Comparison of Selenium Levels and Glutathione Peroxidase Activity in Bovine Whole Blood

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ABSTRACT

Blood glutathione peroxidase activity and selenium levels were found to correlate well, indicating that glutathione peroxidase activity can be used to assess blood selenium levels in beef cattle.

The glutathione peroxidase activity of blood is less stable than is the selenium concentration but when blood was stored at 4°C, the glutathione peroxidase activity remained constant for seven days.

Key words: Selenium, glutathione peroxidase, blood, beef cattle.

RÉSUMÉ

Cette étude a demontré une bonne corrélation entre l'activité de la glutathion-peroxydase du sang et la teneur de ce dernier en sélénium, indice de la possibilité d'utiliser l'activité enzymatique précitée pour déterminer la teneur du sang en sélénium, chez les bovins de boucherie. Même si cette activité est moins stable que la concentration sanguine de sélénium, elle s'est révélée constante pour sept jours, lorsqu'on entreposait le sang à 4°C.

Mots clés: sélénium, glutathion-peroxydase, sang, bovins de boucherie.

INTRODUCTION

Determination of the selenium status of livestock has improved considerably during the past few years with the discovery that selenium in blood closely correlates with glutathione peroxidase (GSH-Px) activity (1-11). Selenium is an essential component of GSH-Px (12,13) and is incorporated into erythrocyte GSH-Px during erythropoeisis (2). Each molecule of GSH-Px contains four atoms of selenium (12). Bovine erythrocytes do not contain detectable amounts of nonselenium-dependent GSH-Px (14).

These studies were designed to determine the efficacy of GSH-Px as a direct correlate of whole blood selenium in beef cattle and the stability of GSH-Px in bovine blood stored at room temperature or 4°C.

MATERIALS AND METHODS

Blood was collected from Hereford cows by jugular venipuncture into tubes containing EDTA (ethylenediamine tetraacetate disodium salt). The blood was kept on ice during transportation to the laboratory and then refrigerated at 4°C.

The initial experiment was to evaluate whole blood GSH-Px activity when blood was stored at room temperature (20°C) or 4°C. Ten blood samples were divided in half, stored at either room temperature or 4°C and assayed for GSH-Px activity on days 5 and 7 after collection.

The second experiment was designed to determine the stability of whole blood GSH-Px activity when blood was stored constantly at 4°C. Blood was assayed for GSH-Px daily for nine days of storage beginning with the day of collection.

The third experiment was performed to evaluate the degree of correlation between the concentration of GSH-Px and selenium in the blood of beef cattle. Each blood sample was divided in half for GSH-Px and selenium analysis. Whole blood selenium was analyzed by using a semiautomated modified fluorometric microdetermination (15) which was performed by the Department of Agriculture Chemistry, Oregon State University.

Whole blood GSH-Px was determined by a modification of a procedure previously described by Paglia and Valentine (7). Briefly, hemoglobin concentration was determined by standard techniques (ZB$_i$ 6 Coulter Electronics Hemoglobinometer, Coulter Electronics, Inc, Hialeah, Florida). Fifty μL of EDTA blood was diluted with 50 μL of isotonic saline. Four hundred μL of double distilled water was added and the samples were freeze-thawed at -70°C (15 min) to hemolyze the red blood cells. After thawing, 0.5 mL of double strength Drabkins reagent (0.0016 M KCN, 0.0012 M $K_3Fe(CN)_6$, 0.0238 M $NaHCO_3$) was added, and the blood-reagent mixture was kept on ice until its addition to the reagent-buffer pool (R-BP).

The R-BP was freshly prepared and a total of 2.8 mL was used for each sample. Each 2.8 mL contained 2.58 mL of 0.5 *M* phosphate buffer, (pH 7), 0.10 mL of 0.0084 *M* NADPH, 0.10 mL of 0.15 *M* glutathione-reduced (GSH) liquid, 0.01 mL glutathione reductase (GR) (used as sup-

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plied) and 0.01 mL of 1.125 *M* NaN_3. All reagents were kept on ice during the analysis.

