Cardiac and Red Blood Cell Glutathione Peroxidase: Results of a Prospective Randomized Trial in Patients on Total Parenteral Nutrition

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ABSTRACT

Oxygen derived free radicals and peroxides result from many antitumor treatments, including radiation and anthracyclines. Doxorubicin cardiotoxicity is thought to result from free radical induced lipid peroxidation. The heart has less active detoxification enzymes than does the liver and depends on selenium dependent glutathione peroxidase (GSH-PX) for this function. We did a sequential prospective trial in patients with totally controlled parenteral diets to examine the activity of red blood cell GSH-PX in patients with and without malignant disease. Decreased GSH-PX activity was found in 54% of the patients on parenteral nutrition and was more common in the older of these patients and in those with the greatest weight loss. In the absence of selenium supplementation, the RBC GSH-PX activity declines steadily, but with supplementation this was prevented or reversed. Because selenium deficiency can manifest as a cardiomyopathy, we measured the enzyme activity in the hearts of five patients who had died. The cardiac enzyme activity correlated strongly with the RBC levels. Significantly decreased GSH-PX has been shown in animals to be associated with changes in other enzymes critical both to activation and detoxification of carcinogens as well as antitumor drugs. Abnormality of selenium status might be a previously unsuspected contributor to interpatient variation in drug effects.

INTRODUCTION

The reduction of molecular oxygen to reactive species such as superoxide, hydrogen peroxide, and the hydroxyl radical occurs as a result of a wide variety of biochemical reactions, both normal and toxic (1). There has been a recent dramatic increase in interest in this area as evidence has accumulated linking the generation of these reactive oxygen species with a wide range of phenomena of importance to medical oncologists. These include such problems as tumor initiation and promotion (2), oxygen toxicity (3), radiation damage (4), and the action of a variety of anticancer drugs including anthracyclines (5). These observations have naturally led to studies on what defenses mammalian cells possess to minimize the toxic consequences of oxygen radical generation.

The enzymatic defense against oxygen radical injury is a two-step process. At first superoxide is converted into hydrogen peroxide; this is followed by the detoxification of that hydrogen peroxide. The latter function is accomplished by two enzymes. Catalase operates at high hydrogen peroxide concentrations and converts peroxide to water and oxygen, while glutathione peroxidase is effective at lower peroxide concentrations and uses glutathione to reduce hydrogen peroxide to water. We have shown previously that certain tissues such as heart muscle lack significant catalase activity and thus appear to depend upon glutathione peroxidase as their sole known mechanism of hydrogen peroxide removal (6). Depletion of the activity of this enzyme resulted in enhanced doxorubicin-induced cardiac toxicity in mice.

Glutathione peroxidase has selenium located at its active site as part of the amino acid selenocysteine. It is now well established in animals that the activity of glutathione peroxidase depends on dietary selenium and that deficiency of this element results in the development of cardiomyopathy as well as other problems (7). In human erythrocytes, although selenium can be found in association with other proteins, it is only the GSH-PX-associated selenium that is reflective of dietary status (8). The essential nature of selenium in humans has been based on two findings (9). People in the Keshan province of China develop a cardiomyopathy which is associated with very low dietary selenium levels. The pathology in this disorder is similar to that seen in selenium deficient animals (10). In the West there have been reports of people in this country on long term hyperalimentation who have developed low selenium or glutathione peroxidase levels with or without cardiomyopathy (11-13). Blood levels of elemental selenium and glutathione peroxidase in cancer patients have been variously reported as low or normal in a variety of reports (14-16). Since the nutritional status is a major determinant of these levels, the lack of appropriately documented and controlled dietary information may explain the confusion.

Selenium status has also been shown in mice to affect other enzymes of importance in both the activation and the detoxification of a number of antitumor drugs including doxorubicin and cyclophosphamide (17-19). Selenium in the form of selenite has been shown to protect mouse renal tubular cells from cis-diaminedichloroplatinum induced toxicity (20). Patients on shorter term hyperalimentation offered a unique opportunity to examine, in a prospective randomized trial, the sequential changes in RBC GSH-PX with and without selenium supplementation. The results confirm the selenium dependence of this enzyme and provide an estimate of selenomethionine required to maintain RBC levels normal. The fact that RBC levels reflect cardiac levels is important to those concerned with the consequences of oxygen radical production in humans.

