Selenium in Prevention of Cancer: evidence and mechanism

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Abstract

Selenium is an essential micronutrient for human and animals. The function of selenium has been mainly attributed to its presence in selenoproteins. Selenium was first proposed as an antitumorigenic trace element in the late 1960s, a decade later than it was identified as a nutritional essential, based on ecological associations of cancer mortality rates and crop selenium contents in the United States. Since then, a large body of scientific evidence indicated that selenium can play a role in cancer prevention. This is supported by an extraordinarily consistent body of discoveries from studies with animal tumor and cell culture models, and by some, but not all epidemiologic studies. Both inorganic and organic selenium compounds can be antitumorigenic at doses greater than those required to support the maximal expression of the selenoenzymes that are generally regarded as discharging the nutritional effects of the element. The evidence for selenium as a cancer preventive agent includes that from geographic, animal, prospective and intervention studies. Newly-published prospective studies on oesophageal, gastric-cardia and lung cancer have reinforced previous evidence, which is particularly strong for prostate cancer. Interventions with selenium have shown benefit in reducing the risk of cancer incidence and mortality in all cancers combined, and specifically in liver, prostate, colo-rectal and lung cancers. The effect seems to be strongest in those individuals with the lowest selenium status. As the level of selenium that appears to be required for optimal effect is higher than that previously understood to be required to maximize the activity of selenoenzymes, the questions has been raised as to whether selenoproteins are involved in the anti-cancer process. However, recent evidence showing an association between selenium, reduction of DNA damage and oxidative stress together with data showing an effect of selenoprotein genotype on cancer risk implies that selenoproteins are indeed implicated. The likelihood of simultaneous and consecutive effects at different cancer stages still allows an important role for anti-cancer selenium metabolites such as methyl selenol formed from gamma-glutamyl-selenomethyl-SeCys and selenomethyl-SeCys, components identified in certain plants and selenium-enriched yeast or garlic that have anti-cancer effects. Several cancer preventive mechanisms have been described and it is likely that selenium acts through multiple pathways including inhibition of cell proliferation, induction of cell apoptosis, inhibition of angiogenesis, the anti-oxidative, and anti-inflammatory effects mediated through activity of selenoenzymes. Genetic variation in selenoenzymes may modify the potential chemopreventive effect of selenium and need to be further investigated. Current primary and secondary prevention trials of selenium are underway in the USA, including the Selenium and Vitamin E Cancer Prevention Trial relating to prostate cancer. It will be important to further evaluate the potential chemopreventive effect of selenium.

