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EFFECT OF SELENIUM SUPPLEMENTATION ON PROTEOMIC SERUM BIOMARKERS IN ELDERLY MEN

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Abstract

Objectives—To determine the effect of selenium supplementation on the human proteomic profile.

Design—Serum samples were collected in this pilot study from a randomized placebo controlled Phase 2 clinical trial (Watchful Waiting (WW)).

Setting—Subjects were followed every three months for up to five years at the University of Arizona Prostate Cancer Prevention Program.

Participants—One hundred and forty men (age < 85 years) had biopsy-proven prostate cancer, a Gleason sum score less than eight, no metastatic cancer, and no prior treatment for prostate cancer.

Intervention—As part of the WW trial, men were randomized to placebo, selenium 200 μg/day or selenium 800 μg/day. For the purpose of the current study, 40 subjects enrolled in the WW study (20 from the placebo group and 20 from Se 800 μg/day group) were selected.

Measurements—Baseline serum samples were collected at each follow-up visit and stored at −80 degrees Celsius. A multiplexed proteomic panel investigated changes in 120 proteins markers simultaneously.

Results—Thirteen proteins (Apolipoprotein J, IL-10, IL-1 alpha, MMP-3, IL-12p70, IL-2 receptor alpha, cathepsin B, eotaxin, EGFR, FGF-basic, myeloperoxidase, RANTES, TGF-beta)

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were determined to be either statistically (p-value < 0.05) or marginally significantly (0.05 < p-value <0.1) changed in the selenium supplemented group as compared to placebo.

Conclusion—Although independent validation of these results is needed, this study is the first of its kind to utilize high throughput fluorescence based protein multiplex panel in analyzing changes in the proteomic profile due to selenium supplementation. Results from this study provide insight into the ability of selenium to modulate numerous protein markers and thus impact various biological processes in humans.

Keywords

Selenium supplementation; proteomic biomarkers; elderly men

Introduction

Selenium is an important trace element that has a role in various biologic processes and disease states (1). Unlike other elements which interact with proteins mainly as co-factors (2), selenium acts by co-translational incorporation into proteins molecules. It has been described as having anti-oxidative, chemopreventive, anti-inflammatory and anti-viral activity (1) and is hypothesized to have a role in multiple systems such as thyroid function, immune function, development of virulence and inhibiting HIV progression to AIDS, sperm motility and reducing the risk of miscarriage (2). Studies have also linked selenium to adverse mood disorders and cardiovascular diseases (1). Due its plausible roles in various important processes such as those mentioned above, it is important to understand the effect that selenium supplementation has on the human proteomic profile. Hence, using serum samples collected as part of a randomized placebo controlled Phase 2 clinical trial, in this pilot study, we investigated the effect of selenium supplementation on 120 proteomic biomarkers in elderly men.

Methods

Data for this study was obtained from samples collected as part of the Watchful Waiting (WW) study, a randomized double-blind placebo-controlled multi-center Phase 2 clinical trial conducted in men with localized prostate cancer. Details of the Watchful Waiting study design and its results have been published earlier (3, 4). All human subjects signed an informed consent and experimental protocols were approved by the University of Arizona Institutional Review Board. The primary aim of the WW study was to assess if subjects who were supplemented with selenium show lower prostate specific antigen (PSA) velocity (rate of PSA change over time) as compared to subjects who received placebo. PSA velocity is a commonly used clinical marker of prostate cancer progression. Study subjects had biopsy-proven prostate cancer within 48 months of enrollment in this study, a Gleason sum score less than eight, no metastatic cancer, and no prior treatment for prostate cancer. PSA level was ≤ 50 ng/ml, age < 85 years, and life expectancy of subjects was at least three years. One hundred and forty men were randomized to placebo (n=46), selenium 200 μg/day (n=47) or selenium 800 μg/day (n=47). Subjects were followed every three months for up to five years. Serum samples were collected at baseline and each follow-up visit for measurement of PSA. Additional serum was collected at baseline and at each follow-up visit for future

