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GENERATION OF REACTIVE OXYGEN SPECIES FROM THE REACTION OF SELENIUM COMPOUNDS WITH THIOLS AND MAMMARY TUMOR CELLS*

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Abstract—Sodium selenite, sodium selenate, selenocystine and selenomethionine were tested for their abilities to generate superoxide by the oxidation of glutathione and other thiols in the absence and presence of cells of the human mammary tumor cell line HTB123/DU4475. Free radical generation was measured by lucigenin- or luminol-amplified chemiluminescence. In the absence of tumor cells, lucigenin-dependent chemiluminescence was observed from the reaction of selenite with the thiols glutathione, 2-mercaptoethanol and L-cysteine, but not with oxidized glutathione. Superoxide dismutase, catalase, and glutathione peroxidase all suppressed the observed chemiluminescence; but when these enzymes were heat inactivated they had little suppressive inhibition on chemiluminescence. Luminol-dependent chemiluminescence from the reaction of selenite with glutathione was much less than that observed by lucigenin-amplified chemiluminescence. In the presence of the HTB123/DU4475 mammary tumor cells, lucigenin-dependent chemiluminescence was observed from the reactions of selenite and selenocystine with glutathione which were 5 and 23 times greater than their respective reactions with glutathione in the absence of tumor cells. The enhanced chemiluminescence generated by selenite and selenocystine in the presence of the tumor cells was also suppressed by superoxide dismutase, catalase and glutathione peroxidase. These data suggest that a free radical, the superoxide anion (O_2^-), and H_2O_2 are produced from the reaction of selenite and selenocystine with glutathione. These free radical reactions may account for the toxicity of selenite and selenocystine *in vitro* in comparison to a near absence of acute tumor cell toxicity and superoxide generation by selenate and selenomethionine with thiols. Enhanced chemiluminescence in the presence of tumor cells may be an expression of cellular selenium metabolism and the capability of cells to form selenium metabolites that more easily oxidize glutathione and other thiols producing reactive free radicals and peroxides.

Selenium is a nutritionally essential [1] and toxic [2, 3] trace element whose role in carcinostatic activity *in vitro* and *in vivo* is presently of interest [4, 5]. While experimentation has documented that some selenium compounds are cytocidal *in vitro* to both normal [6, 7] and tumor [8, 9] cells and are carcinostatic in several animal species bearing different types of tumors, the mechanism(s) of their anti-carcinogenicity remains unknown.

A great deal of research has shown that glutathione (GSH)‡ participates in selenite-induced cytotoxicity. Vernie *et al.* [10] found that selenodiglutathione (GSSeSG) inhibits protein synthesis in intact 3T3-f cells while sodium selenite alone or oxidized glutathione (GSSG) has no inhibitory effect. Frenkel and Falvey [11] reported that sodium selenite has little or no effect on the activity of purified DNA and RNA polymerases in a cell-free system but in the presence of thiols selenite inhibits these enzymes. It is well established that GSSeSG is formed by the reaction of selenite with GSH [12], and Frenkel and Falvey [13] have reported on the ion exchange identification of GSSeSG and other mixed selenotrisulfides from the reaction of selenite with thiols. The instability of this compound under physiological conditions makes it unlikely that the formation of GSSeSG *per se* is responsible for selenite toxicity. In spite of the known instability of GSSeSG, Caffrey and Frenkel [14] have reported that the formation of GSSeSG is the cytotoxic factor in the prevention of HeLa colony formation.

Other research has suggested that oxygen radicals may ultimately be involved in selenium toxicity. Increased lipid peroxidation by selenite but not selenate has been reported both *in vivo* [15] and *in vitro* [16]. Increased oxygen consumption was observed in the reaction of either selenite or selenocystine (SeCys) with GSH [17, 18], and Seko *et al.* [19] were the first to report that the superoxide anion (O_2^-) was likely generated by the oxidation of GSH by selenite.

Previous studies in this [7, 20] and other laboratories [8, 18] have shown that both selenite- and selenocystine-induced cytotoxicity to both normal and tumor cells is accompanied by a loss of intracellular GSH. However, selenate and selenomethionine (SeMet) incubated with cells had no effect in reducing cellular GSH, and these

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‡ Abbreviations: CL, chemiluminescence; CT, catalase; Cys, L-cysteine; GSH, glutathione reduced form; GSHPx, glutathione peroxidase; GSSeSG, selenodiglutathione; GSSG, glutathione oxidized form; MeOH, 2-mercaptoethanol; SeCys, selenocystine; SeMet, selenomethionine; and SOD, superoxide dismutase.

selenium compounds are much less cytotoxic to human mammary or leukemic tumor cells [20, 21].