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Introduction

Selenium was discovered by the Swedish chemist Jons Jacob Berzelius in 1817 and has been recognized as an essential trace element for many life forms including human being since 1957 when the element was found to be the active principle in liver that could replace vitamin E in the diets of rats and chicks for the prevention of vascular, muscular and/or hepatic lesions [1, 2]. Its crucial role is underlined by the fact that it is the only trace element to be specified in the genetic code, as selenocysteine, which when incorporated into selenoproteins, protects
tissues and membranes from oxidative stress and controls cell redox status [3]. This complex insertion machinery for selenoprotein production has implications for the selenium requirements for cancer prevention. Rather than selenocysteine being directly incorporated into selenoproteins such as glutathione peroxidase during that protein’s synthesis, selenium is incorporated via a post translational process involving the modification of a serine residue; the selenium that is inserted is derived from the inorganic selenium pool [4]. More specifically, selenium is obtained from hydrogen selenide, a highly reactive intermediate generated during the detoxification of selenium for its ultimate elimination from the body as either dimethyl selenide via exhalation from the lung or the trimethylselenonium ion excreted in the urine. In humans, plasma selenium concentrations of 1.12 uM are generally considered sufficient to support full tissue expression of glutathione peroxidase [4]. It is around the metabolism of selenium that divergent evidence exists about the probable mechanisms that could account for any effects of supplemental selenium on the carcinogenic process. Evidence is accruing that the level of intake of selenium affects the risk of cancer and may even inhibit its spread from a primary tumors. Interest in selenium as potential anticarcinogenic agent was particularly stirred a decade later based on an inverse relationship of cancer mortality rates and forage crop selenium contents in the United States [5]. In the Nutritional Prevention of Cancer trial, a prospective study of the effects of selenium supplementation (200 ug of selenium as brewer’s yeast, approximately four times the recommended daily value of 55 ug/day) on the occurrence of second primary non melanoma skin cancers, Clark and coworkers reported no effect of selenium supplementation on the incidence of second skin cancers, but these investigators did find evidence of a significant reduction in the incidence of and mortality from prostate, colon and lung cancer [6, 7]. After that, the body of scientific evidence developed since that observation indicates that selenium (as a risk modifier) can, indeed, play a role in cancer prevention for some, but not all, cancers [8-17]. Some studies found selenium status to be inversely associated with cancer risk. The results have strengthened the plausible proposal that selenium, as a nutritional supplement, is a safe and effective preventive agent against the genesis of solid cancers in multiple organ sites, particularly in the prostate, colon, and lung. A series of recently published papers have documented critical interactions between the baseline plasma selenium level and the risk reduction by selenium supplement on cancers of the prostate and lung [18]. It is particularly important to note that the subjects with baseline plasma selenium in the lowest tertile (less than 105 ng/ml, or 1.33 uM) showed the most reduction of prostate and lung cancer and total non-melanoma skin cancers upon selenium-yeast intervention. Subjects entering the trial with higher baseline selenium did not show a reduction of risk for these cancers with selenium-yeast supplement. In addition, hundreds of animal studies showed that selenium-treatment can reduce tumor yields. Selenium was shown to inhibit growth and stimulate programmed cell death in a variety of cell culture systems. The consistent findings are that both inorganic and organic selenium compounds can be antitumorigenic at “supranutritional” doses, i.e., doses greater than those required to support the maximal expression of the selenoenzymes that are regarded as discharging the nutritional effects of the element. Through its role in the selenoprotein, glutathione peroxidase, selenium is generally classified as an antioxidant that works in concert with α-tocopherol residing in cell membranes to control the occurrence of lipid peroxidation and other oxidation reactions mediated by reactive species of oxygen. Specifically, glutathione peroxidase is involved in the detoxification of hydrogen peroxide and organic hydroperoxides. An extensive literature documents that low cellular levels of either glutathione peroxidase activity or α-tocopherol result in increased levels of lipid peroxidation. While indirect, there exists a consideration volume of evidence implicating various types of cellular oxidation including lipid peroxidation as being causally involved in the initiation, promotion, and progression stages of the carcinogenic process. Consequently, it has been argued that selenium inhibits the carcinogenic process by reducing cellular oxidation via increasing glutathione peroxidase activity. The nature of the selenium species involved in anti-cancer processes is still a matter of speculation and much ongoing experimental work. Whether the selenoproteins are crucial to the anti-cancer effects requires some understanding of the biosynthetic machinery involved and of the function of some of the selenoproteins most likely to be relevant to cancer. Against the background, the present discussion focuses on selection of active selenium compounds, pre-clinical evidence and clinical evidence for selenium as a cancer preventive agent, and identifies research needs for expending an information based upon which a health claim to that effect can be considered.
In Vitro Studies
Studies done in vitro have focused on the effects of selenium on cells using cell culture system. It is the first step and easier way for screening the variety of selenium compounds using in vitro cell culture system. The flow chart is shown in Fig. 1. In addition, it is easier to manipulate the experimental condition in vitro to investigate the potential mechanisms of the anti-cancer effect by selenium. The results from the in vitro studies have underscored the difference in dose and mechanism of actions among variable selenium compounds. Inorganic selenium compounds, such as selenite, can induce single-strand breaks in DNA and necrosis [19], which is considered as toxicity to the cells. However, organic selenium compounds inhibited cell proliferation and induced apoptosis without DNA breakage [20-22]. We have reported that methylseleninic acid, a monomethylated form of selenium, effectively inhibited the cell growth of mouse mammary hyperplastic epithelial in vitro by arresting the cells in G1 phase of the cell cycle. The effect was accompanied by a reduction in total cellular levels of cyclin D1, the immunocomplex with cyclin dependent kinase 4 and cyclin dependent kinase 4 activity. In addition, the retinoblastoma was activated by hypophosphorylation to bind E2F1 and insulin-like growth factor 1- Akt pathway was inhibited. This indicates that methylseleninic acid induces a G1 arrest in the cell cycle via its modulation of insulin-like growth factor 1-mediated signal transduction leading to inhibition of Akt activation and limitation of cyclin D1- cyclin dependent kinase 4-mediated phosphorylation of retinoblastoma (Fig. 2) [23, 24]. Redman’s group studied the effect of selenium on three tumor cell lines (breast, melanoma, and prostate cancer cells). Results from their study indicated that selenomethionine inhibits growth in tumor and healthy cell lines in a dose-dependent manner [25]. Selenomethionine also induced apoptosis at concentrations that inhibited 50% growth in all three cell lines. Healthy cells were found to be 1000 times less sensitive than cancer cells to the inhibitory effect of selenomethionine. Methylselenocysteine, has also been found to decrease cell proliferation and enhance apoptosis of cancer cells [26, 27]. Another organic selenium compound, allylselenocysteine has also been shown to inhibit the cell growth in vitro by suppressing cell proliferation and inducing apoptosis [20-22]. When a p53-wild type mouse hyperplastic mammary epithelial cell line was exposed to 50 micromole/L ASC for 3-12 hours, a significant inhibition of cell proliferation, as measured by BrdU incorporation into DNA, was observed within 3 hours of allylselenocysteine treatment.

