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The Effects of Sodium Selenite on the Antioxidative Defence Mechanism of Human Hepatoma G_2 Cells

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Abstract: Human cells that live in biological harmony can become cancerous. As they do so, they engage in anaerobic respiration, undergo rapid cell growth and division according to nonmitotic rules, and important changes take place in their metabolism. We conducted several investigations in an effort to block or change the metabolic characteristics of cancer cells using a typical cancerous cell line. Specifically,we investigated the effects of sodium selenite (Na_2SeO_3) on antioxidant enzyme activities and reduced glutathione levels in Hepatoma G_2 cells, cells that show rapid glutamine uptake and metabolism.

A Hepatoma G_2 cell line was cultured in RPMI 1640 Dutch medium. Sodium selenite solution was added to the culture medium to produce a final concentration of 1µM/ml on the second day of incubation and several measurements of metabolic activity were taken.The selenium treatment increased selenium-dependent glutathione peroxidase and Cu,Zn super oxide dismutase activities by approximately 100% and 21% respectively, yet reduced catalase activity by 20%. There was no Mn-super oxide dismutase activity in the Hepatoma G2 cells, but the existence of an inactive form of Mn-super oxide dismutase was observed. Glutathione S-transferase activity was not affected by selenium. Selenium caused a 56% decrease in reduced glutathione level in a Hepatoma G_2 cell line.

We argue that the observed changes are due to selenium dependent protein as a Se-GSH-Px. The unchanging activity levels of glutathione-S transferase is important because it plays a key role in cellular detoxification mechanisms. We conclude that glutathione metabolism in cancer cells slows with selenium treatment.

Key Words: Hepatoma G_2, Sodium selenite, Glutathione peroxidase, Catalase, Superoxide dismutase.

Introduction

Glutathione peroxidase: GSH-Px, (EC 1.11.1.9) catalyses the oxidation of GSH to GSSG at the expense of H_2O_2: $H_2O+2GSH \rightarrow GSSG+H_2O$. According to selenium (Se) dependency, GSH-Px can be divided into two forms: Se-dependent and Se-independent GSH-Px. Se-GSH-Px is a tetramer of MW 84, 000 with high activity toward both H_2O_2 and organic hydroperoxides, found in both cytosol and mitochondria. The enzyme protects the cells against organic damage of the cell membrane (1). Se-independent GSH-Pxs are the GSH-S transferases: GST, (EC 2.5.1.18). They were observed in the catalysis of the first step in the formation of mercapturic acid. The enzymes are dimerous and their MWs number approximately 50,000, with at least seven different forms of domain and eight isoenzymes (2).

Superoxide dismutase: SOD, (EC 1.15.1.1) dismutases two molecules of $O_2^{\cdot-}$ to form H_2O_2 and O_2. The SOD family consists of four metallo forms; two of them contain copper and zinc, one manganese and one iron. In eucaryotic cells, three forms of SOD are known to exist: Cu,Zn SOD is found in the cytosol, a different form of Cu,Zn SOD is found in the extracellular fluid, where it is called EC-SOD, and Mn SOD is located in the mitochondrial matrix. Cu,Zn SOD has a MW of 32, 000 with two identical subunits. EC-SOD also contains copper and zinc; it has a MW of 135, 000 with four equal noncovalently bound subunits. Mn SOD has a MW of around 88, 000 with 4 equal subunits (3).

Most aerobic cells contain the enzyme catalase: CAT, (EC 1.11.1.6.) which catalyses the reaction: $2H_2O_2 \rightarrow 2H_2O+O_2$. CAT is found mostly in peroxisomes (80%)

and cytosol (20%). The usual form of CAT has a MW of about 240, 000 and consists of four protein domains, each containing a Heme [Fe (III)-protoporphyrin] group bound to its active site (3).

Previous studies have investigated changes in the activity of radical scavengers and the effects of metal ions on cancerous cells (1,3). In this study, the effects of Na-selenite on radical scavenging enzymes of a human Hepatoma G_2 cell line were investigated.

