

Decreased Glutathione Peroxidase Activities with Concomitant Increased Oxidized Glutathione Levels among Residents in an Arsenic Contaminated Community of Southern Thailand

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ABSTRACT

Glutathione peroxidase (GPx) and glutathione are important antioxidants responsible for the scavenging of reactive oxygen species (ROS). It has been shown that changes in GPx activities and glutathione levels are associated with various diseases including toxic chemical related diseases and cancers. The study aimed to determine the levels of GPx activity and glutathione among residents in Ron Phibun district, an arsenic-exposed area. Blood samples were obtained from 32 volunteers in the Thasala group, a nearby nonarsenic-exposed area and 36 residents in the Ron Phibun group. Red cell lysates were subjected to analysis of GPx activity and glutathione. The results showed that GPx activities were significantly decreased among study subjects from Ron Phibun ($p < 0.05$). Interestingly, oxidized glutathione (GSSG) levels were significantly increased compared with those from Thasala ($p < 0.05$). Total glutathione and reduced glutathione (GSH) levels were not different among the two groups. Mean values of GPx activities, total glutathione and GSH tended to decrease among high-exposure subjects compared to low-exposure subjects. This was concomitant with a slight increase in GSSG levels among high-exposure subjects. The levels of GPx activities and GSSG may be early biomarkers for low levels of oxidative stress in a mining area affected with arsenic poisoning.

Keywords: Glutathione peroxidase, glutathione, antioxidants

INTRODUCTION

Glutathione peroxidase (GPx) and glutathione are known antioxidants preventing cells from oxidative damage induced by various conditions. Both of them are biomarkers for the early biological effects of ingested inorganic arsenic [1]. Changes in antioxidant status have been documented in a variety of cultured cells and experimental animals. Arsenic exposure causes a reduction in GPx activities and reduced glutathione (GSH) levels in esophageal cancer cells [2] and in human urothelial cells, respectively [3]. Depletion of GPx, superoxide dismutase (SOD) and catalase (CT) activities were observed in the livers and kidneys of arsenic exposed rats [4]. However, some animal models showed induction in the activities of antioxidant enzymes in arsenic-exposed animals [5,6]. In *in vivo* models, chronic arsenic exposure led to a depletion of GSH and that was accompanied by a significant depletion of hemoglobin, red blood cell (RBC) and hematocrit (Hct) as well as blood SOD activity [7]. Decreased antioxidant nonprotein sulfhydryl (NPSH) levels with concomitant increased plasma levels of reactive oxidants were observed in human populations exposed to arsenic [8]. The levels of arsenic concentration in the blood were positively associated with the levels of reactive oxidants in the plasma and an inversely related to the plasma antioxidant capacity [9].

Ron Phibun district in Nakhon Si Thammarat province, Southern Thailand is well known as an area of arsenic (As) contamination. Arsenic concentrations in drinking water varied within the mean levels of $4.9 \pm 11.0 \mu\text{g/l}$ [10]. Importantly, As guidelines in drinking water in Canada are currently contemplating a further decrease to $5 \mu\text{g/l}$ because of deleterious effects at lower concentrations than was previously thought [11]. Arsenic exposure leads to its accumulation in tissues resulting in various clinical symptoms such as hyperpigmentation and keratosis as well as increased risk of skin, internal organ, and lung cancers. Skin manifestations of arsenic poisoning were found in 9 % of examined adults randomly selected from 58 households [12]. Total arsenic and its metabolites in urine were significantly increased in 10 year old schoolchildren living in high-arsenic exposure areas [10]. The main sources of the exposure are the drinking water and surface soil [10,12]. This indicated that the use of arsenic contaminated water and playing with the soil without the washing of hands afterwards may be the causes for the development of arsenic-related diseases. However, to date no reports on the antioxidant status of GPx and glutathione among residents in an endemic area have been conducted. The objective of this study was to determine GPx activities and glutathione levels among residents in Ron Phibun district.

