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FINAL FOR SUBMISSION

87824

AUG 04 1997

9/7/95

OSTI ANL/CMB/PP--87824

The effects of selenium on glutathione peroxidase activity and radioprotection in mammalian cells.

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Running Title: Selenium effects on GPx and radioprotection.

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ABSTRACT

The media of representative mammalian cell lines were supplemented with low levels of selenium in the form of sodium selenite in order to investigate the effects of selenium on mammalian cells. Following incubation in 30 nM sodium selenite, these cells were assayed for changes in glutathione peroxidase (GPx) activity. The cells examined included NIH 3T3 mouse fibroblasts, PC12 rat sympathetic precursor cells, Supt-1 human lymphocytes, MCF-7^{adr} human breast carcinoma cells and AA8 Chinese hamster ovary cells. Selenium supplementation resulted in a marginal increase in GPx activity for the NIH 3T3, MCF-7^{adr} and Supt-1 cells but stimulated GPx activity approximately 5-fold in PC12 and AA8 cells. AA8 cells were selected to evaluate whether selenium supplementation was radioprotective against ⁶⁰Co gamma irradiation. Protection against radiation-induced mutation was measured by evaluating mutation frequency at the hprt locus. In this assay, pre-incubation of AA8 CHO cells significantly protected these cells from exposure to 8 Gy.

Key words: selenium; glutathione peroxidase; radioprotection

INTRODUCTION

It has been established over recent years that selenium can be an effective chemopreventative agent in the inhibition of cancer [1]. For example, mammary tumor development in mice following exposure to a variety of carcinogens can be inhibited by better than 50% by the inclusion of dietary selenium levels only 5-fold higher than the normal dietary requirement [1]. Similar results have been obtained in other rodent model systems indicating the effectiveness of selenium supplementation of the diet in reducing cancer incidence in a variety of organs after insult with either DNA-damaging agents or oncogenic viruses [1,2]. Selenium has also been shown to inhibit transformation *in vitro* [3], carcinogen metabolism [4] and carcinogen-DNA binding [5]. In humans, numerous epidemiological studies of the general population have indicated an inverse correlation between overall cancer mortality and selenium status [6-10].

Se-dependent glutathione peroxidases (SeGPx) represent a family of related enzymes which are generally believed to constitute a major defense system against toxic peroxides and oxygen free radicals [11]. Given the role of SeGPx in anti-oxidant defense, one might speculate that some of the bioprotective effects of selenium, at least in part, would be due to this enzyme activity. This is generally not believed to be the case as several older studies have indicated that chemoprotective doses of selenium did not result in significant increases in SeGPx activity in experimental animals [12,13]. In this manuscript, it was examined whether the supplementation of culture media with low levels of sodium selenite would have an effect on GPx activity and report that this activity was stimulated in several mammalian cell lines. A representative cell line, CHO AA8 cells, were further examined and shown to be protected from radiation-induced mutation. The implications of this data are discussed regarding the mechanisms by which selenium may provide its bioprotective role.

MATERIALS AND METHODS

Cells and culture conditions. The Chinese hamster ovary (CHO) cell line CHO AA8 was grown in α -minimal essential medium (α -MEM; Gibco, Grand Island, NY) with 10% fetal bovine serum (Biologos, Naperville, IL), NIH 3T3 cells in DMEM (Gibco) with 10% calf serum (BioWhittaker, Walkersville, MD.), MCF-7^{adr} in IMEM (Gibco) with 5% FBS (Intergen Co., Purchase, N.Y.), PC12 in RPMI 1640 containing 10% horse serum (Gibco) and 5% FBS (Intergen) and SupT-1 in RPMI 1640 in 10% FBS (Intergen). Sodium selenite (Sigma Chemical Co., St. Louis, MO) was prepared in H₂O as a 3 μ M stock solution sterilized by filtration. For studies including selenium in the culture medium, cells were maintained in 30 nM sodium selenite for at least three days prior experimental manipulation and maintained at this concentration throughout the experiment. Mutation frequency was determined as described previously [14].

GPx assay. GPx activity was measured by a standard assay which spectrophotometrically measures the oxidation of NADPH in a coupled system containing reduced glutathione, glutathione reductase, cellular extracts and hydrogen peroxide as the substrate as described [15] and expressed as the nmoles of NADPH oxidized per minute per μ g protein. At least 4 assays were performed on independent cultures.