In light of the experimental consistency among various investigators in assessing the cytotoxicity of different selenium compounds to both normal and tumor cells, the purpose of the present investigation was to determine whether O_2^- and/or H_2O_2, as measured by lucigenin- or luminol-dependent chemiluminescence (CL), were generated from the reaction of four chemically different selenium compounds with GSH and other thiols in the absence or presence of mammary tumor cells. Such information may provide general insight into the mechanism of toxicity of the different selenium compounds and their carcinostatic attributes observed *in vitro* and *in vivo*.

MATERIALS AND METHODS

Materials. Roswell Park Memorial Institute Culture Medium 1640 (RPMI 1640), penicillin–streptomycin solution, seleno-DL-cystine (SeCys), seleno-DL-methionine (SeMet), reduced glutathione (GSH), 2-mercaptoethanol (MeOH), L-cysteine (L-Cys), oxidized glutathione (GSSG), catalase (CT), glutathione peroxidase (GSHPx), superoxide dismutase (SOD), bis-*N*-methylacridinium nitrate (lucigenin) and 5-amino-2,3-dihydro-1,4-phthalazinedione (luminol) were purchased from the Sigma Chemical Co. (St. Louis, MO). Iron-supplemented bovine calf serum was from HyClone Laboratories, Inc. (Logan, UT), trypsin from Gibco Laboratories, Inc. (Grand Island, NY), and Dulbecco's phosphate-buffered saline (DPBS) from Hazleton Biochemical, Inc. (Lenexa, KS). Sodium selenite (SeO_3) was purchased from Pfaltz & Bauer, Inc. (Stamford, CT) and sodium selenate (SeO_4) from the Alfa Products Co. (Danver, MA). The HTB123/DU4475 human mammary tumor cells were obtained from the American Type Culture Collection (Rockville, MD).

Chemiluminescence. Lucigenin- or luminol-dependent chemiluminescence from the reaction of the four different selenium compounds with GSH and other thiols either in the absence or in the presence of mammary tumor cells was measured at 37° by a modified method of Lefkowitz *et al.* [22] using a model 1251 luminescence photometer from Los Alamos Diagnostics (Los Alamos, NM). Lucigenin and luminol were both prepared in DPBS (1 mg/mL) containing 0.1% (w/v) globulin-free bovine serum albumin. The solutions were stirred for 10 min and then filtered using a syringe and 0.2 μm filter. The filtrates were aliquoted and stored at 4° in a covered container to prevent exposure to light. Lucigenin- or luminol-dependent CL was used as an indicator of the presence of either superoxide anion or hydrogen peroxide, respectively [23]. The selenium compounds were dissolved in RPMI 1640 (without phenol red, pH 7.6) at various concentrations and kept at 37° in an incubator. Thiols at various concentrations were prepared in RPMI 1640 (without phenol red, pH 7.6) just prior to measurement. The final volume of the total reaction mixture was 1.0 mL. Immediately after a thiol and lucigenin or luminol (50 μL) were added to a selenium compound and/or cell culture tube, CL was measured continuously for 10 min.

Superoxide dismutase, catalase and glutathione peroxidase were prepared in DPBS (10 U/μL). Heat-inactivated enzymes were prepared by heating the enzyme solution at 100° for at least 30 min. For measuring the effect of each enzyme on CL generation from the reactions of the different selenium compounds with GSH, 50 U of each enzyme (active or inactivated) was added to the reaction mixture just prior to the addition of GSH.

Cell cultures. The human mammary tumor cell line HTB123/DU4475 had a passage number of 168. The original cell line was derived from a 70-year-old female patient with advanced breast cancer [24]. The cells were grown in suspension culture in a growth medium containing 80% RPMI 1640, 20% heat-inactivated, iron-supplemented bovine calf serum and a penicillin–streptomycin solution (100 U penicillin and 100 μg streptomycin per mL of growth medium) at pH 7.2. The cells were incubated at 37° in a water-saturated atmosphere of 5% CO_2 in air in a model 3158 incubator from Forma Scientific Inc. (Marietta, OH). The growth medium was changed every 2 days. Tests for Mycoplasma using a modification of the method of Chen [25] were all negative.

