Interference in glucose and other clinical chemistry assays by thiocyanate and cyanide in a patient treated with nitroprusside

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A 3-year-old patient treated with nitroprusside for congestive heart failure had 6.5 mmol/L thiocyanate (toxic, >1.5 mmol/L) and 110 μmol/L cyanide (toxic, >5 μmol/L) present in her blood. At this time a whole-blood glucose concentration assayed on the Nova Stat Profile 5 Plus (Stat Profile) was 25.1 mmol/L. Plasma from that specimen analyzed on a Kodak Ektachem 700 analyzer (E700) indicated 5.2 mmol/L glucose. We investigated the potential interference of dissolved thiocyanate or cyanide on glucose and other routine assays. Toxic concentrations of thiocyanate increased Stat Profile glucose values and E700 total calcium, chloride, and creatinine values. Stat Profile ionized calcium values were decreased by toxic concentrations of thiocyanate. Cyanide (100 μmol/L) decreased alanine aminotransferase activity measured on the E700. Interference with the Stat Profile glucose assay may have been caused by thiocyanate oxidation at the glucose electrode.

INDEXING TERMS: analytical error • toxicology • glucose electrode

Intravenous nitroprusside infusions have become an essential anti-hypertensive therapy for treating congestive heart failure and hypertensive crisis. The action of nitroprusside occurs within seconds of intravenous administration but quickly disappears within 1–2 min after discontinuation of the infusion [1]. For this reason, continuous infusion is required for maintenance of the therapeutic benefit.

The effects of nitroprusside are limited by its rapid biotransformation to cyanide, which becomes concentrated inside erythrocytes. Most of the cyanide (t\(_{1/2}\) = 30–60 min) is metabolized to thiocyanate by rhodanese activity present in the mitochondria of most tissues [2, 3]. Elimination of thiocyanate is by the kidneys; hence significant renal impairment can increase the half-life from 2 to 3 days up to 9 days, thus increasing the risk of accumulating toxic amounts of this metabolite. Toxic accumulations of either cyanide or thiocyanate can occur without obvious clinical signs or symptoms [4, 5].

In this report we present a case that highlights the effects of thiocyanate accumulation on glucose measurements by a glucose electrode method. Although several reports have described cyanide and thiocyanate toxicity in patients after nitroprusside therapy [4, 5], very little information is available regarding the effect that toxic concentrations of these substances have on commonly measured clinical chemistry analytes. Experiments carried out subsequent to our recognition of the effect of thiocyanate on glucose analyses demonstrated that thiocyanate and cyanide also affect the measurement of several other analytes routinely monitored in nitroprusside-treated patients. Therefore, we wish to alert both physicians and laboratory personnel to the effects that high concentrations of thiocyanate and cyanide have on laboratory analyses. This report also demonstrates the importance of careful monitoring of thiocyanate and cyanide whenever nitroprusside therapy is given.

Case Report

The patient, a nondiabetic 3-year-old girl, presented to this hospital for inotrope support while awaiting a heart transplant. The patient had nitroprusside (3 μg/kg body wt. per minute) included in her therapeutic regimen. At 27 days into her nitroprusside therapy, a blood glucose determination performed by the Stat Profile 5 Plus (Nova Biomedical, Waltham, MA) on a sample drawn through the total parenteral nutrition delivery line showed a glucose concentration of 28 mmol/L. This blood specimen was centrifuged and the plasma analyzed on the Kodak Ektachem 700 (E700; Eastman Kodak Co., Rochester, NY) for verification.\(^3\) The E700 glucose value was discordant, indicating

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\(^*\) Nonstandard abbreviations: E700, Kodak Ektachem 700; ALT, alanine aminotransferase; and AST, aspartate aminotransferase.
a plasma glucose concentration of 16 mmol/L. Subsequent review of the patient’s blood glucose analyses performed before that time showed glucose values performed by the Stat Profile glucose electrode to be consistently above the laboratory reference limits (2.8–6.1 mmol/L) for the preceding 11 days. An aliquot of a specimen assayed on both the Stat Profile and E700 was reanalyzed on the AU800 analyzer (Olympus, Dallas, TX) with a glucose oxidase/peroxidase wet chemistry method. This analysis gave a result (5.5 mmol/L consistent with the E700 result (5.2 mmol/L) but widely discordant from the Stat Profile result (25.1 mmol/L).