Se compound treatment - dose & time dependent

- Decrease in cell number (crystal violet 96 well plate assay)
- Without DNA damage
- With DNA damage (toxic)
- Cell proliferation
- Apoptosis
- Necrosis

Ideal compound - high in inhibitory activity and low in toxicity

**Fig. 1** Flow Chart for Screening the Selenium Compounds Using In Vitro Cell Culture System

**Fig. 2** Western blot images of (left box) cyclin D1, CDK4, cyclin D1-immunoprecipitated CDK4 (IP : cyclin D1 and WB : CDK4), cyclin D1-associated kinase activity (IP : cyclin D1 and Rb-GST), and P19 ; of (middle box) retinoblastoma (Rb) and E2F-1 Rb-immunoprecipitated E2F-1 (IP : Rb and WB : E2F-1 ; ppRb, hyper-phosphorylated Rb and pRb, hypo-phosphorylated Rb) ; and of (right box) insulin-like growth factor I receptor (IGF-IR), phosphorylated Akt (ppAkt), and total Akt in synchronously growing TM6 mouse mammary hyperplastic epithelial cells. The cells were wither untreated (control, 0 uM) or treated with 5 uM methylseleninic acid (MSA) for 0.5, 1, 2, and 3 hours after the cells were starved (STV) for 48 hours and released by feeding with regular medium containing growth factors and serum for 6 hours.
The induction of apoptosis was also rapid and progressed from a 1.3-fold increase at 3 hours to a 4.4-fold increase at 12 hours. Consistent with these cellular events, the retinoblastoma was hypophosphorylated and the levels of p53, p21 and p27 were increased. These results demonstrate that allylselenocysteine is able to cause an immediate response in the expression of cell cycle regulatory proteins that favor an arrest in proliferation and an augmentation in apoptosis.

In Vivo Studies Using Animal Models

Hundreds of studies have been done in animals to investigate selenium and cancer. Among them, around 60% studies have reported a decreased incidence in cancer after selenium supplementation, and half of the reductions have involved a decrease of 50% or more [8]. The anticancer activity of selenium in vivo is dependent on the chemical structure of the selenium compounds. Ip has reported that allylselenocysteine at 2 ppm in the animal diet was the most active and caused a reduction in total tumor yield by 86%. While methylselenocysteine and propylselenosysteine, that are similar to allylselenocysteine in their chemical structure, were less effective, and both produced a decrease of about 50% in tumorigenesis [26]. A supplementation study on male beagle dogs, a species that develops spontaneous prostate cancer, has shown that supplementation of the diet of sexually-intact elderly male dogs with selenium, as selenomethionine or high-selenium yeast, at 3 or 6 microgram/kg body weight per day (a dose given is reasonable for man) for 7 months was found to reduce DNA damage and up-regulate epithelial cell apoptosis in their prostates, while no such effects were seen in the dogs that were not supplemented [28]. It appears that selenium sensitizes prostate epithelial cells so that cells with extensive DNA damage undergo apoptosis in vivo.