The mammary tumor cells used to measure free

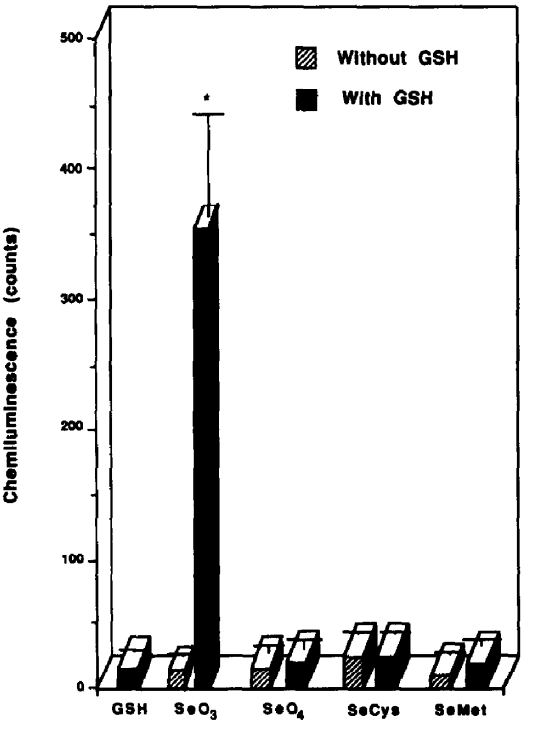


Fig. 1. Lucigenin-dependent CL from the reaction of selenite, selenate, selenocystine and selenomethionine with and without GSH. The selenium concentration was 12.5 μM. The GSH concentration was 0.25 mM. Values are means ± SEM (N = 6). Pairs with an asterisk were significantly different ($P < 0.05$).

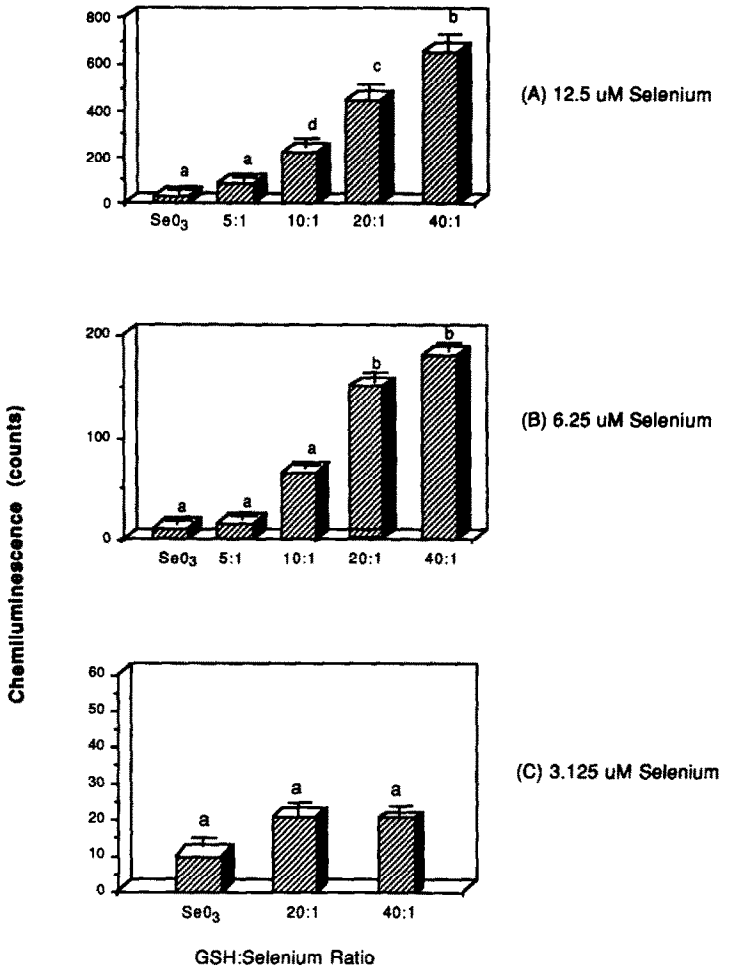


Fig. 2. Lucigenin-dependent CL from the reaction of selenite with GSH at different GSH:selenium ratios. Values are means ± SEM (N = 6). Values with the same letters were not significantly different (P > 0.05). Note: CL scales are not the same and GSH will reduce selenite to Se^0 which is not catalytic.

radical production by CL were harvested just prior to measurement. The cells were washed twice with RPMI 1640 (without phenol red, pH 7.2), trypsinized and counted by trypan blue exclusion using a hemocytometer. The tumor cells were then incubated in RPMI 1640 (without phenol red, pH 7.2) containing 1% (w/v) globulin-free bovine serum albumin at a density of 5×10^6 cells/mL. For measurement of CL from the reaction of the four different selenium compounds with GSH in the presence of tumor cells, 0.25×10^6 cells/50 μL were added to the reaction mixture prior to the addition of GSH. Tumor cells prepared for experimentation and not used within 3 hr were discarded.

Statistical analysis. Analysis of variance and Duncan's new multiple range test were used for the statistical comparisons between the different selenium treatments. Student's *t*-test was used for all paired comparisons. All of the analyses were performed using the SAS program [26] and the level of statistical significance was set at $P < 0.05$.

RESULTS

The present studies using lucigenin- and luminol-enhanced CL for the detection of O_2^- and H_2O_2 suggest that selenite is more reactive than either selenocystine, selenate, or selenomethionine on an equilmolar selenium basis in oxidizing glutathione and producing superoxide (Fig. 1). Over a 10-min CL counting period, the reaction between selenite and glutathione was seen to be generally dependent upon the concentration of selenite in the presence of excess GSH (Fig. 2, A, B and C). Selenite not only reacted with GSH to produce superoxide, but it also reacted with at least two other reduced thiols, MEOH and Cys, to produce CL. When GSSG replaced GSH in the reaction mixture, there was no significant change in CL ($P > 0.05$) as was observed for the reaction between selenite and the other thiols (Fig. 3).