The assay was performed by adding 0.1 mL of the blood-Drabkins mixture to 2.8 mL of the R-BP and allowed to come to room temperature for a minimum of five minutes. The reaction was initiated by adding 0.1 mL of 0.002 M H_2O_2. The change in absorbance (ΔA) was measured at 340 nm (Cary 219 Spectrophotometer) for four minutes, and the first two minutes of nonlinear NADPH consumption was disregarded. To determine the nonenzymatic oxidation of GSH, a blank was prepared with the R-BP, using 0.1 mL of double distilled water to replace the hemolysate.

The GSH-Px activity is reported as mUnits/mg hemoglobin (mU/mg Hgb). Milliunits are defined as the number of nanomoles of NADPH oxidized per minute, calculated on the basis of a molar absorptivity for NADPH at 340 nm of 6.22 x 10^3.

The ΔA of the blank was substracted from the ΔA of the samples. The corrected ΔA was divided by the molar absorptivity (6.22 x 10^3) and multiplied by 0.0017 to correct for the 5 μL of blood in the 3.0 mL of reaction volume. The mUnits of activity were divided by the mg Hgb/5 μL and the activity was reported as mUnits GSH-Px/mg Hgb.

Data were evaluated statistically with a paired t test and regression analysis.

RESULTS

Blood stored at room temperature (RT) for five days had significantly ($P \leq 0.005$) lower GSH-Px activity than its counterpart stored at 4°C. This represented a loss of 32% (91.3 ± 7.5 GSH-Px at 4°C compared to 62.4 ± 5.7 GSH-Px at RT) of the GSH-Px activity on day 5 while 75% of the activity was lost by day 7 after storage at room temperature.

The GSH-Px activity of blood stored at 4°C remained stable for seven days at which time there was a significant ($P \leq 0.025$) decrease in activity (Table I). This decrease was 13% of the original amount. The GSH-Px levels continued to decline on days 8 and 9.

TABLE I. Stability of Glutathione Peroxidase (GSH-Px) Activity of Whole Blood Stored at 4°C

Day	GSH-Px (Units/mg Hgb)	Day	GSH-Px (Units/mg Hgb)
0	52.6 ± 7.8[b]	5	55.1 ± 7.8
1	52.3 ± 7.5	6	52.2 ± 7.5
2	55.3 ± 7.7	7	45.9 ± 6.8[a]
3	53.5 ± 7.6	8	44.8 ± 4.9[a]
4	54.7 ± 6.9	9	44.1 ± 5.1[a]

[a]$P \leq 0.025$ different from day 0 by paired t test, n = 13

[b]Values represent mean ± SE of 13 samples

The relationship between erythrocyte GSH-Px activity and blood selenium concentration is illustrated in Figure 1. A high correlation (r = 0.87, $P \leq 0.0006$) occurred between the two analytical methods.

DISCUSSION

These results indicate that erythrocyte GSH-Px activity can be used to evaluate blood selenium concentrations in beef cattle. A positive correlation (r = 0.87) occurred between GSH-Px and selenium levels. Similar correlations have recently been reported for dairy cattle in which positive linear relationships (r = 0.958 and r = 0.97) (3) existed between blood GSH-Px and selenium. Blood from calves fed selenium supplemented milk diets for 12 weeks also manifested a relatively high GSH-Px-selenium correlation (r = 0.88) (16). A close relationship (r = 0.97) also occurred between blood selenium concentration and erythrocyte GSH-Px activity in cattle of mixed ages (17).

Erythrocyte GSH-Px activity has also been a reliable indicator of selenium status in sheep. Most correlations were high (2,4,18) (r = 0.96) (18) but in one experiment where sheep were supplemented with selenium, the relationship ranged from r = 0.404 to r = 0.915 (19). A direct relationship between blood selenium concentrations and blood GSH-Px activity in cattle and sheep has also been reported by others (1,5,6-8,10,11).

