EFFECT OF BORAX ON GLUTATHIONE LEVELS IN HUMAN RED BLOOD CELLS IN VITRO

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Abstract  Sodium tetraborate or borax is used as a preservative in food and it is toxic to humans depending on dosage. The target organs of borax toxicity include the central nervous system, kidney and liver, and borax can be accumulated in the blood circulation. The aim of this study was to evaluate the effect of borax on human red blood cells (RBCs) of both normal and chronic renal failure (CRF) patients by determining glutathione (GSH) levels and plasma borax levels. Normal packed RBCs were incubated with borax at 0, 0.33, 0.65, 1.30, and 2.60 µM and measured GSH levels to identify the maximal dose effect. Then RBCs of both normal and CRF groups were incubated in borax solution (at 1.3 µM, which was the maximal effect dose) at 0, 60, 120, and 180 minutes and determined for GSH levels by the dithiobisnitrobenzoic (DTNB) method. Plasma borax was measured by using the carminic method in 10 normal and 17 CRF patients. The mean GSH level in normal subjects was found at 53.90± 9.41 mg/dL, and 63.41± 13.64 mg/dL (p>0.05) in CRF patients. GSH levels showed no significant change between healthy and CRF patients (p>0.05) after incubating RBCs with borax solution at different times. The plasma borax levels were not significantly different (p>0.05) between healthy subjects (3.34± 1.05 mg/L) and CRF patients (3.07± 1.34 mg/L). Borax may not be a risk factor of renal failure. It could be concluded that borax is not an oxidant for human RBCs in vitro. Chiang Mai Med Bull 2006;45(3):93-100.

Keywords: borax, glutathione (GSH), chronic renal failure (CRF), erythrocytes or red blood cells (RBCs)

Borax is important in the ceramic industry and used as a reagent in the production of borosilicate glass, which has a high refractive index suitable for the manufacture of lenses. Other applications of borax include the impregnation of textiles and wood to make them fire
resistant. A weakbased, borax is also used in buffer solutions and as photographic developers. It is added to boron poor soils as a fertilizer and also used as a mild disinfectant. Although its toxicity is low, it is not completely harmless. Its use as a food preservative is prohibited in many countries.\(^{(1)}\)

There is a report on the situation of borax abuse in food by a primary screening test from markets chosen randomly. The result indicated that the prevalence of borax abuse in food in Bangkok was 7.2%, with a risk of 49.3% in minced meat, pork, chicken, and fish; 20% in meat, pork, chicken and Thai sweets made from flour; and 10.1% in preserved fruits, significant with \(p=0.00001\).\(^{(2)}\)

The degree of borax toxicity depends on the doses or concentrations that the human body receives. Its toxicity, which involves the kidney mainly, stomach and intestine, will develop signs of inflammation. Other organs affected are the liver, brain, and skin. There would be symptoms of oligouria or anuria in the case of renal failure.\(^{(2)}\)

In an animal model, mice were fed with a high dose of borax (2,000 mg/L that is 5.24 \(\mu\)M) for 60 days and developed testes atrophy, decreased ovulation, weight loss, and decreased spermatogenesis. In severe cases, it caused death and boron was found to have accumulated in the liver, kidney and brain. Toxicity in humans includes carcinogenicity, reproductive and developmental toxicity, neurotoxicity and nephrotoxicity.\(^{(3)}\)

Glutathione (gamma-glutamyl-cysteinyl-glycine) or GSH is an antioxidant found as a small molecule in the red blood cells and other cells in the human body. Thiol moiety on the cysteine is important for its antioxidant property. It also promotes the detoxification process involved in the reaction of glutathione S-transferase (GST). This enzyme conjugates GSH to the functional groups of toxins to increase the polarity of the substances, making them more soluble and allowing excretion via urine. GSH also helps in decreasing oxidative stress in red blood cells (RBCs) to destroy hydrogen peroxide, keep cell membrane integrity and prevent early hemolysis.\(^{(4)}\)

Since borax affects the kidney and can be accumulated in tissue and blood, it is intriguing to measure whether plasma borax is high in chronic renal failure (CRF) patients. The objective of this work was to study the effect of borax on GSH levels in the RBCs of normal humans red blood cells compared to those of CRF patients in an \textit{in vitro} system, and demonstrate the levels of plasma borax in normal and CRF patients.

**Materials and methods**

Dithiobisnitrobenzoic acid, glutathione, and carminic acid were obtained from Sigma (St. Louis, MO, USA).