To determine whether superoxide or hydrogen peroxide or both active oxygen species were produced

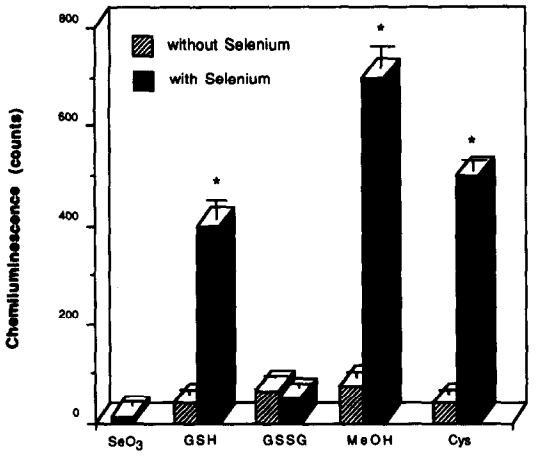


Fig. 3. Lucigenin-dependent CL from the reaction of selenite with glutathione (GSH), oxidized glutathione (GSSG), 2-mercaptoethanol (MeOH) and cysteine (Cys). The concentration of selenium was 12.5 μM and the concentrations of GSH, GSSG, MeOH and Cys were each 0.25 mM. Values are means ± SEM (N = 6). Pairs with an asterisk were significantly different ($P < 0.05$).

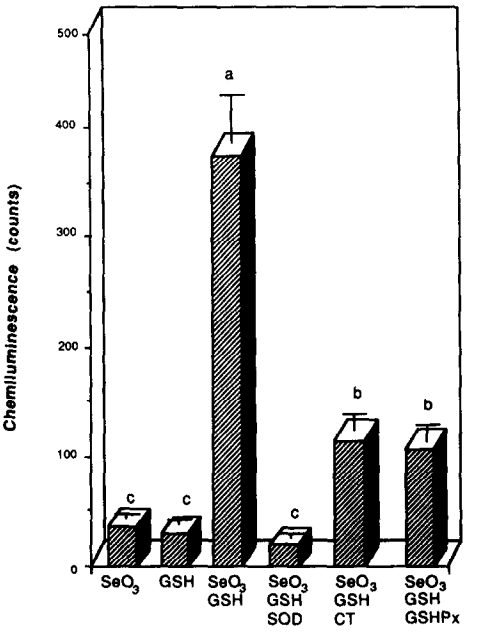


Fig. 4. Inhibitory effects of superoxide dismutase (SOD), catalase (CT) and glutathione peroxidase (GSHPx) on lucigenin-dependent CL from the reaction of sodium selenite with GSH. The concentrations of selenium, GSH and each enzyme were 12.5 μM, 0.25 mM and 50 enzyme units, respectively. Values are means ± SEM (N = 6). Values with the same letters were not significantly different ($P > 0.05$).

from the reaction of selenite with GSH, the enzymes superoxide dismutase, catalase and glutathione peroxidase were added at pH 7.2 to the reaction mixture. All three enzymes significantly ($P < 0.05$) reduced CL from the reaction between selenite and GSH with SOD nearly eliminating any measurable CL (Fig. 4). To assure ourselves that CL was not being reduced excessively by a non-specific protein effect, the reaction between selenite and GSH was measured in the presence of albumin and heat-treated superoxide dismutase and catalase (Fig. 5). As Fig. 5 indicates, protein *per se*, i.e. albumin, and heat-treated SOD and CT were not as effective as the native enzymes in reducing CL.

Figure 6 demonstrates the CL produced by the different selenium compounds and GSH in the presence of 0.25×10^6 mammary tumor cells. All selenium compounds were again not equally effective in oxidizing GSH and producing lucigenin chemiluminescence in the presence of tumor cells. Selenite and selenocystine both oxidized GSH producing superoxide, with the reaction of selenocysteine being less than the reaction of selenite with GSH. In the presence of tumor cells each enhanced total chemiluminescence over the CL produced by the oxidation of GSH alone (see Fig. 1). During the assay period both selenate and selenomethionine appeared not to react with GSH as no significant CL was observed.