Materials and Methods

Cell type and cell culture: Antioxidant enzyme activities were studied using human Hepatoma G_2 cells obtained from the American Cell Culture Collection Centre (U.S.A.). Hepatoma cells were grown in 5ml flasks at 37°C under a humidified atmosphere of 5% CO_2 and 95% air. The cells were maintained in RPMI 1640 Dutch medium supplemented with 1% essential amino acids mixture, 1% Na-pyruvate, 2mM glutamine, 10% Fetal Calf Serum, which was heat inactivated at 56°C for 30 min,1000 U/ml penicillin and 1000U/ml streptomycin (4).

Sodium selenite supplementation: The Hepatoma G_2 cells were treated with 1 μM sodium selenite as the final concentration in 5 ml RPMI 1640 Dutch medium on the second day of the incubation period (4). Supplementation of the selenite was repeated every 3 days, which was chosen as the medium renewing time.

Harvesting of the cells: Cells were detached from the plate by trypsinisation for collecting, counting and homogenisation. They were also detached from the plate with a plastic spatula to analyse the reduced glutathione.

After washing twice with phosphate-buffered saline solution (137 mM NaCl, 8.1 mM Na_2HPO_4, 2.7 mM KCl and 1.5 mM KH_2PO_4, pH 7.4), the cells were treated with 1 ml Trypsin in each 5 ml flask and incubated at 37° C for approximately 5 minutes. Trypsin was inhibited by adding an equal amount of RPMI 1640 Dutch medium to the incubation solution. After centrifugation and removal of the supernatant, the cells were suspended with 0.5 ml RPMI 1640 Dutch medium and resuspended in 1 ml more medium for dilution, and then treated with Trypan Blue for counting.

Sonification of the cells: After centrifugation, the cells were sonicated for 15 seconds in bursts of 30 seconds with cooling at 0° C. The homogenate was then centrifuged at 22 000x g for 20 minutes (5). All enzymatic activities and protein assays were measured in the supernatants.

Enzyme assays: Se-GSH-Px and GSH-S transferase activities were determined by a modified method of Lawrence and Burk (6). SOD activities were measured using modified methods of Rigo et al. (7), Mc Cord and Fridowich (8), Roth and Gilbert (9), Beauchamp and Fridowich (10), and Beyer and Fridowich (11). CAT activity was measured using the method of Luck (12).

Glutathione assay: After collection using a plastic spatula and washing with sodium phosphate buffer, the Hepatoma G_2 cells were centrifuged and damaged by freezing and thawing at a constant repetition time. The proteins of the homogenate were precipitated with sodium metaphosphoric acid at a final concentration of 5% Volume to Weight (W/V). The final solution was centrifuged at 22 000g for 15 min and the supernatant used for the determination of GSH and GSSG using HPLC (13). The curves of the GSH and GSSG mixed standard solutions were measured between 8.8-10.4 min for GSH and 11.1-11.8 min for GSSG determinations.

LDH assay: LDH activity was determined at 25°C in 50 mM of phosphate buffer (pH 7.4) consisting of 0.18 mM NADH and 0.72 mM pyruvate. Absorbance changes were recorded at a wavelength of 340 nm (14).

Protein assay: Protein was determined by the Lowry et al. procedure (15), using bovine serum albumin as a standard.

Isozyme assay: Superoxide dismutase isozymes were observed using a starch gel electrophoresis system. The starch gel was used to determine the existence of Mn-SOD in Hepatoma G_2 cells treated with selenium (16).

Results

The addition of selenium to a Hepatoma G_2 cell line caused a significant increase in Se-GSH-Px activity and total GSH-Px level by 100% and 20% respectively, and reduced CAT activity by 20%. In the same cell line, Mn-SOD activity disappeared after selenium treatment. However, the starch gel electrophoresis showed that an inactive form of Mn-SOD existed in the cells (Table 1, Fig. 1). Selenium caused an increase in Cu,Zn SOD activity by 20.8% and a decrease in the GSH level by 56%. Se-independent GSH-Px activity did not change after selenium treatment. Malonyl aldehyde levels were found to be equal in both selenium-treated and selenium-untreated Hepatoma G_2 cells.

