Selenium Supplementation Enhances Low Selenium Levels and Stimulates Glutathione Peroxidase Activity in Peripheral Blood and Distal Colon Mucosa in Past and Present Carriers of Colon Adenomas

Oliver H. Al-Taie, Jochen Seufert, Serhan Karvar, Christian Adolph, Hubert Mörk, Michael Scheurlen, Josef Köhrle, and Franz Jakob

Abstract: Selenoproteins such as glutathione peroxidases (GPx), thioredoxin reductases (TrxR), and selenoprotein P (SePP) contain molecular selenium in form of selenocysteines within their active center. They are involved in the defense of reactive oxygen species, which otherwise may cause DNA damage and alterations of protein function. Selenium intake has been linked to colon carcinogenesis in epidemiological and interventional studies. In a double-blinded, placebo-controlled trial, we demonstrate that carriers of colon adenomas present with low basal serum levels of selenium and plasma glutathione peroxidase (pGPx) activity before treatment, but both parameters can be normalized by interventional selenium supplementation. GPx activity in colon mucosa was enhanced in the verum group, albeit this had only borderline significance. No change of activity was observed for mucosal TrxR activity on selenium supplementation. In summary, our results confirm the existence of low selenium levels in patients prone to colon adenomas and show that by selenium supplementation this can be normalized. If prospective trials confirm that selenium supplementation reduces colon cancer incidence rates, it may be concluded that selenium supplementation should be recommended for patients at risk.

Introduction

Among a variety of nutrients, the trace element selenium is supposed to play an important role in the prevention of colorectal cancers. Selenium has been shown to prevent chemically induced cancer in animals (1–7). Human studies demonstrated an inverse relationship between plasma selenium levels and the incidence of colon cancer (8–11), low plasma selenium levels in carriers of colon adenomas (12–14), and a protective effect on cancer incidence including colon cancer upon selenium supplementation (15). The molecular mechanisms of these phenomena remain to be investigated.

The average daily uptake of selenium in humans ranges between 20 and more than 600 g per day. The recommended intake is currently defined by the German Society for Nutrition as 1 µg/kg body wt/day (16). The average daily selenium intake in Germany is comparably low and in many cases is below the recommended intake. It ranks, however, still above the threshold of clear-cut deficiency (17).

Selenium is incorporated into proteins as selenocysteine in a regulated fashion (18,19). Many of the selenoproteins known so far are involved in the neutralization of reactive oxygen species such as the families of glutathione peroxidases (20–24) and thioredoxin reductases (25,26) and such as selenoprotein P (27,28), the most abundant secreted selenoprotein in serum (29). Antioxidative selenoproteins and other enzymes like SOD and catalase are important for the antioxidative defense of cells in the organism.

Damage of the cellular genome is an important part of the multistep process of tumorigenesis. One of the most important sources of DNA damage are reactive oxygen species (ROS) such as hydrogen peroxide. The number of ROS-induced hits is estimated at 20,000 per cell per day (30). Antioxidants are suggested to protect cells and the genome against ROS-inducible damage.

We have previously described the expression of several selenoproteins along the intestinal tract and their differential regulation in colon adenomas compared with healthy mucosa (31). According to their antioxidative properties, several selenoproteins such as plasma glutathione peroxidase (pGPx), cytoplasmatic glutathione peroxidases (cGPx), gastrointestinal glutathione peroxidases (Gi-GPx), thioredoxinreductases (TrxR), and selenoprotein P (SePP) are believed to protect cells and the genome against hydrogen peroxide and ROS-inducible DNA damage. Thus, these proteins may be targets for supplementary selenium thereby affecting colon carcinogenesis.

Here, we report on a double-blinded randomized trial, demonstrating that colon adenoma carriers display low serum sele-
nium levels and that selenium supplementation increases systemic glutathione peroxidase activity in patients in vivo.

Materials and Methods

Patients, Biopsies, and Blood Sampling

The study protocol was approved by the ethics committee of the University of Wuerzburg. After written informed consent was obtained, 22 patients, who were referred for colonoscopy because of a history of colon adenoma, were consecutively enrolled. None of the patients displayed any evidence for hereditary colon cancer, for example, familial adenomatous polyposis (FAP) or hereditary nonpolyposis colorectal cancer (HNPPC). At time of enrollment, colonoscopy was negative for colorectal adenoma or carcinoma in each patient. Patients were randomly assigned to receive oral solution containing 500 µg selenium per day (Selenase®) or placebo in a double-blinded fashion for a duration of 12 wk. The amount of selenium supplementation in excess of the daily food-derived intake of 35–55 µg was well within a calculated saturation range yielding an overall intake of 535–555 µg selenium per day. This dose was calculated to be safe, because the “no adverse effects level” (NOAL) was estimated at 600 µg selenium per day (16). During the study, 11 patients were supplemented with selenite (selenium group) and 11 patients received placebo (placebo group). Five to eight biopsy samples of macroscopically normal rectum mucosa were obtained by sigmoidoscopy at the beginning and at Wk 12 of the study period and immediately stored at –80°C. In addition, serum was collected from each patient at the beginning and after 6 and 12 wk and stored at –20°C.

