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Selenium

Mechanistic Aspects of Anticarcinogenic Action

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ABSTRACT

Selenium is increasingly recognized as a versatile anticarcinogenic agent. Its protective functions cannot be solely attributed to the action of glutathione peroxidase. Instead, selenium appears to operate by several mechanisms, depending on dosage and chemical form of selenium and the nature of the carcinogenic stress. In a major protective function, selenium is proposed to prevent the malignant transformation of cells by acting as a "redox switch" in the activation–inactivation of cellular growth factors and other functional proteins through the catalysis of oxidation-reduction reactions of critical SH groups or SS bonds. The growth-modulatory effects of selenium are dependent on the levels of intracellular GSH and the oxygen supply. In general, growth inhibition is achieved by the Se-mediated stimulation of cellular respiration. Selenium appears to inhibit the replication of tumor viruses and the activation of oncogenes by similar mechanisms. However, it may also alter carcinogen metabolism and protect DNA against carcinogen-induced damage. In additional functions of relevance to its anticarcinogenic activity, selenium acts as an acceptor of biogenic methyl groups, and is involved in the detoxification of metals and of certain xenobiotics. In its interactions with transformed cells at higher concentrations, it may induce effects ranging from metabolic and phenotypical changes, and partial renormalization to selective cytotoxicity owing to reversible or irreversible inhibition of protein and DNA synthesis. Selenium also has immunopotentiating properties. It is required for optimal macrophage and NK cell function. Its protective effects are influenced by synergistic and antagonistic dietary and environmental factors. The latter include a variety of

toxic heavy metals and xenobiotic compounds, but they are also influenced by essential elements, such as zinc. The exposure to antagonistic factors must be minimized for the full expression of its anticarcinogenic potential.

INTRODUCTION

The anticarcinogenic properties of selenium (Se) are now well established by numerous experimental studies, but its mechanism of action is still a subject of discussion, as evidenced by several recent reviews (*1–4*). In general, considering the complexity of the carcinogenic process, it should not be expected that a single mechanism can be formulated that would be universally applicable to all tumor model systems in which Se was found to be effective. However, as will be shown in the following, some generalizations are nevertheless possible, on the basis of the available evidence and the known chemical properties of Se. Since Se is known to function as a component of several selenoenzymes, their possible involvement in cancer-protecting activities provides a logical starting point and will be discussed first.

THE ROLES OF SELENOENZYMES IN ANTICARCINOGENESIS

All mammalian cells require traces of Se ($10^{-8}M$) for growth in vivo and in tissue cultures. At these limiting levels, most of the Se is used for the expression of selenoenzymes, especially the glutathione peroxidases (GPx) located in the cytosol or in cellular membranes (*5*), as well as for 4-iodotyronine deiodase (IDT), a newly discovered enzyme that catalyzes the conversion of the thyroid hormone precursor T_4 into its active form, T_3 (*6*). The function of GPx is to protect cellular components against peroxidative damage through the reduction of metabolically generated lipid hydroperoxides and of hydrogen peroxide. GPx thus could play a role in the protection against the effects of some carcinogenic compounds, especially those that either as such, or in the presence of additional agents, inflict peroxidative damage on target tissues. However, it should be kept in mind that the peroxidative damage of a given carcinogen may be unrelated to its carcinogenic action. A case in point is provided by 1,2-dimethylhydrazine (DMH), a colon and liver carcinogen whose toxic effects are attributed to peroxidative damage. Although the acute DMH-induced oxidative damage on the two target tissues was aggravated by Se deficiency (*7*), this does not necessarily mean that its carcinogenic action occurs by the same mechanism. For optimal protection against tumorigenesis, amounts of Se higher than required for saturation of GPx activity are usually necessary. Moreover, the tumorigenic effect of a carcinogen is not necessarily enhanced by Se deficiency. The tumor yield

