Se-Cd interactions and widespread effects on redox systems. Because of such diverse

effects, we used a systems level integrated omics approach [70] to test for effects of Se on changes induced by Cd. This has an advantage over targeted analyses because signals are measured from most transcripts and metabolites from most pathways. Additionally, use of GSEA for transcriptomics and *mummichog* pathway enrichment analysis for metabolomics allows detection of pathway and network effects that are otherwise difficult to detect and/or interpret. Both transcriptomics and metabolomics data showed effects of Cd that were consistent with earlier research, such as studies in which low-dose Cd caused a wide range of oxidation of lung proteins [15] and disruption of zinc finger proteins, with formation of mixed complexes, incomplete coordination and oxidation of cysteine residues [71]. Se could impact these through direct interactions or indirectly through anti-oxidative enzymes, such as glutathione peroxidase and thioredoxin reductase, which modify protein thiols and modulate redox-regulated transcription factors [2,72,73].

The integrated TMWAS network analysis showed that a zinc finger DHHC domain-containing protein, *Zdhhc11*, was present within the structure responsive to both Cd and Se. This protein is in an enzyme family responsible for attachment of different fatty acids onto protein cysteine residues (S-acylation posttranslational modification) [74]. Although more mechanistic research is needed, the results suggest that an antagonistic effect of Se on Cd toxicity could involve deactivation/activation of this zinc finger protein.

Previous research on supplemental Se effects on mouse liver transcriptome and metabolome showed that 0.32 mg/kg body weight per day alters lipid metabolism and energy regulation pathways [30]. Expression levels of 80 genes for cholesterol homeostasis, pancreatic β cell signaling, and fatty acid metabolism were elevated by supplemental Se. The present results with lung show similar effects of Se on activation of fatty acid metabolism pathways (Supplemental Table 6). The changes of the lung transcriptome in the gene set of cholesterol homeostasis were minor, however, and did not reach statistical significance ($P = 0.057$). Additionally, the lung transcriptomic effects showed enrichment of gene sets in immunity regulation such as interferon γ (IFN γ) signaling and Myc target genes ($P < 0.01$). This shows that Se supplementation exerts differential effects in different organs. Such differences could relate to tissue differences in lipid metabolism for energy, surfactant production and/or signaling, either of which could be important in the Se-dependent antagonism of Cd effects. As the roles of lipid mediators in resolution of lung inflammatory responses are being elucidated in clinical and mechanistic research [75], the interaction of transcriptome and metabolome in response to Se supplementation can be better understood to improve the assessment of its health benefits and risks.

Earlier experiments in rats provide a foundation for using Cd-Se interaction to reduce Cd bioavailability [76,77]. Administered Se as selenate or selenite was metabolized to a reduced form of Se (probably hydrogen selenide) and then formed a colloidal Cd-Se complex that contained approximately 1:1 atomic ratio of Cd and Se. The specific forms and stability of the complex may vary, but the evidence indicates that formation of Cd-Se complex in plasma drastically increases Cd content in plasma (30–50 fold over control) and diverts Cd from organ and tissue targets [78]. Although we used lower doses and did not measure plasma Cd concentrations, retention of Cd in plasma by Cd-Se complex could explain the measured decrease in Cd in the lung and contribute to the protective effect of supplemental Se.

Remaining questions exist, however, because urinary Cd excretion with Se supplementation was increased compared to control ($P = 0.05$) but not compared to the Cd group ($P > 0.2$). As Cd and Se form colloidal complex with molecular weight of 130,000 and 330,000 in rat plasma [76], the complex may be poorly filtered and accumulate in kidneys or other tissues.