Discussion

The responses of Hepatoma G_2 cells to selenium treatment, quantified enzymatic activity, and changes in

	Unterated Hepatoma G2 Cells (Control Cells)	Sodium Selenite Treated Hepatoma G_2 Cells
Se-glutathione peroxidase	27.87±0.10µM/mg total prot.	73.23±0.18µM/mg tot.prot
Total glutathione peroxidase	33.28±2.34µM/mg total prot.	77.78±2.60µM/mg tot.prot
Catalase	30.89±0.27nM/mg total prot	30.52±0.19nM/mg tot.prot
Cu,Zn-superoxide dismutase	2.55±0.42µg /mg total prot.	3.22±0.28µg/mg tot.prot.
Mn-superoxide dismutase	4.67±0.41µg /mg total prot.	inactive
Malonyl aldehyde	2±0.001µM	2±0.0001µM
Reduced glutathione	15.91±2.34nM/mg total prot.	9.00±2.84 nM/mg tot.prot.

Table 1. The effects (Means ± 1 sd) of sodium selenite on radical scavenger enzymes and reduced glutathione in Hepatoma G_2 cells.

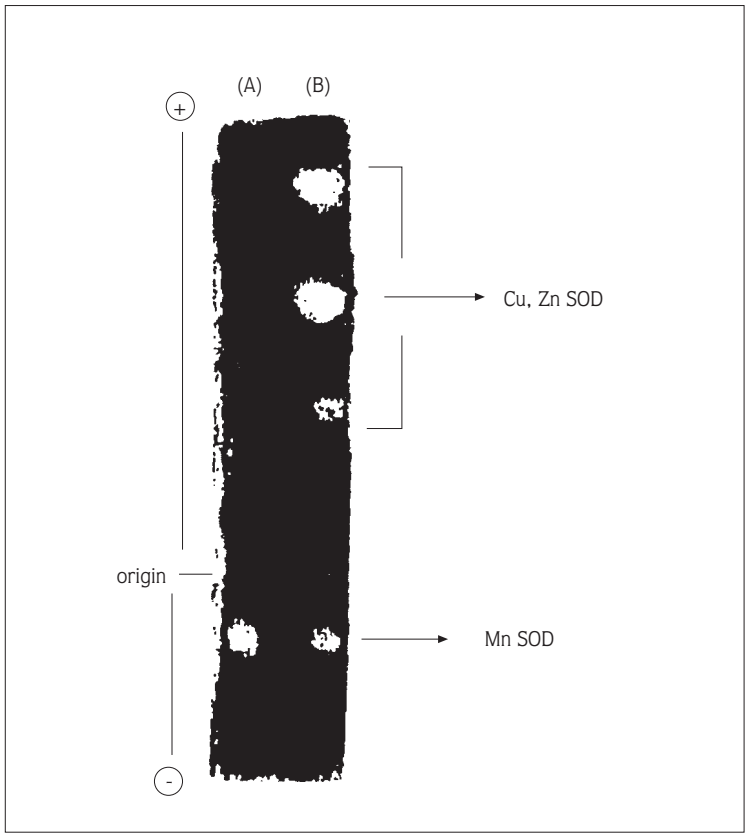

Figure 1. Chromatograms of superoxide dismutase isozymes inhibited with sodium cyanide (A), and not treated with sodium cyanide (B).

GSSG and GSH levels were examined. The decrease in Mn-SOD is of particular significance because it means that the observed changes are due to selenium or selenocysteine dependent protein as a Se-GSH-Px.

The levels of malonyl aldehyde in the selenium-treated and untreated cells (27 nM) were not toxic, a result consistent with that of Ohkawa et al. (17). The Se-GSH-Px activity of the Hepatoma G_2 cells treated with selenite was 2.6 times greater than that of the control cells. However, the Se-GSH-Px activity of the cells did not change when the amount of total glutathione increased by a factor of approximately 2.3. The increase in the Se-GSH-Px activity is interesting for Hepatoma G_2 cells, and there are parallels between these results and those of Simmons et al. (18). The increase in the enzymatic activity of hydroxy radical (OH^{-}) scavengers and membrane peroxidation blockers protects Hepatoma G_2 cells against potential damage from some radicals by

preventing mitochondrial membrane and cellular membrane damage. However, selenium independent GSH-Px, named glutathione-S transferase, did not show any difference.

The unchanging activity levels of this enzyme are significant because glutathione-S transferase plays a key role in cellular detoxification mechanisms. As a result, it requires further research.