research projects and stored at −80 degrees Celsius. Results from the WW study indicated that PSA velocity in men supplemented with selenium was not statistically significantly different from PSA velocity in men on placebo (4). The primary aim of the current study is to determine the effect of selenium supplementation on 120 biomarkers. Sensitivity of this study was increased by comparing the two extremes. 40 subjects enrolled in the WW study (20 from the placebo group and 20 from Se 800 μg/day group) were randomly selected. Serum samples from baseline (pre-supplementation) and two year visits (post-supplementation) were analyzed using a multiplexed panel that measured expression levels of 120 proteins simultaneously (Whatman, part of GE healthcare). Each analyte was analyzed in duplicate and mean fluorescence values were calculated. These fluorescence values were analyzed using parametric (independent t-test) and non-parametric (rank-sum test) statistical techniques to identify analytes that demonstrated statistical ($p<0.05$) or marginal ($0.05<p<0.1$) significant change between the baseline and two year samples in the selenium supplemented group as compared to placebo. Since this is an exploratory study, a liberal approach was used while analyzing data and hence subjects demonstrating statistical or marginal significance were included in the analysis. Statistical analysis was carried out using Stata12 analytical software (Statacorp, College Station, TX).

Results

Table 1 demonstrates the distribution of baseline characteristics for the subjects selected for this study as compared to subjects in its parent study. This table shows that the study sample for the current study is consistent with the rest of the subjects in the WW study. In addition to the t-test, linear regression was also conducted. Parametric analysis indicated the following markers to be statistically ($p< 0.05$) or marginally significantly ($0.05 < p < 0.1$) different between the selenium supplemented group and placebo (table 2): Apolipoprotein J, IL-10, IL-1alpha, MMP-3, IL-12p70, IL-2 receptor alpha, PDGF (all isoforms). While non-parametric analysis indicated the following markers to be statistically ($p< 0.05$) or marginally significantly ($0.05 < p < 0.1$) different between the selenium supplemented group and placebo (table 3): Apolipoprotein J, Cathepsin B, eotaxin, EGFR, FGF-basic, IL-10, IL-1 receptor alpha, MMP-3, myeloperoxidase, RANTES, TGF-beta.

Discussion

In this systematic experiment designed to analyze the effect of selenium supplementation on proteomic markers in elderly men using a novel multiplexed assay, results indicated that as compared to placebo, selenium supplementation altered the serum levels of thirteen markers (Apolipoprotein J, IL-10, IL-1alpha, MMP-3, IL-12p70, IL-2 receptor alpha, cathepsin B, eotaxin, EGFR, FGF-basic, myeloperoxidase, RANTES and TGF-beta).

These analytes play important roles in multiple systems. Apolipoprotein J, also known as clusterin, is a glycoprotein that serves multiple functions based on its interactions between different molecules (5). These functions include sperm maturation, tissue remodeling, apoptosis, lipid transport, and defense against damaged neurons (6). Interleukin-10 (IL-10) is a cytokine with multiple effects in immune and inflammatory response regulation. It inhibits the function of T cells, monocytes and macrophages. Additionally, IL-10 regulates

proliferation of B cells, natural killer cells, cytotoxic and helper T cells, mast cells, granulocytes, dendritic cells, keratinocytes, and endothelial cells (7). Interleukin-1alpha (IL-1alpha) is one of the many cytokines represented by the Interleukin-1 family. High levels of IL-1alpha are found in keratinocytes and necrotic cells (8). IL-1alpha, along with its agonist (IL-1beta), is released during programmed cell death; however only IL-1alpha plays a role in malignant cell death activation (9). Matrix metalloproteinases (MMPs) are a family of proteases which deconstruct extracellular matrices. MMPs, especially MMP-3, play an important role in tumor development (10). Interleukin-12p70 (IL-12p70) is a cytokine that promotes T cell responses and macrophage activation for tumor rejection. It increases dendritic cell ability to induce T helper 1 cells and cytotoxic T lymphocytes (11). Interleukin – 2 receptor alpha (IL-2R alpha) is an inflammatory marker (12). It functions to regulate the sensitivity of lymphocytes to Interleukin-2 (13). Cathepsin B is a cysteine involved in the proteolytic processing of peptides and proteins that are endocytosed or phagocytosed (14). Eotaxin is a chemokine that has been shown to induce allergic responses by selectively recruiting eosinophils (15). Cell growth is mediated by members of the epidermal growth factor family, which bind to receptors on the surface of the cell to induce cell proliferation. Epidermal growth factor receptor (EGFR) becomes dimerized which leads to the autophosphorylation of tyrosine (16). Neuron growth and survival in the central nervous system is influenced by basic fibroblast growth factor (FGF-basic) (17). Myeloperoxidase is a heme protein that is secreted by neutrophils, monocytes and macrophages. It has been shown that myeloperoxidase promotes lipid peroxidation by serving as an enzyme in vitro (18). RANTES (Regulated on Activation, Normal T cell Expressed and Secreted), as the name suggests, is a crucial part for normal T cell function. Monocytes and T cells are also recruited by RANTES at inflammation sites (19). Transforming growth factor-beta (TGF-beta) plays a role in immunity by inhibiting cytokine production and T cell proliferation (20).