MATERIALS AND METHODS

Subjects and blood samples

Blood samples were collected from 20 and 16 volunteers who have attended for regular check up at Thasala and Ron Phibun Hospitals, respectively. Additionally, 12 students from Walailak University in Thasala district and 20 residents in Ron Phibun district were recruited. Thus, a total of 32 volunteers, the Thasala group and 36 volunteers, the Ron Phibun group, participated in the study. Subjects of the Thasala group were residents from Muang, Thasala and Sichon Districts of Nakhon Si Thammarat and have never lived in Ron Phibun district. In the case of the Ron Phibun group, residents who have been living in Ron Phibun district for more than 1 year were chosen for the study. All the subjects consented to take part in the study. Face-to-face interviews were undertaken to rule out volunteers who had health problems. All subjects recruited for this study were free of any clinical symptoms such as inflammatory diseases. For all assays, a 3 ml blood sample was collected in EDTA (ethylenediaminetetraacetic acid) tubes and kept at 4 °C throughout the process. For long term storage, the lysates were kept at -80 °C.

Glutathione peroxidase activity assay

For determination of GPx activity, an enzyme kinetic method was performed [13]. Briefly, 1.34 mM GSH (Sigma) and 1.33 U/ml glutathione reductase (GR) (Sigma) were prepared in a potassium phosphate buffer at pH 7.0 containing 1.1 mM EDTA and 1.1 mM NaN₃. Lysates of 1.5 g Hb/l was added to the prepared buffer. GSH and GR at a final concentration were 0.94 mM and 0.93 U/ml, respectively. NADPH (Sigma) and H₂O₂ were added to obtain a final concentration of 0.4 mM and 0.25 mM, respectively. Reduction of NADPH was measured every 30 seconds at 340 nm. The results were expressed as U/g Hb.

Glutathione assay

Fresh red cells were lysed and the lysates were determined for hemoglobin content. Equal hemoglobin concentrations were used for the analysis of glutathione using colorimetry [14]. Briefly, an equal volume of 5 g Hb/l and 5 % 5-sulfosalicylic acid (SSA) were mixed to precipitate proteins. The supernatant was obtained by centrifugation and an aliquot of 50 µl was added into each separated microcentrifuge tube for total glutathione and GSSG assays. GSSG was prepared by adding 2 µl of a 4-Vinylpyridine (4-VP) (Sigma) solution containing ethanol (ratio 1:1) to the aliquot and the mixture was incubated for 90 min at 4 °C. Both tubes were centrifuged at maximum speed for 5 min at room temperature. The supernatant was subjected to analysis in the presence of NADPH, 5,5'-dithio-bis-(2-nitrobenzoic acid) (Sigma), and GR at final concentrations of 0.2 mM, 0.6 mM, and 0.5 U/ml, respectively. The kinetic reaction was measured every 30 seconds following color development at 412 nm. GSH was obtained by subtraction of GSSG from the total glutathione. The results were expressed as µmol/g Hb.

Statistical analysis

Means values from each analysis were statistically tested using Student's t-test for independent samples.

RESULTS AND DISCUSSION

In total there were 68 study subjects. There were 32 and 36 people in the Thasala and Ron Phibun group, respectively (**Table 1**). The ages of residents in the Thasala group were 38 ± 13 years while in the Ron Phibun group this was 30 ± 12 years. The present study revealed that GPx activities in the Ron Phibun group (27.8 ± 9.8 U/g Hb) were statistically lower in comparison with those from Thasala group (64.4 ± 35.2 U/g Hb) ($p < 0.05$; **Table 2**). The levels of GSSG, a marker of oxidative stress, were significantly increased in Ron Phibun group (0.9 ± 0.5 $\mu\text{mol/g}$ Hb) compared to those from Thasala group (0.5 ± 0.2 $\mu\text{mol/g}$ Hb) ($p < 0.05$; **Table 2**). Environmental exposure to toxic chemicals in a mining area may play an important role in oxidative stress as shown by the reduction of the enzyme activities and increased GSSG levels. Total glutathione and GSH levels were slightly decreased in Ron Phibun group compared to the Thasala group (**Table 2**).