RESULTS

In order to evaluate whether low levels of selenium above that present in standard culture media could influence GPx activity, several mammalian cell lines were incubated with 30 nM sodium selenite. The cells selected for examination represent a diverse group with respect to both cell type and species of origin. These included mouse NIH 3T3 fibroblasts, rat PC12 sympathetic precursor cells, human Supt-1 T cells and Chinese hamster AA8 ovary cells. The culture medium were supplemented with sodium selenite for 3-10 days and the GPx activity was measure as described in the Methods section. The dose and time for incubation with the selenite was based on GPx effects and time course experiments previously published by Chu et al. [16]. Maximal GPx stimulation occurred at 3 days and did not decline over extended culturing. As seen in Table 1, comparison between cells incubated with and without added selenite yielded diverse results for the different cell lines tested. For example, NIH 3T3 cells, which exhibited the highest GPx activity of the cells tested, demonstrated a marginal increase in GPx activity of 1.3-fold. Similarly, human T-cells and breast carcinoma cells exhibited a 1.5- and 1.6-fold stimulation of GPx activity. In contrast, two of the cell lines tested displayed significant increase in activity. Addition of 30 nM sodium selenite stimulated the GPx activity of CHO AA8 cells by 4.1-fold and maximal stimulation was observed for rat PC12 cells whose GPx activity increased by 6.3-fold. Thus, the GPx activity of the cell lines tested exhibited a diverse response to added low levels of selenium.

CHO AA8 cells were selected for mutation analysis in order to evaluate whether the low-level supplementation of culture media with selenite could offer protection from the mutagenic effects of gamma irradiation. Mutation frequency was assessed by examining mutation frequency at the hprt locus following irradiation of cells. Mutation at this locus is one of the most widely studied markers for mutagenesis.

CHO AA8 cells were selected for these studies. Less than $2 \pm$ mutations per 10^6 surviving were observed when AA8 cells were examined by this assay. Following exposure to 8 Gy gamma irradiation this number rose to $117 \pm$ mutations/ 10^6 cells. Inclusion of sodium selenite for three days prior to irradiation reduced this value to $73 \pm$ mutations/ 10^6 surviving cells, representing a protection factor of 1.602. Therefore, incubation of these cells with low levels of sodium selenite capable of stimulating GPx activity by better than 4-fold was able to significantly protect CHO AA8 cells from radiation-induced mutation.

DISCUSSION

In this manuscript, the effects of low-level supplementation of tissue culture media with sodium selenite on mammalian is described. It is reported that the diverse mammalian cell lines examined herein responded differently with regard to the degree of stimulation of GPx activity. These results did not appear to be related to the amount of selenium present in the culture media prior to selenite addition. If this were the case, one would anticipate that the cells maintained in the least serum concentration would be most responsive to added selenium. This is clearly not the case as the minimally responsive breast cells were maintained in 5% FBS and the maximally stimulated PC12 were incubated in media containing 5% FBS as well as 10% horse serum. Similarly, both SupT-1 cells and CHO AA8 cells, which responded quite differently to selenite supplementation were both maintained in 10% FBS. The molecular basis for the differential response to selenite remains unknown. Several *in vivo* and *in vitro* studies have indicated that levels of GSHPx-1 mRNA are elevated under conditions of selenium adequacy compared to selenium deprivation [17-19]. In contrast, other studies have shown GSHPx-1 expression is elevated under conditions of selenium

deprivation [20]; still others have shown that GSHPx-1 expression is unaffected by selenium status [21]. Other experiments have clearly demonstrated that post-transcriptional mechanisms, i.e. effects on RNA stability, are involved [22].

The results presented in this manuscript also establish the efficacy of low level selenium supplementation in the reduction of radiation-induced mutations. Numerous studies have indicated the ability of selenium to protect cells against mutation induction, toxicity or both following exposure to a wide range of insults, including radiation [1-5,23]. A variety of mechanisms of action have been suggested, including stimulation of a repair processes, induction of apoptosis and its role in GPx proteins. Several studies have indicated that the protection afforded by selenium was likely to be by mechanisms independent of effects on GPx activity [12,13,24,25]. The results with CHO AA8 cells indicated that the same dose of selenium that resulted in a better than 4 fold increase in GPx activity significantly protected these cells from radiation-induced mutation. This data cannot distinguish between GPx-dependent vs. -independent mechanisms of protection. Future studies using GPx expression constructs to genetically increase GPx activity independent of selenium status will be required to establish the protective role of this enzyme.

Acknowledgement: This work was supported by Grant # CN-133 from the American Cancer Society (to A.M.D.).

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<u>Cell Type</u>	<u>GPx Activity*</u>	<u>Manipulation</u>	<u>% Stimulation</u>
NIH 3T3 fibroblasts (mouse)	29.1 (n=11)	30 nM Sodium selenite	1.3X (n=11)
PC12 sympathetic precursor (rat)	4.1 (n=5)	"	6.3X (n=5)
MCF-7(adr) Breast carcinoma (human)	20.3 (n=7)	"	1.6X (n=7)
Supt-1 T-cells (human)	8.5 (n=4)	"	1.5X (n=4)
AA8 ovary (Chinese hamster)	20.7 (n=5)	"	4.1X (n=5)

Table 1. Effect of Selenium Supplementation on GPx Activity in Mammalian Cells. The indicated cell types were incubated with 30 nM sodium selenite and GPx activity measured as indicated in the text. *GPx activity represents the average of the indicated independent experiments and is expressed as the nmoles of NADPH oxidized per μg protein.

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