In addition to this critical question concerning the fate of Cd-Se complexes, the current study does not address whether Se can protect against or reverse effects of pre-existing Cd burden in lungs. This question is important because of the potential benefit to removing lung Cd in former smokers. Prior research shows that the interaction of Cd^{2+} and Se^{2-} is sufficiently strong for Se to scavenge albumin-bound Cd but not for Cd to bind to Se when Se was bound to plasma proteins before addition of Cd [76]. Whether Se can bind and reverse effects caused by pre-deposited lung Cd are currently unknown. A comparison of results in Fig 5D and 5A suggest that this could occur, but more detailed studies will be needed to determine whether such effects could be beneficial and/or have adverse effects due to re-distribution of Cd to other tissues.

5. Conclusion

Concurrent Se supplementation with low-dose Cd in mice prevented accumulation of Cd in the lung, alleviated Cd-induced gene dysregulation in inflammation, myogenesis and other pathways, and diminished Cd-associated changes in metabolic pathways. Cd and Se alone induced opposite correlations of transcripts and lipid metabolites, respectively, indicating that the protective effects of Se on lung transcriptome are linked to responses in lipid metabolism. Integrated transcriptome-metabolome wide association study (TMWAS) further showed that Cd caused a more condensed network structure that was prevented by supplemental Se. Central hub genes included *Zdhhc11* (protein-cysteine S-palmitoyltransferase) and *Ighg1* (immunoglobulin heavy constant gamma-1), reinforcing the interpretation that Cd toxicity in lung involves complex systems responses that are counteracted by Se. Future studies are needed to determine whether Se supplementation could be used as a strategy to protect against or reverse toxic effects of high Cd burden in lung.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

Cd	cadmium
Cys	cysteine
CySS	cystine

ECM	Eigenvector centrality measure
FDR	false discovery rate
GSEA	gene set enrichment analysis
GSH	glutathione
GSSG	glutathione disulfide
HCA	hierarchical clustering analysis
HMDA	human metabolome database
ICP-MS	inductively-coupled plasma-mass spectrometer
Ighg1	immunoglobulin heavy constant gamma 1
MS	mass spectrometry,
mTORC1	mammalian target of rapamycin complex 1
MWAS	metabolome-wide association study
<i>m/z</i>	mass over charge
NOAEL	no-observed-adverse-effect-level
PCA	principal component analysis
RDA	recommended dietary allowance
RMA	robust multi-array average
RSD	relative standard deviation
RT	retention time
Se	selenium
sPLS	sparse partial least squares
TMWAS	transcriptome-metabolome-wide association study
UL	tolerable upper intake level
Zdhhc11	zinc finger DHHC-type containing 11

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Highlights

- Se supplementation with low-dose Cd in mice prevented accumulation of Cd in the lung
- Se supplementation alleviated Cd-induced gene dysregulation in inflammation, myogenesis and other pathways, and diminished Cd-associated changes in metabolic pathways
- Se counteracted Cd toxicity pathways in lung involving complex systems responses

Fig. 1. Effects of co-administration of Cd and Se on mouse lung and urinary contents of Cd and Se

Lung Cd (**A**), urine Cd (**B**), lung Se (**C**) and urine Se (**D**) concentrations in male C57BL/6 mice that consumed water without (Control) or with 18 μ M CdCl₂ only (Cd) or 20 μ M Na₂SeO₄ only (Se), or with both Cd and Se (Cd+Se) for 16 weeks. Values are means $\pm$ SEMs, n=10 per group.

Fig. 2. Impact of co-administration of Se on gene sets altered by Cd

A. Gene sets that were enriched in the lung transcriptome of Cd and Cd+Se groups, respectively, compared to control ($P < 0.01$) ($n=5$). **B.** Heat map shows genes that contributed to the enrichment of pathways. Cluster 1: genes downregulated by Cd; cluster 2: genes upregulated by Cd. Gene identities are provided in Supplemental Table 2.