Table 1 Demographic data of the subjects.

| Groups | Total number (n) | Sex | | Age (years) |
|------------|------------------|-------|---------|-------------|
| | | Males | Females | |
| Thasala | 32 | 9 | 23 | 38 ± 13 |
| Ron Phibun | 36 | 10 | 26 | 30 ± 12 |

Table 2 Levels of GPx activities, total glutathione, GSH, and GSSG among residents in the Thasala and Ron Phibun group.

| Parameters | Thasala (n = 32) | Ron Phibun (n = 36) |
|---|------------------|---------------------|
| GPx (U/g Hb) | 64.4 ± 35.2 | $27.8 \pm 9.8^*$ |
| Total glutathione ($\mu\text{mol/g}$ Hb) | 7.6 ± 2.8 | 7.3 ± 2.60 |
| GSH ($\mu\text{mol/g}$ Hb) | 7.1 ± 2.8 | 6.4 ± 2.80 |
| GSSG ($\mu\text{mol/g}$ Hb) | 0.5 ± 0.2 | $0.9 \pm 0.5^*$ |

Abbreviation: Hb = hemoglobin, U = unit. Values shown are mean \pm SD (* $p < 0.05$).

Toxic chemicals including arsenic induced free radicals with concomitant interfering antioxidant capacity [15]. Decreased GPx activities were observed in various animal models including arsenic-exposed rats [4,16] as well as *in vitro* cultured cells [17]. It was suggested that GPx is more important than CT in defending against arsenite toxicity in chinese hamster ovary (CHO) cells [17]. The results obtained from this preliminary study indicated that the levels of GPx activities were significantly decreased among residents in Ron Phibun district compared to those from Thasala [18]. Reduction in the activities of GPx by arsenic may be the result of binding to –SH groups of the enzymes and the oxidation of As(III) to As(V) resulting in H₂O₂ production [15]. Various conditions including diet, drugs, toxic chemicals and diseases may affect GPx activities. The subjects were free of diseases and medication use. Other conditions (e.g. vitamin deficiency and oxidative stress) affecting the specific enzyme may lead to low activity levels. As Ron Phibun district was previously a mining area affected with arsenic poisoning, the present results may be the result of arsenic toxicity. However, other toxic chemicals in the endemic area cannot be excluded although such chemicals have not been documented.

It has been shown that chronic arsenic exposure leads to a ROS-mediated apoptosis in hepatic cells with a significant increase in GSSG levels and decreased GR activities [19]. The GSH:GSSG ratio was significantly decreased in hepatic and renal cells suffering from chronic arsenic poisoning in rats [7]. Significant increases in GSSG levels may implicate oxidative stress induced by environmental factors in a mining area. However, the results show a slight decrease in total glutathione and GSH levels and that may depend on various factors of the exposure.

Thirty six subjects from Ron Phibun group were divided into high and low exposure groups according to whether they lived in a high exposure area (subdistricts 2, 12, and 13) or a low exposure area (subdistricts 1, 3, 4, 5, 6, 7, 9, 10, 11 and 15) as previously described [10]. The high exposure area in Ron Phibun district is composed of subdistricts where tin mining and tin smelting had been active in the past. Mean values of arsenic levels in drinking water were 4.9 ± 11.0 µg/l and in soil were 93.3 ± 70.3 mg/kg. A zone on the perimeter of the high exposure area was designated as a low exposure area where lower concentrations of arsenic were found. Mean values of arsenic levels in the low exposure area were 1.7 ± 2.0 µg/l in drinking water and 16.9 ± 10.3 mg/kg in soil. To compare among residents who have been living in high and low exposure areas, all parameters were statistically tested. The results showed that GPx activities, total glutathione and GSH levels were slightly decreased among high-exposure subjects when compared to low-exposure subjects (**Table 3**). High-exposure subjects also showed slight increases in GSSG levels (**Table 3**). The results obtained from high- and low-exposure subjects were not significantly different. This may be due to various factors including the duration and magnitude of exposure, source of exposure, nutrition, age and general health status of the individuals. Measurement of the arsenic concentration will provide valuable information as to which factors cause deterioration in the antioxidant defense system.