Since as much as 98% of GSH-Px activity in peripheral blood is associated with erythrocytes (20), analysis of red blood cells for GSH-Px activity is effective for determining the selenium status of livestock. Further, the high linear relationship (r = 0.87) observed in these investigations confirms the positive correlation between blood GSH-Px activity and selenium concentration, particularly when selenium intake remains fairly constant.

The GSH-Px analysis does have some limitations. The activity of this enzyme in erythrocytes depends upon availability of selenium during the period of development of the whole population of erythrocytes (2). For instance, when animals were treated with selenium, the blood selenium concentrations increased and decreased more rapidly than did the corresponding GSH-Px activity (17,19). Since GSH-Px is dependent on rates of erythropoeisis and erythrocyte turnover, incorporation of selenium into GSH-Px is regulated by this normal

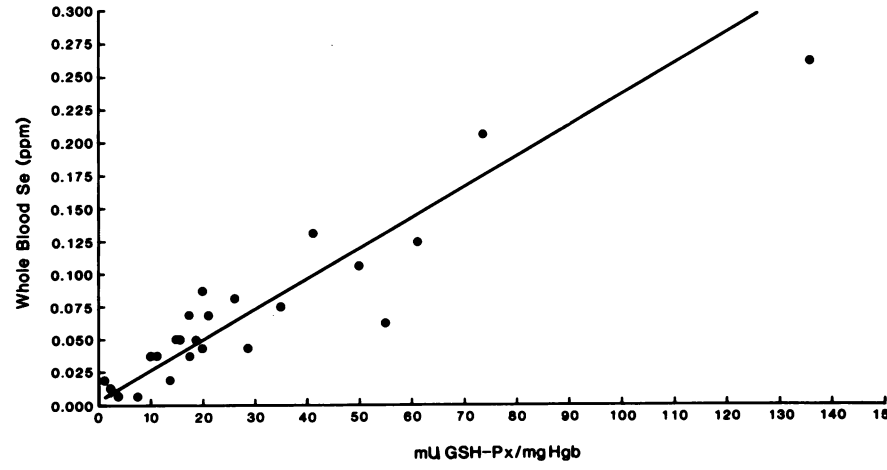

Fig. 1. Correlation between selenium (ppm = μg selenium/mL blood) and glutathione peroxidase (GSH-Px) levels in whole blood of beef cattle.

biological process while selenium transported in the plasma can fluctuate according to daily intake. The lag period for GSH-Px activity to reach comparative peak selenium concentrations after dietary selenium supplementation has been reported to be approximately 30 days for sheep (18) and 35 days for cattle (20). Therefore, recent changes in selenium content of feeds which determine the bioavailability of this element may result in a reduced correlation between blood selenium concentration and GSH-Px activity. Nevertheless, even under these conditions, GSH-Px provides a reliable test for determination of selenium status of cattle and sheep. Further, the high positive correlation which exists between these two methods suggests that assessment of erythrocyte GSH-Px activity will serve as a substitute for selenium analysis and accurately predict blood selenium concentrations.

Another limitation of GSH-Px compared to selenium is that the GSH-Px activity will deteriorate in a relatively short time while selenium remains fairly constant for a prolonged period. We found that 32% of the erythrocyte GSH-Px activity was lost by day 5 when blood was stored at room temperature. However, when blood was stored at 4°C, the GSH-Px levels remained constant for seven days at which time they became significantly ($P \geqslant 0.025$) reduced. This decrease amounted to 13% of the original sampling.

These investigations and others demonstrate that a high positive linear correlation exists between erythrocyte GSH-Px and blood selenium concentrations. Therefore, GSH-Px is an acceptable method of evaluating the selenium status of cattle. This procedure is relatively simple to perform and does not require the specialized laboratory equipment necessary for selenium analysis. The degree of enzyme activity in erythrocytes provides a better estimate of long-term selenium availability than does whole blood selenium concentration which fluctuates with daily intake. The equivocal values for deficient, marginally deficient, or normal blood selenium versus erythrocyte GSH-Px have been published elsewhere (21).

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