The normal blood was collected from the blood bank at Maharaj Nakorn Chiang Mai Hospital and screened for infectious diseases (hepatitis viruses and HIV). The patient blood was collected from the CRF patients who attended a special clinic at the Department of Surgery for an arterovenous fistula operation. The erythrocyte GSH was measured in 20 healthy subjects whose age range was 18-38 years old, and 10 CRF patients whose age range was 35-59 years old. Ten milliliters of whole blood was collected and stored at 4 degrees Celsius until use or for a maximum of 1 week. ethylenediaminetetraacetic acid (EDTA) was used as an anticoagulant. The CRF patients had a mean creatinine level of more than 10 mg/dL. Neither normal subjects nor CRF patients received any drugs.
The normal subjects and CRF patients were screened by methemoglobin reduction assay to exclude the status of glucose 6-phosphate dehydrogenase (G-6PD) deficiency. Briefly, whole blood (0.2 mL) was added to glucose/sodium nitrite (0.01 mL) and methylene blue (0.01 mL), mixed well, and incubated at 37 degrees Celsius for 3 h. Distilled water (5 mL) was added and the color was observed. For G-6PD deficient patients, the solution was brown in color and for normal subjects, red.

**Treatment of RBCs with borax**

Normal packed red cells at 1.2 mL, were mixed with borax solution (0, 0.33, 0.65, 1.30, 2.60 µM) (final concentration) and incubated at 37 degrees Celsius. The GSH levels were measured at 0, 60, 120, and 180 min.

**Determination of glutathione by the DTNB method**

Whole blood (0.4 mL) was added to distilled water (1.6 mL) together with 3 mL of precipitating solution (1.67% glacial meta-phosphoric acid, 0.7 mM ethylenediaminetetraacetic acid (EDTA) and 5.13 M sodium chloride). Then, the filtrate (1 mL) was added to 0.05 M phosphate buffer, pH 6.4 (4 mL). Finally, 1 mM DTNB (0.5 mL) was added, mixed well, and the absorbance was read at 412 nm within 4 min.

**Determination of plasma borax**

Ammonium sulfate solution (4 g%) at 5 mL, was added to plasma (1 mL), mixed well and boiled for 15 min. The solution was centrifuged at 600 gX for 10 min. The supernate was separated and then distilled water was added to the precipitate, mixed well and recentrifuged. The second supernate was added to the previous one and distilled water was added, and mixed when the volume reached 10 mL. The solution, at 1 mL was added to concentrated sulfuric acid at 5 mL and mixed well. Carminic acid (20 mg% in concentrated sulfuric acid) was added. The mixture was incubated at room temperature for 10 min and the absorbance was read at 600 nm.

**Statistical analysis**

The borax levels in normal subjects were compared to those of CRF patients by using the Student-t test. The other data were analyzed by two way ANOVA. Statistical differences were considered at \( p < 0.05 \).

**Results and discussion**

The blood obtained was checked for G-6PD deficiency and excluded from further experiments if positive. G-6PD patients having low levels of glucose 6-phosphate dehydrogenase would cause a low level of nicotinamide adenine dinucleotide phosphate; reduced form (NADPH). NADPH in red blood cells functions as coenzymes in changing oxidized GSH to a reduced form. In our experiments, reduced GSH was measured. In G-6PD patients, the reduced GSH levels in RBCs were lower than in normal individuals and the response to oxidative stress was more retarded than in normal RBCs.

The mean GSH level in normal subjects was found at 53.90± 9.41 mg/dL, and 63.41± 13.64 mg/dL (\( p > 0.05 \)) in CRF patients as shown in Fig. 1. When incubating normal RBCs with phosphate buffered saline (PBS), there was no significant alteration in GSH levels. Borax, at various concentrations, affected the GSH levels with a good response (i.e. GSH is decreased when treating RBCs with borax and then is increased to near the starting level when
followed up after 90 min) at 1.3 μM (Fig. 2). Therefore, we chose this concentration for further experiments. GSH levels after incubating normal and CRF RBCs with borax solution (1.3 μM) at different times showed no significant change between healthy (n=20) and CRF patients (n=10) ($p>0.05$) (Fig. 3). After incubating normal and CRF RBCs with borax

**Figure 1.** Mean glutathione levels in RBCs of normal (n=20) and CRF patients (n=10). The values represented as mean±SEM.

**Figure 2.** The effect of various concentrations of borax (0, 0.33, 0.65, 1.3, and 2.6 μM) on GSH levels in normal RBCs at different time points. The response at 1.3 μM is quick with a highest point and return to the original level in a short period of time.
Solution (1.3 µM) at different times (0, 60, 120 and 180 min). The plasma borax levels were also not significantly different ($p > 0.05$) between healthy subjects (3.34±1.05 mg/L) and CRF patients (3.07±1.34 mg/L) as shown in Fig. 4. Normal plasma levels of borax were within the range of 0-7 mg/L (0-18.4 nM). It was worth noting that plasma borax could be measured by using carminic acid in a colorimetric method. The toxic level of borax was 20-150 mg/L.\(^{(6,7)}\)

Sodium tetraborate (borax) contains many advantages and also has hazardous effects on human beings. It could be used with alum to reduce the fluid requirement in acute diarrhea patients,\(^{(8)}\) or treat Candida paronychia and onychomysis.\(^{(9)}\) There is a report of chronic boric acid poisoning in infants with seizure development,\(^{(10)}\) and adults to having alopecia due to occupational exposure.\(^{(11)}\)

Contrary to our work, there were reports that erythrocyte GSH in CRF patients was lower than that in normal individuals.\(^{(12-14)}\) This might be caused by the effect of other antioxidants in food or other antioxidant enzymes that could influence the oxidative stress in RBCs. Further experiments are required to measure the antioxidants and/or antioxidant enzymes, such as vitamin C or glutathione reductase in the RBCs, to confirm such an explanation.