Human Clinical Trials and Epidemiological Studies

As the strongest treatment effect in the Nutritional Prevention of Cancer trial has been observed in subjects in the lowest tertile of plasma selenium at baseline [29]. The study, carried out by Clark and co-workers, involved 1,312 subjects with a history of non-melanoma skin cancer and were randomized to placebo or 200 ug selenium (as selenium-enriched yeast)/day [6, 7]. After four and half years of treatment and six and half years of follow-up, no effect was found on the primary end point of non-melanoma skin cancer. However, in those receiving selenium, significant secondary end-point effects of 50% lower total cancer mortality and 37% lower total cancer incidence were observed, that is, fewer prostate, colorectal and lung cancers. Follow-up analyses to the end of the blinded treatment period, a further 25 months, showed a reduced effect on total cancer, while the protective effect on prostate cancer was maintained there was no longer a protective effect on lung and colorectal cancers [18, 29-31]. Despite the striking cancer risk reduction observed in the prostate, the above findings were secondary endpoints of the trial. Because non-melanoma skin cancers are rarely life-threatening, the potential benefit of preventing lethal solid cancers of the prostate has stimulated great research interests that culminated into a large prospective intervention human trial in the United State to validate the preventive efficacy of selenium for prostate cancer, that is, Selenium and Vitamin E Cancer Prevention Trial funded by the National Cancer Institute, National Institute of Health in USA. This is a phase III randomized double-blind placebo-controlled trial designed to test the efficacy of selenium (200 ug L-selenomethionine, which is a principal selenium component of selenium-yeast) and vitamin E (400 mg DL-α-tocopherol), both alone and in combination, in the prevention of prostate cancer among healthy men [32]. The target accrual of 32,400 male volunteers has been achieved and final results are expected in 2013. Another phase III randomized chemopreventive study of selenium in participants with previously resected stage I non-small cell lung cancer has been initiated by the Eastern Cooperative Oncology Group with several oncology groups participating in Canada. Eligibility criteria include at least 18 years old, 6 weeks but no more than 3 years since surgery to remove lung tumors, no evidence of lung cancer after surgery, and no previous chemotherapy or radiation therapy for lung cancer. Patients will be randomly assigned to selenium supplementation (200 ug of selenium per day in the form of selenium yeast) or placebo group and the treatment will continue for up to 4 years (see http://www.cancer.gov/clinicaltrials/, protocol ECOG-5597). These trials are billed as the definitive tests for validating the preventive efficacy of selenium, in the form of selenium-yeast or selenomethionine, for prostate and lung cancers. Several small-scale clinical trials and pilot studies concerning prostate cancer prevention have also been either completed or initiated in the United State [33-35] and other countries including the Prevention of Cancer by Intervention by Selenium (PRES- CISE) Trial pilot studies in the United Kingdom and Denmark [36]. The protective effects of selenium supple-
ment on cancer risk in different organ sites were also seen in a few other studies, including a protective effect against liver cancer [17, 37, 38], summarized in the Table 1.

### Potential Mechanisms of Selenium Cancer Prevention

A better understanding of the mechanisms will provide novel insights for interpreting the results of these new selenium trials and for guiding the design of future trials to fully exploit the beneficial effects of selenium for cancer prevention. It has been suggested that there are a number of mechanisms to interpret the effects of selenium cancer prevention. There is fairly general acceptance that methyl selenol is involved in the effects of selenium cancer prevention at supra-nutritional doses [39]. Until recently, the investigation of the cellular and molecular effects of selenium as a chemopreventive agent has been hampered by the lack of a compound that delivers and rapidly releases to the cell a form of selenium, methylselenol, which has been reported to be a proximal chemopreventive metabolite of the element. A recently introduced compound, methylseleninic acid, overcomes this limitation and was used to explore the potential mechanisms and to probe in depth the origins of selenium’s effects on cell cycle progression. It has been found that methylseleninic acid modulates a specific stage of the cell cycle, that is, the phosphorylation of retinoblastoma by cyclin D1-dependent kinase [24]. In addition, evidence is reported that indicates that cyclin D1 kinase activity may be reduced because of the down-regulation of the insulin-like growth factor 1 signaling pathway through the phosphoinositide 3/Akt kinase cascade of events that promote cyclin D1-mediated phosphorylation of retinoblastoma. Induction of apoptosis is considered an important cellular event that can account for the cancer preventive effects of selenium [20-22]. Prior to occurrence of apoptosis, selenium compounds alter the expression and/or activities of signaling molecules, mitochondria-associated factors, transcriptional factors, tumor suppressor genes, and cellular reduced glutathione. Mechanistic studies have demonstrated that the methylselenol metabolite pool has many desirable attributes of chemoprevention, whereas the hydrogen selenide pool with excess of selenoprotein synthesis can lead to DNA single-strand breaks. Either methylselenos metabolite or hydrogen selenide can be the initiator of the apoptosis. The cancer chemopreventive ef-