from azoxymethane, a carcinogen related to DMH, was lower in Se-deficient as compared to Se-adequate rats (*8*). Although this argues against a major role of GPx in the anticarcinogenic action of Se, an indirect protective action of this enzyme is still possible under conditions where the peroxidative damage of cell membranes or other cellular constituents is caused by factors other than the carcinogen, for example, by unsaturated fats or inhaled reactive oxygen species. However, in such instances, Se acts as an *anticocarcinogenic* factor, rather than anticarcinogenic agent, which is a mechanistically important distinction. In the 7,12-dimethylbenz(a)anthracene (DMBA) mammary carcinogenesis model, dietary fat could be viewed to function as a cocarcinogen increasing the peroxidative damage of mammary tissue, and this damage could be reduced by GPx. Accordingly, as shown by Ip (*9*), in rats exposed to DMBA and maintained on Se-adequate diets containing 25% corn oil, the mammary tumor yield was significantly lower than in rats receiving the same percentage of corn oil in Se-deficient diets. GPx may be especially important in the protection against lung cancer development, which is well known to be accelerated by prooxidants and inhibited by antioxidants. As dietary fats stimulate the production of lipid hydroperoxides, insufficient dietary intakes of Se may lower the resistance to pulmonary carcinogens. In accordance with this hypothesis is the fact that human lung CA mortalities in different countries are inversely correlated to blood-Se levels, and directly associated with the dietary fat consumptions (*10*). GPx may furthermore play a role in the protection of radiation-induced carcinogenesis, but since it is also expressed in malignant cells, GPx may also render them more resistant to radiation or protect them against the action of cytotoxic drugs that function by stimulating oxygen radical production. The increased doxorubicin resistance of a human sarcoma cell line was interpreted on this basis (*11*). In such cases, lowering the GPx activity, e.g., by dietary Se depletion, may induce selective damage or destruction of the tumor cells. Thus, although feeding an Se- and vitamin E-deficient diet is known to produce necrosis of the liver in normal mice or rats, in mice with Ehrlich ascites carcinoma, Baumgartner (*12*) was able to induce necrosis of the tumors instead. Growing tumors also deplete the Se storage organs of the host; Ehrlich ascites carcinoma in mice actually had the same Se-depleting effect on the liver as a necrogenic Se-deficient diet in the tumor-free controls (*12*). However, growing tumors may also diminish Se retention in the host organism. Thus, in rats with DMBA-induced mammary tumors, L'Abbe et al. (*13*) found the urinary excretion of Se to be accelerated compared to that in animals without tumors. The reason for this effect is as yet unknown; it may be hypothesized that the decreased retention of Se is accompanied by altered levels of glutathione (GSH) and/or the increased retention of zinc.

As for ITD, the only other presently known Se-dependent mammalian enzyme (*6*), too little is known at the moment to permit a detailed

assessment of its potential role in Se-dependent anticarcinogenesis. Since Se deficiency could lead to a diminution of the concentrations of active thyroid hormone T_3, an effect on certain immune functions may be expected. It has been suggested that Se protects against the development of CA of the thyroid (*14,15*). In as yet unpublished studies from our laboratory, subtoxic levels of supplemental iodine in the drinking water were found to retard mammary tumorigenesis in MMTV-infected female mice. It remains to be demonstrated whether the joint administration of iodine and Se could produce synergistic effects in the prevention of mammary tumorigenesis. As iodine deficiency is known to produce hyperplastic foci in the mammary gland (*16*), the effect of combined iodine and Se deficiency on mammary tumorigenesis also needs to be investigated. Initial studies (*17*) indicate that a deficiency of both trace elements produces specific effects, such as a reduction of the level of uncoupling protein required for thermogenesis in brown adipose tissue of rats.