In these selenium reactions, lucigenin is oxidized by the superoxide anion and chemiluminescence is observed. Luminol is most susceptible to oxidation by H_2O_2 and its CL is amplified by peroxide generation [23]. Figure 7 shows that the reaction between selenite and glutathione not only produced superoxide but also hydrogen peroxide. The amount of CL produced in this reaction with luminol was less than the amount of CL produced in the presence

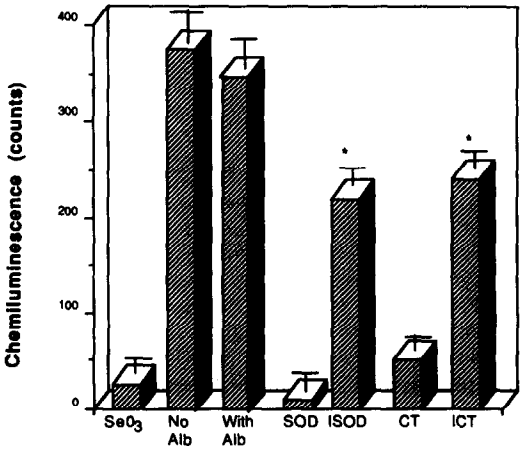


Fig. 5. Effects of albumin (Alb), SOD and CT on luciginin-dependent CL from the reaction of selenite with GSH. The concentrations of selenium, GSH and each enzyme were 12.5 μM, 0.25 mM and 50 enzyme units, respectively. Albumin contained twice the amount of protein provided by 50 U of catalase or superoxide dismutase. ICT and ISOD represent heat-treated CT and SOD, as described in Materials and Methods, with a small amount of residual activity. Values are means ± SEM (N = 6). Values with an asterisk were significantly different ($P < 0.05$).

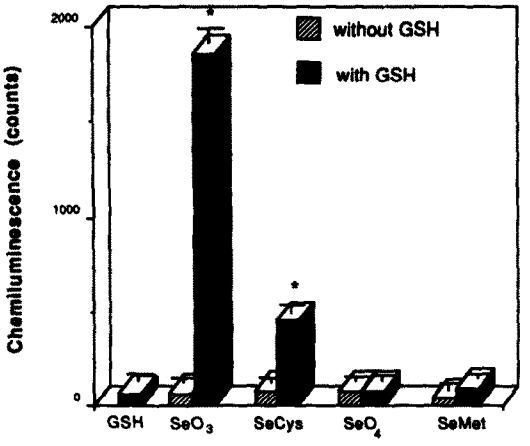


Fig. 6. Lucigenin chemiluminescence of different selenium compounds in the presence of 0.25×10^6 cells of the human mammary tumor cell line HTB123/DU4475. The concentration of selenium was 12.5 μM, and the concentration of GSH was 0.25 mM. Values are means ± SEM. Each data point represents 5–9 observations. Pairs with an asterisk were significantly different ($P < 0.05$). Note: Scale is four times that of Fig. 1 and CL values have been corrected for the CL of cells alone which was 723 ± 8 counts (N = 6) over 10 min.

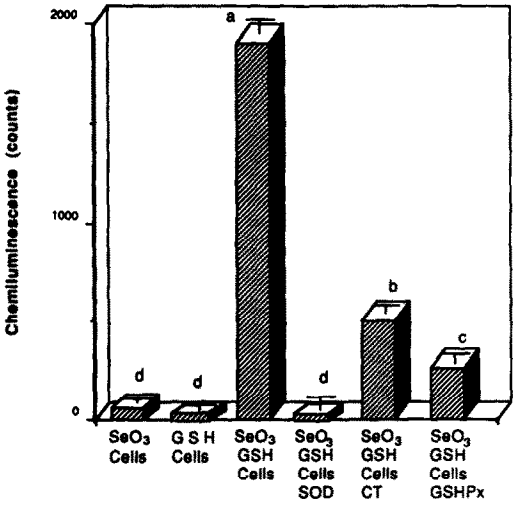


Fig. 8. Inhibitory effects of SOD, CT and GSHPx on lucigenin-dependent CL from the reaction of selenite with GSH in the presence of 0.25×10^6 cells of the human mammary tumor cell line HTB123/DU4475. The concentrations of selenium, GSH and each enzyme were 12.5 μM, 0.25 mM and 50 enzyme units, respectively. Values are means ± SEM (N = 6). Values with the same letters were not significantly different ($P > 0.05$). CL values have been corrected for the CL of cells alone which was 723 ± 8 counts (N = 6) in 10 min.

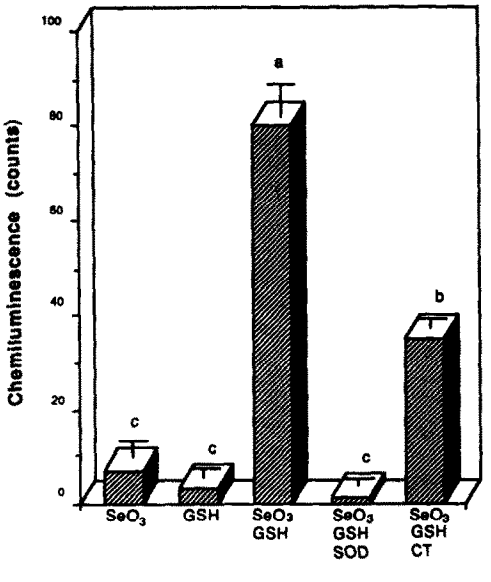


Fig. 7. Luminol-dependent CL from the reaction of sodium selenite with GSH and inhibition by SOD and CT. The concentrations of selenium, GSH and each enzyme were 12.5 μM, 0.25 mM, and 50 enzyme units, respectively. Values are means ± SEM (N = 5). Values with the same letters were not significantly different ($P > 0.05$). GSHPx was not tested for inhibitory activity.

of lucigenin. Nevertheless, SOD and CT both reduced the amount of CL observed between the reaction of selenite and GSH in the presence of luminol.