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### Table 1 A summary of human studies in selenium and cancer prevention

<table>
<thead>
<tr>
<th>Study type</th>
<th>Country</th>
<th>Cancer type</th>
<th>Agent</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>and incidence of oesophageal and gastric-cardia cancers</td>
<td></td>
</tr>
<tr>
<td>Intervention</td>
<td>China</td>
<td>liver</td>
<td>Selenium (200 ug)-enriched yeast, 4 years</td>
<td>0 vs 4 liver cancer in Se vs placebo group</td>
<td>Yu et al (1997)</td>
</tr>
<tr>
<td>Prospective</td>
<td>USA</td>
<td>Prostate</td>
<td>200 ug selenium</td>
<td>Reduce the risk of the prostate cancer</td>
<td>Yoshizawa et al (1998)</td>
</tr>
<tr>
<td>Prospective</td>
<td>USA</td>
<td>Prostate</td>
<td>200 ug selenium and/or 400 mg Vit. E</td>
<td>On going</td>
<td>Klein (2004)</td>
</tr>
</tbody>
</table>
fect of selenium may also in part be mediated by its antiangiogenic activities and that methylseleninic acid can target both cancer cells and vascular endothelial cells [40]. The assumed paradigm based on a consideration of the post-initiation biology of avascular early lesion expansion microenvironment, physiochemistry of selenium delivery, and the obligatory need for angiogenesis to sustain lesion progression. Evidence is accruing that the selenoenzymes do play a role, particularly at nutritional levels of intake. Selenium in selenoproteins can reduce oxidative stress and limit DNA damage, both of which have been linked to cancer risk [39]. For a more thorough treatment of the literature, the reader is referred to recent comprehensive reviews [8, 16, 17, 23, 40, 41].

Summary

The anticarcinogenic effects of selenium compounds constitute intermediate mechanisms with several underlying chemical/biochemical mechanisms such as redox cycling, alteration of protein-thiol redox status and methionine mimicry. The results from all of three different levels of research, clinical trial, animal model and cultured cells, have confirmed that selenium can be recognized as a cancer preventive agent to reduce the risk of solid cancers of several organ sites. The significant interaction between baseline selenium and the efficacy of the selenium-yeast for reducing cancer risk, if validated, has important implications for the tailor-designing of delivery of selenium of target populations for cancer prevention. Studies in mechanisms have indicated that the metabolite pool of methylselenol has a lot of desirable attributes for chemoprevention by targeting both epithelial and vascular endothelial cells of cancers. Recent studies in a variety of model systems have increased the understanding of the anticarcinogenic mechanisms of selenium compounds. These include effects on gene expression, DNA damage and repair, signaling pathways, regulation of cell cycle and apoptosis, metastasis and angiogenesis. These effects would appear to be related to the production of reactive oxygen species produced by the redox cycling, modification of protein-thiols and methionine mimicry. Three principle selenium metabolites appear to execute these effects: hydrogen selenide, methylselenol and selenomethionine. The fact that various selenium compounds can be metabolized to one or more of these species but differ in anticarcinogenic activity indicates competing pathways of their metabolic and chemical/biochemical disposition. Increasing knowledge of selenoprotein polymorphisms has shown that at least some are related to cancer risk and may affect carcinogenesis indirectly by influencing selenium metabolism. Future researches may focus on selenium metabolite profiling and status assessment, role of selenoenzymes, define optimal selenium dosage and interactions with baseline selenium, speciation of selenium compounds, and randomized cancer prevention trials with methylselenol precursors and organoselenium.

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Selenium in Prevention of Cancer: evidence and mechanism