ANTIPROLIFERATIVE PROPERTIES

The antiproliferative effects of Se are observed at concentrations from slightly above the minimum dietary requirements to surprisingly high levels. In HeLa S3 cells, for example, macromolecular synthesis starts to be inhibited at intracellular Se levels of above 0.5 ppm; DNA synthesis proceeds with 40% activity at Se concentrations of about 2 ppm; recovery from intoxication is still possible after exposures to 130 ppm Se (*18*). However, other cell types are more sensitive, and with some human tumor cell lines, impressive selective cytotoxic effects of Se have been observed in vitro (*19*). Se has a delaying effect on all mitotic phases, consistent with the inhibition of protein and DNA biosynthesis; Se also prolongs the premitotic resting phase, G-2, and thus may create conditions favorable for DNA repair (*20–22*). Relevant examples demonstrating the antiproliferative properties of Se were provided by the studies of liver regeneration in mice after partial hepatectomy (PH). These studies showed that the growth of hepatocytes after PH was abnormally accelerated at low dietary Se intakes. This was interpreted as a pathological response to compensated Se deficiency, since the hepatocytes produced under these conditions were of inferior quality and had a lower life-span. Supplemental Se normalized the rate of liver regeneration process (*22–24*). Se may similarly inhibit or modulate the proliferation of cells subjected to a proliferative stimulus, such as the exposure to a viral agent or chemical carcinogen, and in this manner lower the probability of malignant transformation.

The consensus seems to be emerging that Se, in its antiproliferative functions, operates in close conjunction with biogenic thiols or disulfides. Since selenite was used in the majority of anticarcinogenesis experiments, its reaction with various thiols (RSH) was studied in some detail.

Selenite is reduced by thiols to the selenotrisulfides, RSSeSR, $RSSe_2H$, and selenodisulfides RSSeH (*25,26*). In the reaction of selenite with glutathione (GSH), the selenotrisulfide GSSeSG was sufficiently stable to be isolated. The compound was found to inhibit protein biosynthesis in rat liver (*27*) and to exhibit antitumor activity (*28*). Other authors have linked the antitumorigenic action of Se to specific selenoproteins, but this produced contradictory results. For example, a 60-kDa selenoprotein in mouse cells was suggested to be responsible for the inhibition of DNA biosynthesis, but in other studies with cells in which this protein was entirely absent, selenite elicited similar inhibitory effects, suggesting that in this case either other selenoproteins were involved or perhaps none at all (*29*). However, in the meantime, a mouse liver protein of 56 KDa has been characterized (*30*) that does not contain selenocysteine, but binds Se and is believed to be involved in growth-regulatory processes. It contains nine cys residues, which could be the sites of interaction with Se. If Se exerts its inhibitory effects on cell growth by modulating the properties of growth-regulatory proteins, it remains to be discussed how the growth modulation is achieved and what the driving force might be.

Several lines of evidence suggest that the anticarcinogenic effects of Se are ultimately linked to its ability to *stimulate cellular respiration* and to oxidize SH groups of regulatory proteins to SS bonds selectively. That Se promotes such reactions is well established. In studies with minimum deviation hepatoma cells, LeBoeuf et al. (*22*) showed that the addition of selenite to the culture media increased the GSSG/GSH ratios and the levels of oxidized pyridine nucleotides. The same effect was also observed in vivo, as evidenced by the increased hepatic GSSG/GSH ratios in Se-supplemented rats compared to unsupplemented controls. Although factors that determine cellular GSH levels and their changes during pathological conditions are not yet fully understood processes, they are known to decline in tumor-bearing animals with increasing tumor size; in tumor tissue, progressively increasing GSH levels correlate closely with tumor growth (*31*).

The changes of the cellular GSH or GSSG may be expected to affect the equilibrium concentrations of oxidized and reduced forms of cellular growth factors and of other biologically active proteins with SH groups and SS bonds. By catalyzing the oxidation reduction of SH groups and SS bonds of cellular growth factors and of related functional proteins, Se could thus function as a "redox switch," turning growth processes either off or on, depending on the oxidizing or reducing properties of the intracellular medium.

A model of such a compound is Elongation Factor 2 (EF2), which regulates protein biosynthesis in rat liver polyribosomes (*32*). EF2 is a protein that contains SH groups in the active form and is inactive in the oxidized form with SS bonds. By (directly or indirectly) catalyzing the oxidation-reduction of EF2, Se could regulate protein biosynthesis, allowing it under anerobic, and inhibiting it under aerobic, conditions.