To determine if the discordant glucose results were unique to this patient, we reanalyzed with the E700 plasma from patients whose whole-blood glucose was >10 mmol/L measured on the Stat Profile. This amounted to >35 specimens over a 10-day period with blood glucose concentrations ranging from 9.7 to 28.5 mmol/L by the Stat Profile. For these specimens, the plasma glucose value by the E700 differed from the corresponding Stat Profile value by no more than 15%.

We also reviewed the patient’s chart for concurrent drugs or treatments that might interfere with the assay methods. Published literature [6–8], a Medline search, and manufacturer-provided materials were used as references. These sources failed to reveal any factors likely to affect either the E700 or Stat Profile glucose assays for this patient. After identification of this problem, all glucose requests for this patient were performed with the E700.

On day 25 of the nitroprusside therapy, 2 days before identifying the discordant glucose measurements, a blood sample had been drawn for cyanide determination. A whole-blood glucose analysis performed on that day with the Stat Profile indicated that glucose concentrations were 26.8 mmol/L. The cyanide assay report revealed 88 μmol/L cyanide (therapeutic, <5 μmol/L), though no clinical signs or symptoms of toxicity were apparent in the patient. Subsequent cyanide and thiocyanate determinations showed 110 μmol/L cyanide and 6.5 mmol/L thiocyanate (toxic, >1.7 mmol/L; therapeutic, <0.5 mmol/L). Although her vasodilator therapy was changed, the patient developed significant renal function impairment; her plasma urea rose to 35–40 mmol/L and her creatinine to >200 μmol/L. Thiocyanate and cyanide concentrations also remained high, as did the blood glucose values determined with the Stat Profile. When her renal function failed to improve, renal dialysis was used. Interestingly, this treatment also removed thiocyanate and cyanide, reducing their plasma concentrations to within therapeutic limits. The discordance between glucose measurements on the E700 and Stat Profile also disappeared.

The patient’s condition stabilized somewhat after dialysis, and she eventually received a donor heart. We emphasize that at no time did the patient experience signs or symptoms that could be specifically attributed to cyanide or thiocyanate toxicity.

The discovery of toxic amounts of cyanide and thiocyanate in the patient’s blood highlighted the possibility that either cyanide or thiocyanate was interfering with the glucose measurements by the Stat Profile. Retrospective evaluation of the patient’s results revealed that values for several other analytes, including ionized and total calcium, were also outside reference limits during the period that toxic amounts of thiocyanate and cyanide were present. Plasma total calcium concentration determined during this time was 2.83 mmol/L (albumin 44 g/L) by the E700. Ionized calcium measured in whole blood from the same specimen was 0.93 mmol/L (pH 7.56). It is possible that either thiocyanate or cyanide could interfere in these assays, masking the true concentrations and the true level of renal function.

All procedures involving the patient were in accordance with local medical ethics committee guidelines and also with the Helsinki Declaration of 1975, as revised in 1983.

Materials and Methods

All reagents and chemicals were of the highest grade commercially available. Potassium cyanide was purchased from Fisher Scientific Co. (Chemical Manufacturing Division, Fair Lawn, NJ). Potassium thiocyanate was purchased from BDH (Toronto, Canada).

The Stat Profile 5 Plus blood gas analyzer was used and maintained according to the manufacturer’s instructions. It carried lot nos. 31A, 44C, and 3CA for the glucose, calcium, and sodium electrodes, respectively.

Assays on the E700 were performed with the appropriate clinical chemistry slides. Additional assays for glucose (glucose oxidase-peroxidase coupled reaction), creatinine (modified Jaffe method), urea (urease-glutamate dehydrogenase-coupled reaction), and alanine aminotransferase (ALT; lactate dehydrogenase-coupled reaction) were performed on the Olympus AU800. Creatinine was also measured by an HPLC method [9]. Thiocyanate was measured in the patient’s specimen by the Bowler method [10], and cyanide was measured in heparinized whole blood by photometric analysis after microdiffusion [11].

Pooled heparinized plasma was used to prepare stock solutions containing precisely weighed amounts of either potassium thiocyanate (20 mmol/L) or potassium cyanide (10 mmol/L). Working concentrations were prepared by diluting the stock solutions with plasma from the same pool. These diluted samples were assayed for the various analytes.