There are very few studies currently in the literature that have investigated the effect of selenium supplementation using a proteomic approach. Mahn et al investigated the effect on proteome by supplementing rats with sodium-selenate (21). Six rats were fed a basic diet supplemented with sodium-selenate and a control group consisting of six rats was fed a diet that met the minimum selenium requirements for ten weeks. Matrix-assisted laser desorption/ionization-time-of-flight mass spectrometer (MALDI-TOF) was used to both quantify the changes in the abundance of some plasmatic proteins and to identify them. Fibrinogen, apolipoproteins, haptoglobin, and transthyretin changed their abundance significantly due to selenium administration. In another study, Sinha et al using mass spectrometry, identified 1496 proteins in serum samples from 17 normal adult men after being supplemented with 247 μg/day selenized yeast for nine months as compared to serum samples of 19 men on placebo. Eight proteins were upregulated (clusterin isoform 1 [CLU], transthyretin, α-1B-glycoprotein, transferrin, complement component 4B proprotein, isocitrate dehydrogenase, haptoglobin, and keratin 1) and three proteins were downregulated (α-1 antitrypsin [AAT], angiotensin precursor, and albumin precursor) by selenized yeast. The unbalanced study groups and small sample size could lead to reduction in the confidence for these results (22). Standardized assays for proteomic assessment are needed in order to compare results between these studies.

Although the influence of small sample size and multiple comparisons cannot be ignored in our study, this study is the first of its kind to utilize high throughput fluorescence based protein multiplex panel in analyzing changes in the proteomic profile due to selenium supplementation. Validation of these results by a separate platform is needed. This study gives crucial insight into the ability of selenium to modulate important protein markers in humans. Markers identified as modulated by selenium have important roles in various biological processes in humans. Thus, selenium has the ability to influence numerous biologic processes through these proteins.

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Table 1

Distribution of baseline variable for subjects chosen for this study as compared to its parent population (WW study)

Variable	Parent Study Population (WW study) (mean, SD) (N = 140)	Current Study population (mean, SD)			p-value
		Placebo (N = 20)	800 μg/day (N = 20)	Total (N = 40)	
Baseline selenium	134.50 (41.49)	124.9 (18.7)	127.2 (15.8)	126.0 (17.0)	0.21
Age	72.8 (6.7)	72.9 (7.8)	72.2 (6.3)	72.5 (7.0)	0.80
Body mass index	26.9 (4.0)	27.0 (3.3)	28.3 (4.3)	27.6 (3.8)	0.32
Pack-years of smoking	23.8 (29.8)	20.8 (27.6)	23.5 (32.9)	22.2 (30.0)	0.77
Gleason score	5.7 (1.0)	5.9 (0.6)	5.8 (0.79)	5.8 (0.7)	0.55

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Table 2

Analytes in which the mean difference (between baseline and 2yr samples) was statistically significantly ($p<0.05$) or marginally significantly ($0.05 < p < 1.00$) different in the selenium group as compared to placebo

Analyte	Coefficient	p-value
Apolipoprotein J	723.35	0.032
IL-10	146.44	0.048
IL-1-alpha	349.26	0.043
MMP3	150.86	0.026
Eotaxin	474.01	0.086
IL-12-p70	207.70	0.088
IL-2 receptor alpha	121.31	0.065
PDGF	283.40	0.096

Table 3

Results from non-parametric analysis (Wilcoxon-rank sum test) to determine analytes that are statistically significantly ($p<0.05$) or marginally significantly ($0.05 < p < 1.00$) different in the selenium group as compared to placebo

Analyte	z-value	p-value
Apolipoprotein J	1.66	0.09
Cathepsin B	1.69	0.09
Eotaxin	2.04	0.04
EGFR	1.93	0.05
FGF-basic	1.88	0.06
IL-10	1.80	0.07
IL-1alpha	1.90	0.05
IL-2 receptor alpha	1.90	0.05
MMP-3	1.77	0.07
Myeloperoxidase	1.66	0.09
RANTES	1.79	0.07
TGF-beta	1.69	0.09