In addition, Se could catalyze the activation–inactivation of inhibitors or of negative regulators of cell growth. It is of interest that some of these factors are active in the "oxidized" forms containing SS bonds. These factors would thus be activated by Se under oxidizing conditions. Examples of two such factors are somatostatin, the growth-hormone-release inhibiting factor, and tumor necrosis factor (TNF), both of which contain SS bonds (*33,34*). The known (*35*) inhibition of growth hormone release by Se could be linked to an increase of somatostatin production by a process involving the Se-mediated oxidation of SH groups. The assembly or excretion of TNF or of related factors could also be mediated by Se, and this could be the mechanism by which Se increases the cytotoxicity of monocytes against tumor cells.

In a related manner, Se could also regulate the differentiation and proliferation of tumor cells. At the cellular level, as was demonstrated with hepatoma cells, Se stimulates c-*fos* gene expression and suppresses c-*myc* gene expression (*36*); c-*fos* is a highly inducible gene in response to mitogens, or differentiation-specific agents or stimuli, including partial hepatectomy (*37*); c-*myc* is most frequently expressed in human tumors (*38*). The modulating effects of Se on oncogene expression are consistent with partial retransformation toward normal and are also directly observable by phenotypical changes of the cells, as well as normalization of enzyme activities, as observed by Shu-Yu Yu (*19*).

The postulated catalytic role of Se as an electron-transfer catalyst in the oxidation reduction of SH groups and SS brings to mind earlier studies and concepts that associated malignant transformation with a "disturbance of cellular respiration," and showed that the malignancy of transformed cells increases in parallel with the increase of SH groups and the decline of SS bonds in the mitochondria and in other cellular components (*39–41*).

Oxidation-reduction reactions of protein-SH or SS bonds are well known to occur during mitosis, and take place in a kinetically and sequentially controlled fashion. Perturbations of these processes may lead to mutagenesis and the activation of oncogenes. Alterations of SH/SS equilibria may also involve receptor sites at the surface of cells and affect the immune functions.

The glucocorticoid hormone receptor sites of rat hepatocytes, for example, contain at least four protein-SH groups in the active form (*42*). Hormone binding by this receptor is irreversibly inhibited by low levels of Se in the form of selenite; activity is restored by dithiothreitol. Accordingly, receptor-site inactivation represents another mechanism that could account for some of the anticarcinogenic properties of Se.

EFFECTS OF SELENIUM ANTAGONISTS

The protective functions of Se are subject to inhibition by numerous elements that may be encountered in foods, in the environment, or at

the work place. An incomplete list of elements includes As, Cu, Zn, Cd, Hg, Sn, Pb, Ni, Co, Sb, Bi, Ag, Au, Mo.

The interactions of Se with many of these elements occur spontaneously in vivo and in part represent natural detoxification processes. Elements with the highest affinities for Se combine with Se to yield metal selenides or protein complexes thereof. Such interactions lead to the physiological inactivation of Se. The best-known example for this type of interaction is provided by mercury.

The interactions between Se and Zn are thermodynamically weaker than those between Se and Hg, preventing the formation of insoluble ZnSe and allowing Se to function in biological systems even in the presence of a 10–100-fold excess of Zn. However, the antagonism between the two elements becomes manifest under nutritionally imbalanced conditions. For example, Se as present in foods has an antagonistic effect on Zn absorption in Zn-depleted rats, and Zn has an antagonistic effect on Se absorption by Se-adequate rats (*43*). Excessive Zn abolishes the anticarcinogenic effects of Se (*44*), promotes tumor growth, and reduces the survival time of tumor-bearing experimental animals (*45*). Correlational studies revealed the dietary Zn intakes to be directly correlated with the mortalities from major forms of cancer, and suggest that the high incidence of certain cancers in the developed countries may be associated with excessive zinc and insufficient Se intakes (*46*). The antagonism between Se and Zn is probably indirect. Zinc could protect the critical SH groups of growth factors against Se-catalyzed oxidation, for example; conversely, Se could inactivate zinc-binding proteins by catalyzing the oxidation of their SH groups.