The apparent production of superoxide by selenite's oxidation of GSH was enhanced in the presence of cells of the human mammary tumor cell line HTB123/DU4475. As shown in Fig. 8, the presence of 0.25×10^6 viable tumor cells enhanced selenite-induced CL nearly 5-fold over that produced by selenite and GSH alone (Fig. 3). The enzymes SOD, CT and GSHPx also significantly reduced the chemiluminescence from the reaction mixture containing tumor cells. In the presence of GSH and tumor cells, CL from the reaction of selenocystine with GSH was enhanced *ca.* 23-fold (without cells 21 ± 6 and with cells 571 ± 41) (Figs 9 and 10). SOD and CT (GSHPx was not tested) both significantly reduced the CL produced by selenocystine in the presence of GSH and the human mammary tumor cells.

Figure 10 depicts the significant differences ($P \leq 0.05$) in lucigenin CL produced in the absence and presence of tumor cells for the four selenium compounds studied: selenite, selenocystine, selenate and selenomethionine. The presence of tumor cells dramatically enhanced the CL from the reaction of GSH with selenite and selenocystine. Chemiluminescence was even significantly enhanced with selenate and selenomethionine by the presence of tumor cells and GSH. Most lucigenin CL was produced between the reaction of selenite and GSH followed by selenocystine and GSH both alone and

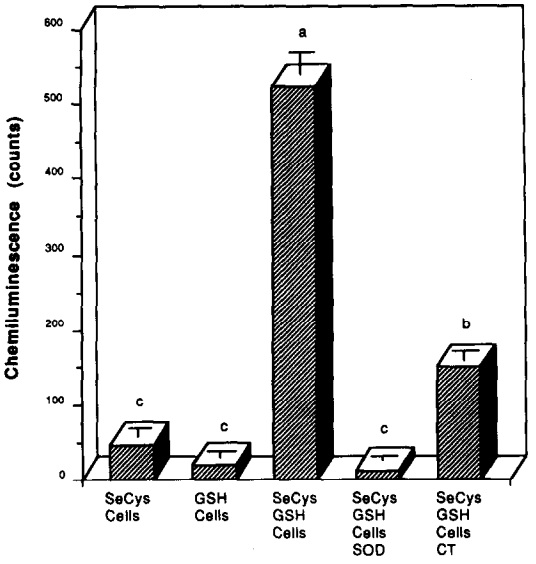


Fig. 9. Inhibitory effects of SOD, CT, and GSHPx on lucigenin-dependent CL from the reaction of selenocystine (SeCys) with GSH in the presence of 0.25×10^6 cells of the human mammary tumor cell line HTB123/DU4475. The concentrations of selenium, GSH and each enzyme were 12.5 μM, 0.25 mM and 50 enzyme units, respectively. Values are means ± SEM (N = 5). Values with the same letters were not significantly different ($P > 0.05$). Inhibition by GSHPx was not tested with selenocystine-generated CL. CL values have been corrected for CL of cells alone which was 718 ± 7 counts (N = 8) in 10 min.

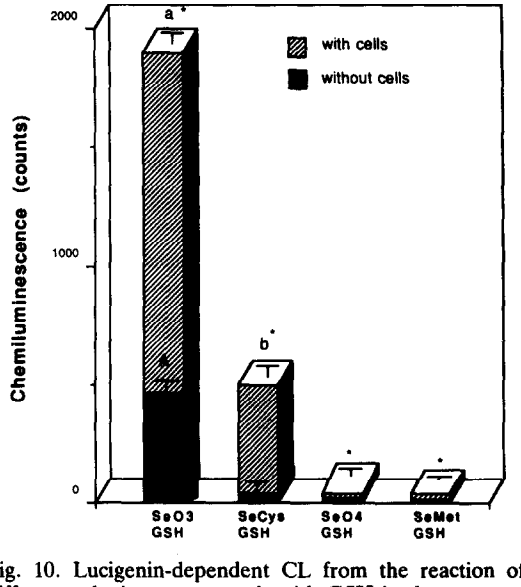


Fig. 10. Lucigenin-dependent CL from the reaction of different selenium compounds with GSH in the presence (total bar) and absence (black bar) of 0.25×10^6 cells of the human mammary tumor cell line HTB123/DU4475. The concentration of selenium administered was 12.5 μM as selenite, selenocystine, selenate or selenomethionine. The concentration of GSH was 0.25 mM. Values are means ± SEM; each value represents 5–9 observations. An asterisk represents a significant difference in CL generated by selenium and GSH in the presence and absence of tumor cells. A lower case represents a significant difference between selenium treatments and GSH in the presence of tumor cells. A capital letter represents a significant difference in CL between selenium and GSH in the absence of tumor cells. The significance level was set at $P < 0.05$.