The erythrocyte is a highly specialized cell, which has main functions such as oxygen transportation and the mediation of carbon dioxide transport. Energy production in the mature RBC depends on glycolysis, with glucose as the principal substrate. Glycolysis and oxidative pentose phosphate pathway generate nicotinamide adenine dinucleotide; reduced form (NADH) and NADPH to reduce methemoglobin (which is continuously
produced) and the antioxidant GSH (which is present in high concentrations). RBCs are equipped with a highly effective antioxidant defense even without the GSH system.

The influence of a low protein diet in CRF patients might also be another confounding factor. Borax is used as a preservative in minced pork/fish/beef/chicken balls and CRF patients are advised to take a low protein diet. The protein intake in such patients was low in general. However, it was found that anemic status rather than nutritional status determines RBC and blood GSH in CRF patients. The treatment with Selenium (an antioxidant metal) as well as Selenium and erythropoietin might be of clinical use.

**Conclusion**

There is no difference in mean plasma GSH levels between normal and CRF RBCs. The response of GSH levels in normal and CRF RBCs at various times of borax treatment does not change significantly. Plasma borax levels measured in normal and CRF patients are also not different. Borax may not be the risk factor of renal failure. It could be concluded that borax is not an oxidant and has no effect on thiol antioxidants such as GSH in RBCs, which is an in vitro model.

**References**

ผลของบอแร็กซ์ต่อระดับกลูตาไธโอนในเซลล์เม็ดเลือดแดงมนุษย์ในหลอดทดลอง

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บทคัดย่อ โซเดียมเตตราบอเรทหรือบอแร็กซ์ถูกใช้เป็นสารถนอมอาหารและเป็นพิษต่อร่างกายขึ้นกับขนาดอวัยวะเป้าหมายในการเป็นพิษของบอแร็กซ์ได้แก่ระบบประสาทกลางไตและตับและบอแร็กซ์สามารถสะสมในกระแสเลือด วัสดุประสงค์ของการศึกษานี้เพื่อศึกษาการทดลองของบอแร็กซ์ต่อเซลล์เม็ดเลือดแดงมนุษย์ในคนปกติและผู้ป่วยโรคไตวายเรื้อรังโดยวัดระดับกลูตาไธโอนที่เวลาต่างๆและระดับบอแร็กซ์ในพลาสมาซึ่งระดับผลตอบสนองมากที่สุดส่วนบอแร็กซ์ที่ความเข้มข้น 0.33, 0.65, 1.30 และ 2.60 ไมโครโมลาร์ วัดระดับกลูตาไธโอนในเซลล์เม็ดเลือดแดงปกติถูกบ่มด้วยบอแร็กซ์ที่ความเข้มข้นที่ระดับ 0, 60, 120, 180 นาทีและวัดระดับบอแร็กซ์ในพลาสมาโดยวิธีการวัดไม่มีการเปลี่ยนแปลงอย่างมีนัยสำคัญระหว่างคนปกติและผู้ป่วยโรคไตวายเรื้อรัง ที่เวลาต่างๆระดับกลูตาไธโอนในเซลล์เม็ดเลือดแดงที่บอแร็กซ์ไม่มีการเปลี่ยนแปลงอย่างมีนัยสำคัญระหว่างคนปกติและผู้ป่วยโรคไตวายเรื้อรังระดับกลูตาไธโอนของ pessoaปกติเท่ากับ 53.9 ± 9.4 มิลลิกรัม/ดีซิลิตรและผู้ป่วยโรคไตวายเรื้อรังเท่ากับ 63.41 ± 13.64 มิลลิกรัม/ดีซิลิตร (p > 0.05) ระดับกลูตาไธโอนเมื่อบาบบอยเรื้อรังไม่มีการเปลี่ยนแปลงอย่างมีนัยสำคัญในคนปกติ (3.34 ± 1.05 มิลลิกรัม/ลิตร)และผู้ป่วยโรคไตวายเรื้อรัง (3.07 ± 1.34 มิลลิกรัม/ลิตร) บอแร็กซ์อาจไม่เป็นปัจจัยเสี่ยงของการเกิดโรคไตวายเรื้อรังในหลอดทดลอง เชียงใหม่เวชสาร 2549;45(3):93-100.

คำสำคัญ: บอแร็กซ์ กลูตาไธโอน โรคไตวายเรื้อรัง เม็ดเลือดแดง