Table 3 Levels of GPx activities, total glutathione, GSH and GSSG of high- and low-exposure subjects in Ron Phibun district.

| Parameters | Ron Phibun group | |
|-------------------------------|------------------|--------------|
| | High (n = 12) | Low (n = 24) |
| GPx (U/g Hb) | 25.0 ± 10.5 | 28.8 ± 9.4 |
| Total glutathione (μmol/g Hb) | 6.7 ± 1.70 | 7.6 ± 3.0 |
| GSH (μmol/g Hb) | 5.6 ± 1.90 | 6.8 ± 3.0 |
| GSSG (μmol/g Hb) | 1.0 ± 0.40 | 0.8 ± 0.5 |

Abbreviation: Hb = hemoglobin, U = unit. Values shown are mean ± SD

CONCLUSIONS

This is the first report on the activity of GPx and level of glutathione among residents in a mining area. Decreased GPx activities with concomitant gain of GSSG levels may be determinants of early biomarkers for environmental exposure to toxic chemicals in a mining area. The long-term goal of this research is to look for early biomarkers which may be used as clinical parameters for human populations exposed to toxic chemicals in this area.

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บทคัดย่อ

วรางคณา จุ่งลก

การลดลงของระดับ glutathione peroxidase activities และการเพิ่มสูงขึ้นของระดับ oxidized glutathione ในกลุ่มตัวอย่างที่อาศัยในบริเวณที่มีการปนเปื้อนของสารหนูภาคใต้ของประเทศไทย

เอนไซม์ glutathione peroxidase (GPx) และ glutathione เป็นสารต้านอนุมูลอิสระที่มีความสำคัญในการกำจัดสาร reactive oxygen species (ROS) การเปลี่ยนแปลงของระดับสารต้านอนุมูลอิสระดังกล่าวพบว่ามี ความสัมพันธ์กับการเกิดโรคต่างๆ มากมาย ที่มีสาเหตุมาจากสารพิษรวมถึงโรคมะเร็ง การศึกษาครั้งนี้มี วัตถุประสงค์เพื่อศึกษาระดับ GPx activities และระดับ glutathione ของกลุ่มตัวอย่างที่อาศัยในอำเภอรัตนวาปีซึ่ง เป็นพื้นที่ที่พบการปนเปื้อนของสารหนู โดยการเก็บตัวอย่างเลือดจากกลุ่มทำศาลาซึ่งมีจำนวนอาสาสมัครที่เข้าร่วม 32 คน และจากกลุ่มรัตนวาปีจำนวน 36 คน ผลการศึกษาพบว่าระดับ GPx activities ลดลงอย่างมีนัยสำคัญทาง สถิติในกลุ่มรัตนวาปีเมื่อเปรียบเทียบกับกลุ่มทำศาลา ($p < 0.05$) ระดับ oxidized glutathione (GSSG) ก็พบว่าสูงขึ้น อย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับกลุ่มทำศาลา ($p < 0.05$) ส่วนระดับ total glutathione และ reduced glutathione (GSH) ไม่มีความแตกต่างกันทางสถิติระหว่างสองกลุ่ม เมื่อศึกษาเปรียบเทียบเฉพาะกลุ่มรัตนวาปี ระหว่างกลุ่มที่พบว่าการปนเปื้อนของสารหนูสูง และในกลุ่มที่พบว่าการปนเปื้อนของสารหนುದำพบว่าระดับ GPx activities ระดับ total glutathione และ ระดับ GSH มีค่าต่ำลงเพียงเล็กน้อยในกลุ่มที่พบว่าการปนเปื้อนของสารหนู สูง ส่วนระดับ GSSG พบว่าสูงขึ้นเพียงเล็กน้อยในกลุ่มดังกล่าว ดังนั้นการลดลงของระดับ GPx activity และ GSSG อาจใช้เป็นตัวชี้วัดภาวะ oxidative stress ระดับต่ำๆ ที่เกิดขึ้นภายในร่างกาย อันเนื่องมาจากสิ่งแวดล้อมที่อยู่ อาศัยของกลุ่มตัวอย่างในพื้นที่อำเภอรัตนวาปีที่พบรายงานการเกิดพิษจากสารหนู