in the presence of tumor cells. Even though selenate and selenomethionine produced almost no lucigenin CL with GSH alone, in the presence of tumor cells even these selenium compounds produced statistically significant ($P < 0.05$) lucigenin chemiluminescence.

DISCUSSION

The toxic attributes of selenium had been known for a long time before the discovery of its essentially for the three presently known selenium enzymes containing the amino acid selenocysteine: glutathione peroxidase [27], phospholipid hydroperoxide glutathione peroxide [28] and iodothyronine-5'-deiodinase [29]. Selenium toxicity in animals is thought to have been first observed by Marco Polo while traveling in China but the first written report in 1860 of selenium toxicity in livestock is attributed to T. C. Madison [30]. Selenium is also known to have carcinostatic activity in experimental animals and humans [31], and some chemical forms are cytotoxic to normal and cancer cell lines *in vitro* [7, 8, 20, 21].

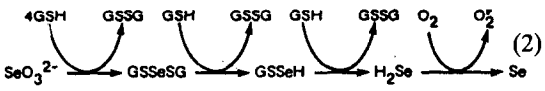
In 1941, Painter first proposed that selenium was toxic due to its chemical interaction with sulfur compounds [32]. Ganther was to demonstrate later that the reaction product of selenious acid formed from selenite with GSH resulted in the formation of a selenotrisulfide (RSSeSR) [12] and GSSG according to the reaction originally proposed by Painter:

$$4\,GSH + H_2SeO_3 \rightarrow GSSeSg + GSSG + 2H_2O \quad (1)$$

Under physiological conditions GSSeSG is unstable and the major product of Equation 1 is a selenopersulfide (GSSeH) believed to be formed by either the direct reduction of GSSeSG in the presence of GSH reductase and NADPH or by a nonenzymatic reduction in the presence of excess GSH. Kice [33] has studied extensively the reactions of these bis-alkylthioselenides, i.e. selenotrisulfides *in vitro*. Whichever way the selenopersulfide is formed, numerous *in vitro* experiments have shown a consistent and concentration-dependent loss of cellular GSH by increasing additions of selenium (usually selenite, SeO_3). GSH is the major thiol in tissues in millimolar concentration and is often found at even higher tissue concentrations in tumor cells [34].

Garberg *et al.* [35] have shown that selenite can induce cellular DNA fragmentation in normal hepatocytes *in vitro* but only in the presence of oxygen. Single-strand DNA breaks in their experiments correlated to oxygen uptake by cells in the presence of selenite and were inhibited in hepatocytes equilibrated with nitrogen gas. Seko *et al.* [19] have shown that reactions between selenite

and GSH, GSSeSG and GSH, and H_2Se and oxygen produce the free radical, (O_2^-). The reaction sequence proposed by Seko *et al.* for the production of the superoxide anion from the oxidation of GSH by selenite is shown below in Equation 2.



(2)

The present experiments using lucigenin-amplified chemiluminescence seem to confirm the generation of superoxide by the oxidation of GSH by selenite as shown by Seko *et al.*, and extend their observations to other selenium compounds: selenate, selenocystine and selenomethionine. These selenium compounds produced little, if any, *in vitro* evidence for superoxide generation in the presence of GSH (Fig. 1). Selenite was also the most effective selenium compound in oxidizing the other thiols, mercaptoethanol and cysteine, that resulted in slightly more superoxide being produced as observed by lucigenin CL. No CL was observed by the mixing of GSSG and selenite. The active enzymes, superoxide dismutase, catalase, and glutathione peroxidase, all reduced lucigenin CL (Fig. 4), whereas the heat-treated enzymes, SOD, CT and GSHPx, or albumin did not reduce the CL (Fig. 5) as much as the native proteins. These results suggest that selenite can oxidize a variety of thiols including GSH and that the superoxide anion, as reported by Seko *et al.*, is a product of the selenium reaction. The other selenium compounds (selenate, selenocystine and selenomethionine) in the presence of GSH did not produce superoxide significantly *in vitro* as measured by lucigenin chemiluminescence (Fig. 1).