MATERIALS AND METHODS

Patients. Ten normal volunteers from our laboratory (four females and six males) served as the laboratory control population. The mean age (±) is 23.7 ± 2.7 years.

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1The abbreviations used are: GSH-PX, glutathione peroxidase; TPN, total parenteral nutrition; GST, glutathione-S-transferase.
The tissue was homogenized in a buffer solution containing sucrose, mannitol, and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid. Protein was conducted in accordance with the principles for human experimentation on April 15, 2017. © 1985 American Association for Cancer Research. cancerres.aacrjournals.org Downloaded from cancerres.aacrjournals.org on April 15, 2017. © 1985 American Association for Cancer Research.

analytical studies demonstrated the stability of GSH-PX upon freezing. Between the time from death to harvesting and the enzyme activity, and water. Hemoglobin was determined by a colorimetric technique (Sigma). Than platelets, since this method has been reported elsewhere (11–13) and all patients on TPN either gained weight or else had stabilization of SD) was 47.1 ± 16.7 years. The patients studied were referred to the Nutrition Support Service at the Clinical Center of the NIH for i.v. feeding. The time on TPN was 36 ± 26 days (range, 5–113). The study was conducted in accordance with the principles for human experimentation as defined in the Declaration of Helsinki. The initial 10 patients simply had weekly RBC enzyme assays without supplementation to establish the incidence and degree of decrease in GSH-PX without symptoms that could be expected. The subsequent 22 patients were randomized to either receive selenomethionine (200 µg i.v. daily) or not. One patient was on nutritional support for only 1 week and was therefore included in analysis of the initial RBC GSH-PX level only, but not in the sequential measurements. Of the 32 patients 23 (72%) had a diagnosis of cancer; all were recently admitted and either about to begin or had just begun anticancer therapy. Table 1 lists the tumor types and stage in these patients. There were approximately equal numbers of patients with localized versus more extensive tumor.

Enzyme Assays. GSH-PX was assayed in RBC hemolysates rather than platelets, since this method has been reported elsewhere (11–13) and because many of our patients had significant thrombocytopenia as a result of treatment for their cancers. Glutathione peroxidase can use a variety of substrates, including organic hydroperoxides (e.g., cumene, and tert-butyl hydroperoxides), as well as hydrogen peroxide. The RBC hemolysates were treated with Drabkin’s reagent (Sigma Chemical Co., St. Louis, MO) to prevent interaction of hydrogen peroxide with oxyhemoglobin. The initial 15 patient samples were assayed using all three substrates; however, the patterns of change were found to be virtually identical, and for this reason hydrogen peroxide alone was used for the studies in this report. Further, selenium-independent peroxidase activity has been reported due to GST with organic hydroperoxides but not with hydrogen peroxide as substrate (21). We measured GSH-PX with H2O2 as substrate as well as GST activity.

Weekly samples of blood were drawn into heparinized tubes and placed on ice. RBC were separated, washed, and lysed with distilled water. Hemoglobin was determined by a colorimetric technique (Sigma). The heart tissue was obtained at autopsy performed from 6 to 16 h after death and was kept frozen until assayed. There was no relationship between the time from death to harvesting and the enzyme activity, and analytical studies demonstrated the stability of GSH-PX upon freezing. The tissue was homogenized in a buffer solution containing sucrose, mannitol, and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid. Protein was determined according to the method of Lowry et al. (22). Enzymes were measured spectrophotometrically; glutathione peroxidase was assayed using the method of Paglia and Valentine (23), and glutathione S-transferase was measured with chlorodinitrobenzene (Sigma) as substrate as described previously (24). Results are expressed as IU, or 10⁻³ nmol NADPH oxidized/min/g hemoglobin (or nmol/g protein).

Statistical Analysis. Data are presented as mean ± SD and are analyzed using the Wilcoxon nonparametric test and the Spearman correlation analysis.

RESULTS

Pretreatment Evaluation. The 10 normal volunteers had a mean RBC glutathione peroxidase level of 27.2 ± 3.3 IU/g hemoglobin (range, 22–31 IU/g hemoglobin). The initial RBC GSH-PX activity was below the normal range in 17 patients. These patients were significantly older (P = 0.033) and had greater weight loss (P = 0.036) than did the patients with initially normal GSH-PX activity (Table 2). There was no difference in the incidence of cancer between these two groups.