Since many of the Se-antagonistic elements are toxic and some are suspected or proven environmental or occupational carcinogens, it must be of concern that the combined background exposures to these elements of the populations, especially in some industrialized countries, may significantly exceed the total dietary Se intakes. In these instances, organ- or blood-Se determinations provide reliable information on Se status only if complemented by determinations of the Se-antagonistic elements. In the organs of chronically mercury-exposed subjects, for example, Se levels were high, but the Se was actually present in the form of protein-bound, physiologically inactive HgSe (*47*).

Working along these lines, Nordberg and his colleagues (*48*) reported that the ratios of As to Se were higher in lung, liver, and kidney tissue of Swedish smelter workers who died from malignancies than in workers who died from other causes. In a subsequent paper (*49*), these authors introduced an empirical "protective quotient," P_{tot}, defined as the ratio of the weighted sum of the concentrations of metals and the Se concentration in lung tissue:

$$P_{tot} = [(Sb) + 3(As) + 2(Cd) + 4(Cr) + (Co) + (La)]/(Se) \quad (1)$$

The coefficients of the metals in P_{tot} reflect their relative carcinogenic or cocarcinogenic potency. Among the elements considered, Sb, As, Cd,

and Co are known to possess Se-antagonistic properties; the status of La is uncertain; Sb and As have been linked to lung Ca development; Cr is carcinogenic in the +6 oxidation state. Lung tissue from workers who died from lung cancer showed significantly higher P_{tot} values than that from workers who died from other causes. Similarly defined quotients involving additional elements could become useful as prognostic indices of cancer risk, utilizing scalp hair or toenails as indicators of exposure.

Since the Se-antagonistic elements have specific effects on tumor development and growth, as well as on the immune system, exposures to individual elements may induce different metabolic imbalances. Low-dose lead or arsenic exposures, for example, abolished the cancer-protective effects of Se in MMTV-infected mice (*50,51*), but As and Pb had different effects on tumor growth and mortality.

SELENIUM, ARSENIC, AND METHYL GROUPS

The interactions between Se and As provide an example for the complexity of the problems that are encountered in attempts to elucidate the effects of both elements in carcinogenesis and tumor development. Both elements in general behave as metabolic antipodes; each can be used to alleviate the symptoms of poisoning of the other. Se stimulates and As inhibits cellular respiration. Se is antimutagenic; As is mutagenic. Se has immunopotentiating properties; As is immunosuppressive. Both elements occur in multiple-oxidation states and have comparable affinities for protein-SH groups, but in contrast to Se, As does not catalyze oxidation-reduction reactions of thiols and disulfides. Feeding an As-containing diet to mice caused a reduction of the levels of nonprotein-SH and SS groups in the liver and the brain. An Se-containing diet increased SH and SS levels in the brain; the joint administration of both elements abolished the effects of each (*50*). As inhibits enzymes involved in DNA repair; Se promotes DNA repair and, at least under some conditions, stimulates interferon production (*51,52*). Both elements furthermore interact with each other directly and compete for methyl groups. In vivo, As in the form of $NaAsO_2$ inhibits the exhalation of $Se(CH_3)_2$ by rats exposed to Na_2SeO_3 (*53,54*). Although $NaAsO_2$ had no effect on the urinary excretion of $Se(CH_3)_3^+$, recent studies by Ip and Ganther (*55*) show that $Se(CH_3)_3^+$ methylates As, thus causing it to be converted into lower methylated selenium species of higher toxicity, which the authors believe to be the actual anticarcinogenic agents in the DMBA induction of mammary tumors. The latter suggestion requires confirmation, e.g., by tests with CH_3SeH or $CH_3SeSeCH_3$ in other tumor model systems.

The fact that Se, in addition to its other functions, is also an acceptor of biogenic methyl groups suggests a possible regulatory role of Se in methyl group metabolism. Supplemental Se could prevent the methylation of DNA in the early stages of carcinogenesis and would be physi-

ologically inactivated in the process. This could constitute an additional "stoichiometric" mechanism of anticarcinogenic action. Indeed, Se has been shown to inhibit DNA methylase activity (*56*).

It would now be of interest to compare this function of Se at pharmacological dosage levels with the action of folic acid antagonists on tumor cells and to investigate the joint deployment of both in cancer chemotherapy; Se could potentiate the effects, e.g., of methotrexate, which is an inhibitor of dihydrofolate reductase.