Analysis of TrxR Activity in Intestinal Mucosa

TrxR activity was measured according to the 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) method as described by Holmgren and Björnstedt (25). Tissue samples were washed with cold phosphate-buffered saline and sonicated in buffer containing 50 mM Tris, pH 7.5, and 1 mM EDTA. For determination of TrxR activity after selenium addition, extracts were prepared using the buffer described above or a buffer containing 250 mM sucrose, 20 mM N-2-hydroxyethylpiperazine-N′-2ethane sulfonic acid, and 1 mM EDTA, pH 7.4. GPx activity was measured by the method of Beutler using tertiary butylhydroperoxide as substrate. Cytosols of the biopsies as well as serum probes were added to the reaction mixture in a final volume of 1 ml containing 0.1 M Tris, 0.5 mM EDTA, pH 8.0, 200 µM NADPH, 2 mM glutathione, and 1 U/ml glutathione reductase type IV form baker’s yeast (Sigma Chemical, Deisenhofen, Germany). The reaction was started by the addition of 7 µM tertiary butylhydroperoxide. After 1–1.5 min, the oxidation of NADPH was measured at 340 nm for 2–3 min. The activity of GPx was expressed as nanomoles of NADPH oxidized per minute normalized to milligrams of protein, as determined by the BioRad protein assay. Nonspecific NADPH oxidation was measured by complete inhibition of GPx in the presence of 100 mM mercaptosuccinate. These background values were subtracted from the results obtained in cellular extracts and serum.

Analysis of Glutathione Peroxidase (GPx) Activity in Intestinal Mucosa and Serum

Analysis of Selenium Concentrations in Serum

Serum samples were prepared by H2O2/HNO3 acid pressure disintegration in a microwave with a high-pressure rotor. Selenium concentration was determined by electrothermal graphite tube atomic absorption spectrometry with matrix modifier (32). Seronorm™ Trace Elements Serum Level 1 (Sero AS, Asker, Norway) was used as reference serum for the measurements of selenium levels in patients.

Statistical Analysis

The Student’s t-test for paired observations was used for statistical analysis (software SPSS). Results are given as mean values ± SEM.

Results

Supplementation of 500 µg selenite per day was safe and well tolerated. No adverse effects have been observed during the study period.

Serum Selenium Levels Are Low in Former Carriers of Colorectal Adenomas

The average basal level of serum selenium was 57 (±3.97) µg/l in patients with a history of colorectal adenomas as com-
pared with 71 (±3.92) µg/l in a control group of persons without a history of colorectal adenomas living in the same geographical area (P < 0.05) (33).

Increased Plasma Selenium Levels after Oral Sodium Selenite Supplementation

Six and 12 wk after supplementation, serum selenium levels of colorectal adenoma patients increased to 87 (±5.4) and 86 (±6.3) µg/l, respectively, in the selenium group, whereas no significant changes were observed in the placebo group (Fig. 1).

Oral Selenium Supplementation Increases Serum Glutathione Peroxidase Activity

Average serum GPx activity was 2.93 (±0.15) nmol NADPH/min/mg protein in the selenium group and 3.17 (±0.16) nmol NADPH/min/mg protein in the placebo group at the beginning of the study. Selenium supplementation for 6 wk led to a significant increase of average serum GPx activity by 11.04% (±5.24%) in the verum group as compared with 5.72% (±2.26%) in the placebo group. The differences were even more pronounced after 12 wk of supplementation, when average GPx activity increased by 17.17% (±5.92%) in the verum group as compared with 5.03% (±2.95%) in the placebo group (Fig. 2).

Oral Selenium Supplementation Induces GPx but Not TrxR Activity in Distal Colon Mucosa

Average basal GPx activity of the rectum mucosa was 23.5 (±1.94) nmol NADPH/min/mg protein in the selenium group versus 24.4 (±1.48) nmol NADPH/min/mg protein in the placebo group (no significant difference). Oral selenium supplementation for 12 wk induced average GPx activity to 28.8 (±1.98) nmol NADPH/min/mg protein in the verum group compared with 25.7 (±1.29) nmol NADPH/min/mg protein in the placebo group (P > 0.05) (Fig. 3).

Average basal TrxR activity of the rectum mucosa was 4.00 (±0.15) nmol NADPH/min/mg protein in the placebo group versus 5.2 (±0.42) nmol NADPH/min/mg protein in the selenium group. After 12 wk of selenium supplementation no significant changes of TrxR activity in the rectum mucosa was observed.

Discussion

Selenium supplementation is linked to reduced cancer incidence in epidemiological studies and intervention studies. Incidence of cancer of the colon, lung, and prostate was significantly reduced in the selenium intervention study of Clark et al. (15). The cellular targets for selenium and the molecular mechanisms mediating such results are largely unknown.