The *in vitro* experiments described above showing that only selenite reacted significantly with GSH to produce superoxide were extended to an *in vitro* system containing cells of the mammary tumor cell line HTB123/DU4475. In the presence of 0.25×10^6 tumor cells, selenite as well as selenocystine, selenomethionine and selenate produced chemiluminescence that was significantly higher in the presence of tumor cells than in the presence of GSH alone. The chemiluminescence generated by selenite and GSH in the presence of tumor cells was 4-fold higher in the presence than in the absence of tumor cells. We believe that this higher chemiluminescence in the presence of tumor cells, along with the increase in CL generated by selenocystine, selenomethionine and selenate in the presence of tumor cells, is probably due to cellular metabolism of the selenium compounds. The tumor cells likely provide for a rapid reduction of selenate, selenomethionine and selenocystine to SeO_3, H_2Se or other reactive metabolites by the tumor cells.

Previous studies in this laboratory have compared the cytotoxicities of selenite, selenate, selenocystine and selenomethionine towards this human mammary tumor cell line HTB123/DU4475 *in vitro* [20]. The results showed that selenite and selenocystine are much more cytotoxic to these tumor cells in culture than either selenate or selenomethionine. Our results [20] are in excellent agreement with the selenium-induced cytotoxicity studies of human leukemic cells of Batist *et al.* [18]. These authors reported that only selenite and selenocystine were cytotoxic towards their leukemia cells and that selenate and selenomethionine were not cytotoxic.

Both of these *in vitro* studies of selenite and selenocystine cytotoxicity were accompanied by a loss of cellular GSH from the target tumor cells. The cytotoxicity of both selenite and selenocystine towards the tumor cells was enhanced by the addition of exogenous GSH. In our tumor cell experiments [20], only selenite and selenocystine showed acute toxicity. Selenate or selenomethionine were also toxic towards the HTB123/DU4475 mammary tumor cells, but toxicity was greatly delayed and much less severe. Figures 8 and 9 show that both selenite and selenocystine in the presence of tumor cells and GSH produced chemiluminescence that was significantly reduced and greatly retarded by superoxide dismutase, catalase or glutathione peroxidase.

It is clear that the presence of tumor cells resulted in an amplification of CL and that the three selenium compounds that did not appreciably produce much superoxide with GSH alone, selenocystine, selenate and selenomethionine, produced significantly more CL in the presence of tumor cells (Fig. 10). We do not yet know the specific reason for the enhancement of CL by tumor cells but in the case of selenate and selenomethionine, metabolism of these compounds must result in the reduction of selenium to selenite or possibly H_2Se which then produces the chemiluminescence. The tumor cells added to the reaction mixture are, of course, themselves a source of GSH and carry with them GSH reductase and NADPH which may sustain the culture concentration of GSH contributing to the total amount of chemiluminescence.

The toxicity of different selenium compounds has been known for many years without any substantial theory that accounts for their toxicity. The paper of Seko *et al.* [19] showing that selenite and GSH undergo a redox reaction that produces the superoxide anion (O_2^-) may provide the rational for selenite and selenium toxicity in general. *In vivo*, selenite toxicity in animals is essentially equal to that of selenate [36] as well as to selenocystine but not selenomethionine [37]. The near equality of toxicity in animals among the three selenium compounds, selenite, selenate and selenocystine, is understandable based upon their metabolism as described by Magos and Webb [38] and Young *et al.* [39]. Briefly, selenite, selenate and selenocystine are reduced via cellular enzymatic and nonenzymatic steps to H_2Se. H_2Se is reduced further to dimethylselenide or the trimethylselenonium ion which is then excreted in urine. It is reasoned that when these reductive pathways are overwhelmed by dietary excesses of these selenium compounds, selenotrisulfides and selenopersulfides accumulate and oxidize GSH and other cellular thiols to produce superoxide and H_2O_2.

Selenomethionine, however, fed to animals does not possess toxicity [37] comparable to the other selenium compounds nor is selenomethionine very toxic to various tumor cells *in vitro* [18, 20, 21, 40]. Unlike other selenium compounds, selenomethionine can be incorporated directly into the

primary structure of proteins [37, 41]. Not until selenomethionine is released from muscle and other protein tissues, together with an excessive intake, is it metabolized and only then does it produce selenium toxicity analogous to other selenium compounds.

The experiments of Seko *et al.* [19] and the present studies using human mammary tumor cells demonstrate that selenium compounds can produce O_2^-, H_2O_2 and very likely other hydroperoxides. The differences in their abilities to generate O_2^- and H_2O_2, however, offer an explanation as to why there is a wide variation in the *in vitro* toxicity of different selenium compounds ranging from selenite to the Ebselen [42] compounds which are non-toxic. The present experiments also provide a plausible explanation for the carcinostatic activity of selenium compounds *in vivo* as well as for their cytotoxicity *in vitro*. Should it be found possible to selectively concentrate selenium in tumor cells or establish a significant difference in the thiol concentrations between cancer and normal tissues, a means may be found to eliminate cancer cells selectively using selenium-generated free radicals without a systemic selenium toxicity [43].

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