EFFECTS OF OXYGEN ON SE METHYLATION AND ANTICARCINOGENIC ACTIVITY

If, as has been suggested above, Se modulates cell growth by catalyzing the oxidation/reduction of critical SH groups and SS bonds of growth-regulating proteins and related factors, the effects of Se should be strongly dependent on oxygen tension. Studies by Garberg and Hoegberg (*57*) already indicate that Se metabolism is profoundly affected by the amount of oxygen available to cells. Thus, dimethylselenide formation by rat hepatocytes from added [^{75}Se] selenite was delayed in carbogen gas (93.5% O_2, 6.5% CO_2) compared to air and was stimulated under hypoxic conditions. Furthermore, incubation of the cells with selenite in the presence of limiting amounts of oxygen led to an accelerated decrease of oxygen tension, and Se volatilization owing to the formation of dimethylselenide occurred only when the oxygen tension was low. Based on these observations, the anticarcinogenic effects of Se are expected to be abolished under hypoxic conditions and augmented at high oxygen tensions.

CONCLUDING REMARKS

After the completion of this article, several papers have appeared that further address the role of thiols in relation to the anticarcinogenic effects of Se. Frenkel et al. (*58*) reported on the products of the reaction of selenite with intracellular sulfhydryl compounds and concluded that selenotrisulfides could have direct cytotoxic effects. Yan et al. (*59*) investigated the effects of selenite, selenocystine, sodium selenate, and selenomethionine on viability and growth rates of a human mammary tumor cell line in the presence of GSH, 2-mercapotethanol, and L-cystine. These authors found that a depletion of intracellular GSH by a pretreatment of the cells with buthionine sulfoximine (BSO) increased the cytotoxicity of selenite, as have others (*60*). Thompson and Ip (*61*) investigated the effects of selenite and of selenomethionine on liver GSH levels in rats, and concluded that Se-induced changes of GSH or GSSG are probably unrelated to the chemopreventive effect of Se, since they occurred in a transient manner and only at close to toxic dosages of sele-

nite. In another recent study, the anticarcinogenic effects of Se were attributed to diminished energy utilization as measured by reduced food utilization and weight gains of Se rats exposed to 4 and to 6 ppm of Se (*62*). However, these Se dosages are in the chronically toxic range; the true anticarcinogenic actions of Se occur well below the levels of any form of toxicity, are possibly limited to cells subjected to an intense proliferative stimulus, and may involve participation of the immune system. Studies of cytotoxic effects of Se in the presence of thiols and other agents are at best of interest in relation to possible chemotherapeutic applications of Se.

In view of the high cytotoxicity of GSSeSG, a promising combination is that of selenite with GSH. In Wistar rats with benzo(a)pyrene-induced tumors, favorable responses were observed on oral administration of a solution of 0.1% GSH containing 5 ppm of Se in the form of sodium selenite, for example (*63*). Se could also be used in conjunction with chemotherapeutic agents to reduce their toxic side effects, e.g., the cytotoxicity of *cis*-platinum (*64*) or the cardiotoxicity of adryamycin (*65,66*). In rats with transplanted prostate tumor cells, a protective effect of selenite against the most toxic combination of polyamine synthesis inhibitors (ARA-A/EHNA, MGBG) has also been observed (*67*). Also in rats, the joint administration of tamoxifen, tocopherol, retinyl acetate, amino-glutethimide, ergocryptine, and Se as sodium selenite (1 mg/kg) protected against DMBA-induced mammary tumorigenesis more effectively than when these modulators were given separately (*68*). Supplemental Se thus should be considered as an adjuvant for the prevention of recurrences in treated CA patients. Last but not least, oral and topical applications of Se in the form of L(+)-selenomethionine protected against UV-induced skin cancer (*69*). In this study, topical selenomethionine was only as active as orally administered selenomethionine (1.5 ppm Se in the drinking water), even though the Se concentrations on the skin on topical application were significantly higher. This clearly shows that the protective mechanism of Se is systemic and not a localized cytotoxic effect.

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