The initial GSH-PX activity was examined as a function of weight loss. Mild to moderate weight loss is associated with a minimal decline in the enzyme activity to the low normal range. With massive weight loss of 25% or greater of usual body weight, there is a further and dramatic decline in GSH-PX. The Spearman correlation coefficient is 0.521 (P = 0.002).

There was no significant difference between the mean GSH-PX activity in the whole group of patients with cancers versus those with benign diseases (22.4 ± 9.7 versus 20.5 ± 5.4). They had similar amounts of weight loss. Some tumor types appeared to be associated with lowered enzyme activity not due to disproportionate weight loss. Both lymphoma patients had a mean value of 16.5 IU/g hemoglobin, both esophageal cancer patients had a mean value of 12.8 IU/g hemoglobin, and the eight patients with pancreatic tumors had a mean value of 18.1 IU/g hemoglobin. There was no relationship between initial RBC and GSH-PX. RBC transfusions were given to seven patients in the placebo group and five in the selenium supplemented patients during the course of TPN. There was no apparent significant or persistent effect on the GSH-PX in either group.

Effect of Selenomethionine on GSH-PX. Thirty-one patients were evaluable for the effect of selenium supplementation. Twelve patients were randomized to receive selenomethionine. Four of these had normal base-line levels which remained normal during TPN. The other eight patients had low initial GSH-PX activity. The values in five of these patients increased to within the normal range, from 13.8 ± 4.7 IU at base line to 23.4 ± 1.5 IU in 11.4 ± 9 days. Two additional patients had significant increases but did not reach the normal range while on nutritional support. The single patient whose level remained low despite supplementation was unique only for a severe bacterial septicemia.

Nineteen patients received no selenium supplementation. Eleven had base-line GSH-PX activity in the normal range. Ten of these had significant decreases in GSH-PX during the study.

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>No.</th>
<th>Local</th>
<th>Extensive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoma (DHL)</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Pancreas</td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Esophagus</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Brain</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Ovarian</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Testicular</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1

Patients with malignant disease

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>No.</th>
<th>Local</th>
<th>Extensive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoma (DHL)</td>
<td>2</td>
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<td>0</td>
</tr>
<tr>
<td>Ovarian</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Testicular</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2

Characteristics of patients with initial GSH-PX activity above and below the mean of the normal control

<table>
<thead>
<tr>
<th>GSH-PX activity (IU/g hemoglobin)</th>
<th>No.</th>
<th>Age (yr)</th>
<th>Wt loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low 14.42 ± 5.5*</td>
<td>17</td>
<td>54.44 ± 11.5</td>
<td>20.11 ± 9.5</td>
</tr>
<tr>
<td>Normal 29.22 ± 6.5</td>
<td>15</td>
<td>38.36 ± 16.8</td>
<td>12.61 ± 5.5</td>
</tr>
</tbody>
</table>

| | P | 0.033 | 0.036 |

* Mean ± SE.
eight to below the normal range, during 44.7 ± 21 days on parenteral nutrition. Chart 1 demonstrates the mean weekly percentage of control values for these 11 patients. Without selenium supplementation there is a progressive decline in mean RBC GSH-PX activity. Eight patients receiving placebo had low initial RBC GSH-PX activity (12.2 ± 4.5). These remained low or declined at a slow rate in the absence of selenium. There was no detectable clinical evidence for selenium deficiency. Clinical and chemical monitoring of the patients receiving selenomethionine during the study did not show any evidence of toxicity, including unexplained nausea and vomiting, hair loss, or nail abnormalities.

**Glutathione-S-transferase.** The initial RBC GST in these patients was 1.66 ± 0.9 IU/g hemoglobin, within the normal range of the control population. There was no correlation of GST activity with GSH-PX activity, weight loss, diagnosis, or selenium supplementation.

**Pathology.** Postmortem examination was performed on five of the patients included in this study, all with low GSH-PX values prior to death (16.5 ± 4.0 IU/g hemoglobin). Only one had been receiving selenium supplementation. Death was in no case related to cardiac dysfunction. All five of these patients had a cancer and all five received a wide variety of antineoplastic drugs. Two were given Adriamycin (total doses, 365 and 425 mg/m², respectively). There was no discernible effect of the chemotherapy on the RBC GSH-PX activity in these patients or in those supplemented with selenium.