Selenium was shown to affect carcinogen metabolism, cell division and detoxification processes, apoptosis, and specific functions of the immune system (34). In addition, antioxidative selenoproteins may contribute to reduced cellular oxidative burden, reduced mutation rate and consecutively reduced cancer incidence. However, the amount of selenium intake necessary for optimal function of selenium-dependent enzyme systems is not known. Also, clinical intervention studies to date are lacking molecular parameters for the analysis of optimized selenium supplementation.
Current and past carriers of colon adenomas participating in this study displayed lower serum selenium levels as compared with the serum concentration of a control group from the same countryside. Low serum selenium levels have been reported mainly in patients with active cancer disease, but the reason for this phenomenon is unknown at present. Recently, similar plasma selenoprotein P or selenium levels and extracellular glutathione peroxidase activity were found in patients with adenomatous colon polyps or colorectal cancer and controls in a study in the United States (35). However, in contrast with North America and several other parts of the world, daily selenium intake and supply in Europe and Germany is two to four times lower. This fact may contribute to the findings different from this in our study. The predominant carrier of selenium in the peripheral blood is selenoprotein P. Thus, the explanation for low basal serum selenium levels could be that persons at risk to develop colon adenomas may produce less selenoprotein P for unknown reasons. A recent study from Sweden actually found such an association (36).

SeP expression is known to be inhibited by proinflammatory cytokines such as interleukin I (37) and TGF-β (38), and the promoter of the human SeP gene contains functionally relevant polymorphisms, which are unstable under conditions of mismatch repair deficiency (39). The fact, however, that oral selenium supplementation is capable of increasing serum selenium to normal levels and serum GPx activities in this study suggests that there may exist a subclinical selenium deficiency in these patients. Alternatively, an unknown constitutive factor may be overcome by selenium supplementation. Because these patients carry a substantial risk for the development of colon adenomas, suboptimal selenium status may represent an additional risk factor for manifestation of this within years or decades.

In rectal mucosal biopsies, tissue GPx activity, representing mainly the activity of the intestinal isozyme gastrointestinal glutathione peroxidase (GI-GPx), was also stimulated by oral selenium supplementation, but less pronounced as stimulation of serum GPx activity. The difference between serum GPx activity in former colon adenoma carriers during oral supplementation. Sodium selenite (500 µg) or placebo was supplemented orally for 12 wk. Results are expressed as percentage of increase ±SEM at Wk 6 and 12 compared with average baseline values (*P < 0.05).

Figure 2. Average changes in serum GPx activity in former colon adenoma carriers during oral supplementation. Sodium selenite (500 µg) or placebo was supplemented orally for 12 wk. Results are expressed as percentage of increase ±SEM at Wk 6 and 12 compared with average baseline values (*P < 0.05).

Figure 3. Average GPx activity in rectum mucosa during oral supplementation 500 µg sodium selenite or placebo was supplemented orally for 12 wk. A borderline increase (P > 0.05) of GPx activity in rectum mucosa at Wk 12 of selenium supplementation was observed.
and mucosal changes may be explained by the hierarchy of selenium supply for various selenium-dependent enzymes. This hierarchy is based on the affinity of the individual 3'-untranslated hairpin structure (Secis element) to proteins involved in the selenoprotein translation machinery. It has been well demonstrated that TrxR ranks higher than GI-GPx and GI-GPx higher than plasma GPx in the hierarchy for selenium supply (40,41). Thus, enhanced levels of GPx activity in rectum mucosa group in this study are remarkable and indicate that also tissue-specific selenium-dependent enzymes may suffer from borderline selenium deficiency.

The difficulty of interpreting measurements in intestinal tissue heavily exposed to changes in the micro- and macroenvironment is that unspecific effects of, for example, changing microbial flora or subclinical inflammatory reactions on selenoprotein expression levels are largely unknown. If these unknown factors would influence the basal expression of GPx and/or TrxR in the mucosa, we would miss changes in consequence to selenium supplementation. Moreover, subtle changes in enzyme activities might not be detectable for statistical significance, because the number of patients in our trial is comparably low.

In summary, we conclude from this interventional trial that carriers of colon adenomas display reduced serum selenium levels and that oral selenium supplementation is capable of normalizing both serum selenium levels and serum GPx activity. In turn, selenium supplementation leads to enhanced GPx activity in the rectal mucosa of these patients. This would suggest that selenium supplementation in a population of low but (as generally believed) sufficient selenium intake targets selenoproteins in the organism with measurable consequences. This fact may represent one of possibly several phenomena responsible for the reduced cancer incidence in selenium-supplemented populations. If the ongoing clinical intervention trial confirms results of reduced cancer incidence in selenium-supplemented patients, one would have to conclude that selenium supplementation has to be recommended in patients at risk.

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