Selenium did not cause any changes in CAT activity, but it caused an increase in Cu,Zn SOD activity by approximately 21% in Hepatoma G_2 cells. Selenium treatment depressed Mn-SOD activity, so the disappearance of this enzyme activity was investigated using electrophoresis. Inactivation of Mn-SOD, which is a mitochondrial enzyme, occurs either at transcriptional or post–translational level. Cu,Zn SOD did not exhibit activity in the medium supplemented with sodium cyanide using the polarographic method. On the other hand, a Mn-SOD band was observed in starch gel, as shown in Fig 1. This finding shows that Mn-SOD was found in Hepatoma G_2 cells but this activity may have been depressed in these cells by the selenium treatment. We are continuing our studies to determine if selenium has an inhibitor effect on Mn-SOD.

It has been shown (Table 1) that selenium caused a significant decrease in the GSH level, by 43%. In addition, chromatographic analysis showed that there was no GSSG in the Hepatoma G_2 cells (Fig 2). Therefore, we conclude that glutathione metabolism in cancer cells slows with selenium treatment. Although this idea seems to be contradictory, it is supported by the increase in the substrate oxidation, and the decrease in the metabolic oxidation in the cancer cells. It was considered that the increases in GSH-Px and Cu,Zn SOD activities played a more effective role in sweeping away free radicals which occur in Hepatoma G_2 cells. For this reason, studies on the control mechanism of glutathione reductase in cancer cells have become necessary.

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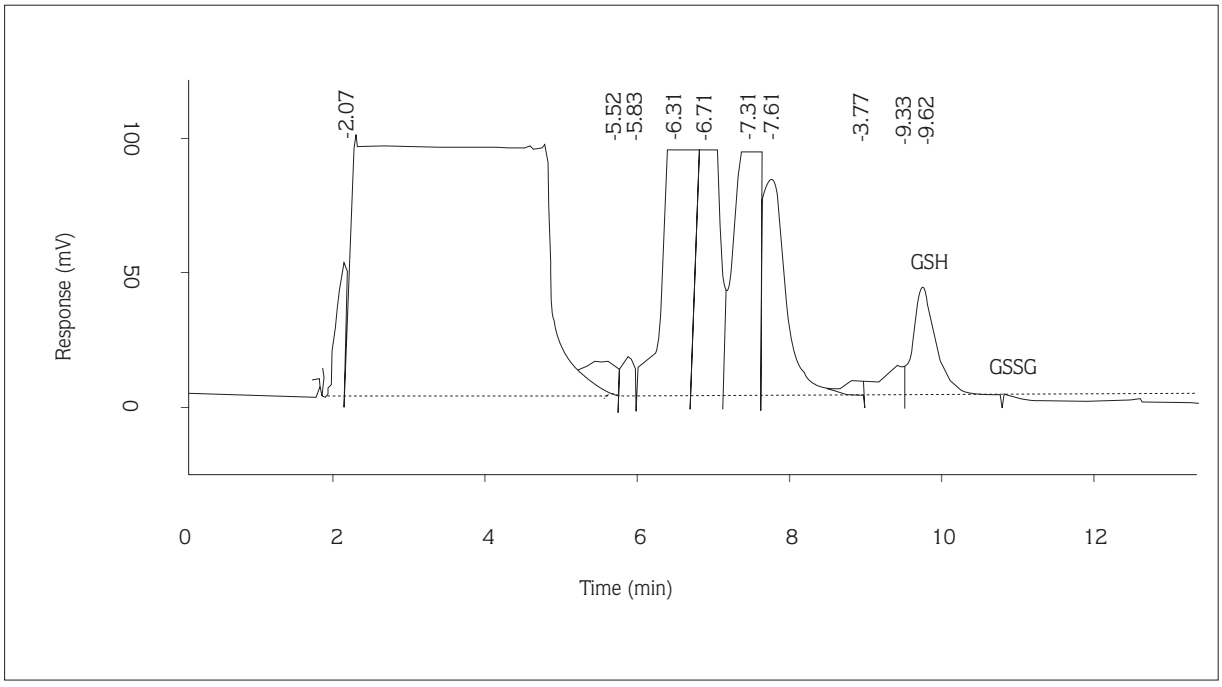

Figure 2. High Performance Liquid Chromatography curve of reduced (GSH) and oxidised (GSSG) glutathione prepared from a Hepatoma G_2 cell line treated with sodium selenite.

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