Fig. 3. Effect of co-administration of Se and Cd on abundance of representative transcripts for complement, TNF- α /NF- κ B, inflammation, myogenesis and fibrosis pathways

A. Ubiquitin carboxyl-terminal hydrolase 16 (*Usp16*) is a specific tag for epigenetic transcriptional repression in the complement cascade. **B.** Atypical chemokine receptor 3(*Cxcr7*) is a chemokine receptor that controls chemokine levels and localization in TNF- α signaling. **C.** Cyclin-dependent kinase inhibitor 1 (*Cdkn1a*) is an inflammation response gene. **D.** Adenylate cyclase type 9 (*Adcy9*) catalyzes the formation of cAMP in response to activation of G protein-coupled receptors in myogenesis. **E.** Epidermal growth factor receptor (*Egfr*) is a critical signaling controller in onset and progression of lung fibrosis. Values are Robust Multi-array Average (RMA) normalized expression levels (n=5). Significance was tested by limma t-test. * $P < 0.05$ for comparison between control (Cont) and Cd groups; # $P < 0.05$ for comparison between Cd+Se and Cd groups.

Fig. 4. Cd and Se effects on lung metabolism in mice

A. *Mummichog* pathway enrichment analysis for 301 mass spectral features that differed between control and Cd treatment groups. **B-E.** Representative features for each pathway. **B.** Lysine is a basic amino acid with relatively high nitrogen content for ureagenesis. **C.** N-Acetylglutamate is an enzyme cofactor of ureagenesis and activated by arginine. **D.** Dopa is a metabolite of tyrosine. **E.** 3-Hydroxyanthranilate is metabolite of tryptophan. Significance was tested by limma t-test. * $P < 0.05$ for comparison between control (Cont) and Cd groups. For E, results showed a trend † $P < 0.05$. Additional information is available in Supplemental Tables 1A and 3.

Fig. 5. Integrated Metabolome-Transcriptome-wide association study (TMWAS) of mouse lung following Cd and Se treatments

Central network structures were obtained for 10,428 metabolic features and 10,654 transcripts using xMWAS with a high stringency ($|\rho| > 0.8$). **A.** Control mouse lung showed relatively loose distribution of network structures suggestive of high flexibility and adaptability. The transcript for immunoglobulin heavy constant gamma 1 (*Ighg1*) was present within the large central hub, suggesting a central relationship in transcriptional and metabolic regulation. **B.** The network structure in mice treated with low-dose Cd was relatively condensed compared to controls, indicating a more rigid and less adaptable structure. The zinc finger DHHC-type containing 11 gene (*Zdhhc11*) encoding a protein-cysteine S-palmitoyltransferase, which was peripheral to the central hub in the control, was present along with *Ighg1* in the condensed central hub. **C.** The network structure in the Cd +Se group showed a more dispersed structure with separate hubs for *Zdhhc11* and *Ighg1*. Although not fully restoring the control structure, the general appearance was more reflective of the control and the Se treatment than that of the Cd group. **D.** The Se group had two central hubs, with the *Zdhhc11* hub being greater than the hub containing *Ighg1*. The results suggest that Se has an effect on the transcriptome-metabolome interaction structure that is opposite to that of Cd. This raises the possibility that Se is not functioning simply to block effects of Cd but rather to elicit opposing network responses. Orange squares,

metabolites; green circles, transcripts; red edges, positive associations between metabolites and transcripts; blue edges, negative associations.

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Fig. 6. Se and Cd caused opposite correlation patterns for selected transcripts and metabolites Metabolites selected were annotated using xMSannotator [33] with confidence levels of 2 or higher. Values for the transcripts represent RMA expression levels, and values for the metabolites represent $\log_2$ transformed, quantile normalized mass spectral intensities. **A.** Correlations between the abundance of ganglioside and the expression levels of *IgHg1* (Immunoglobulin heavy constant gamma 1) differ between the Cd group and the other treatment groups (control, Cd+Se, Se). **B.** Correlations between the abundance of cardiolipin and the expression levels of *IgHg1* (Immunoglobulin heavy constant gamma 1) differ between the Cd group and the other treatment groups (control, Cd+Se, Se).