Sections of left and right ventricular tissue of the five patients were examined grossly and histologically, looking specifically for lesions consistent with selenium deficiency. The amount of lipofuscin pigment present in the myocytes was consistent with the patients' respective ages. Only one patient had evidence of myocytolysis which involved only two small foci, and no other patients showed any lesions of the type described in this deficiency syndrome. Two patients had mild fibrosis, a nonspecific finding. Three patients demonstrated atrophy and edema of the myocardium. These changes were thought to be consistent with the respective patients' histories of poor nutritional status. There were none of the signs of Adriamycin-induced cardiomyopathy. Examination of samples of skeletal muscle showed no changes suggestive of selenium deficiency.

**Cardiac Tissue Glutathione Peroxidase.** Samples of ventricular muscle were obtained at autopsy performed on the five patients. Since a previous report of selenium deficiency related cardiomyopathy reported GSH-PX activity in whole tissue homogenates, we present our results in the same form (11). In that report the authors used tert-butyl hydroperoxide as the substrate in the assay and reported a mean value for heart tissue homogenates in three patients who died of noncardiac causes (27 nmol/g protein) (11). Chart 2 demonstrates the relation between GSH-PX activity in RBC hemolysates versus heart tissue homogenates, using tert-butyl hydroperoxide. The mean "normal" value of Cohen et al. is shown. The Spearman correlation coefficient is 0.85. Since we did most of the RBC measurements using H₂O₂ as substrate to control for GST activity, the values of heart versus RBC GSH-PX with this substrate are presented as well (r = 0.87).

**DISCUSSION**

Previous reports have shown diminished GSH-PX activity in long term parenteral nutrition patients and in persons living in areas with low soil selenium content (11–13). We wished to study the sequential effects of selenium supplementation in a
prospective and randomized trial. In the absence of selenium supplementation 10 of 11 patients had a decline in RBC GSH-PX over only 4 weeks, while supplementation maintained normal levels in those with initially normal levels. Selenium supplementation increased those with low levels within 20 days.

In animal studies of selenium deficiency, the decline in erythrocyte glutathione peroxidase is paralleled in the various tissues including liver and muscle (7). As discussed previously certain tissues such as the heart appear to lack significant catalase activity. Thus a decline in selenium dependent glutathione peroxidase leaves the heart with no known means for detoxification of hydrogen peroxide. For this reason we sought to establish the relationship between RBC hemolysate and cardiac tissue GSH-PX. Since hydrogen peroxide is known to be a by-product of cardiac mitochondrial metabolism, these findings provide an explanation for the cardiac damage which results from prolonged selenium deficiency. Pathological examination of these hearts failed to demonstrate any of the light microscopic changes classically described with selenium deficiency. This may be due to the fact that the selenium deficiency was of brief duration. Indeed most of the reported cases of symptomatic selenium deficiency were in cases where the deficiency was of much longer duration. Studies are underway in this laboratory to establish the relationship between the duration of the selenium deficiency and the evolution of cardiac injury using a newly established animal model.

Since glutathione peroxidase is important in the removal of reactive peroxides, the toxic consequences of depressed enzyme activity may be made more evident by any agent or event that would accelerate peroxide generation. Doxorubicin has been demonstrated, at clinically relevant doses, to dramatically enhance superoxide and hydrogen peroxide production in cardiac sarcoplasmic reticulum and mitochondria (25). It is thus not surprising that selenium deficiency has been shown to increase the susceptibility of mice to Adriamycin injury (6). In addition recent studies in mice have demonstrated both increases and decreases in a variety of liver enzymes important in both activation and detoxification of a number of anticancer drugs as well as carcinogens (19). Furthermore the changes in these enzymes in selenium deficient animals occur after the GSH-PX has significantly declined. Since cancer patients will receive therapies associated with oxygen radical production or requiring metabolic activation or detoxification, these data suggest the importance of assessing selenium status and the utility of GSH-PX as marker of selenium status.

The small numbers of patients receiving any one particular form of therapy make it difficult to recognize a consistent effect of the treatment on RBC GSH-PX. This will require further prospective testing of a larger group with similar treatment.

REFERENCES

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