Results

The effect of increasing concentrations of thiocyanate on plasma glucose measurements on the Stat Profile is shown in Fig. 1. The measured concentrations of plasma glucose were substantially increased even with thiocyanate as low as 1 mmol/L. The measurement of plasma glucose by the E700 and AU800 was not affected by thiocyanate concentrations up to 20 mmol/L (results not shown). Interestingly, cyanide at concentrations up to 100 μmol/L, a concentration similar to that found in the patient’s blood but possibly much higher than that actually present in her plasma, had no effect on the glucose determinations by all three analyzers.

The effects of thiocyanate on the Stat Profile ionized calcium and the E700 chloride and creatinine values are shown in Fig. 2. Decreases in the values for ionized calcium were observed at thiocyanate concentrations >1 mmol/L. However, no trend in the measured pH values was seen that could otherwise explain this result. In three different plasma pools the chloride values on the E700 increased by 14% to 22% in the presence of 5 mmol/L
thiocyanate, but the ion-selective electrode measurement of sodium was not affected by thiocyanate. In contrast to its effect on ionized calcium measurements, thiocyanate at 20 mmol/L increased the measured total calcium value on the E700.

An examination of the effect of thiocyanate on the E700 creatinine assay showed a false increase in results (Fig. 2). In three other plasma pools, creatinine values ranging from 131 to 160 μmol/L increased by 20–32% in the presence of 5 mmol/L thiocyanate. To determine whether this interference by thiocyanate was specific to the E700 assay for creatinine, we assayed plasma samples containing thiocyanate with the AU800, using a modified Jaffe method. No significant change in creatinine results was observed in two pools containing 141 and 166 μmol/L creatinine and as much as 10 mmol/L thiocyanate. For the patient’s plasma, however, agreement between creatinine determinations by the two methods was not good and varied by as much as 20–60% for E700 creatinine values ranging from 96 to 240 μmol/L. For this reason aliquots of the patient’s plasma, taken at key points throughout her treatment, were analyzed for creatinine by an HPLC method [9]. The results of this analysis showed some discordance with the E700 results but confirmed the increased creatinine values.

Table 1 summarizes the Stat Profile, E700, and AU800 assays examined for interference by thiocyanate or cyanide. The only E700 assays affected by the presence of cyanide were those for ALT and aspartate aminotransferase (AST; Fig. 3). ALT activity was inhibited in a dose-dependent manner in the presence of cyanide, whereas AST activity was only modestly reduced. Cyanide also decreased ALT activity on the AU800.
two plasma pools containing 20 and 377 U/L ALT, the presence of cyanide (100 μmol/L) reduced the ALT activity to <5 and 102 U/L, respectively.

Discussion

These results strongly suggest that thiocyanate but not cyanide was responsible for the interference in the patient's blood glucose measurements by the Stat Profile. One explanation for the interference by thiocyanate on the glucose electrode involves oxidation of thiocyanate at the platinum anode, producing a false glucose signal. This glucose electrode relies on the oxidation of glucose by glucose oxidase to form gluconic acid and hydrogen peroxide. The formation of hydrogen peroxide is monitored at the electrode surface by application of a constant potential of 0.700 V with respect to the Ag/AgCl internal reference electrode. Under these conditions the oxidation of hydrogen peroxide at the surface of the platinum anode yields a current proportional to the concentration of glucose in the sample. If thiocyanate penetrates the membrane surrounding the electrode, it would be oxidized with hydrogen peroxide at the surface of the anode. Interestingly, the redox potential for oxidation of thiocyanate is 0.77 vs 0.682 for the hydrogen peroxide reaction. The applied potential relative to a standard hydrogen electrode would be 0.922 V, or 0.222 V (the reduction potential of the Ag/AgCl reference electrode) plus the 0.700 V applied to the electrode. This voltage is sufficient to oxidize thiocyanate and generate a current that can be measured by this method, along with the current generated by the hydrogen peroxide.

The method used by the E700 differs in that the hydrogen peroxide generated by glucose oxidase is utilized by horseradish peroxidase to convert 7-dihydronaphthalene and 4-aminoantipyrine to a colored product that can be measured by reflectance at 540 nm. The AU800 glucose assay reactions are similar to that of the E700 but use different chromogens. The interference by thiocyanate on glucose electrodes may also occur with electrodes produced by other manufacturers using similar methodology.

A recent study by Wang et al. [12] described the sensitivity to thiocyanate interference of chloride electrodes used on the Hitachi Model 737 analyzer. The observed increase in chloride signal was believed to have arisen from interactions of thiocyanate with quaternary ammonium salts on the electrode membrane. Interestingly, that report showed little interference in the E700 chloride electrode in a patient's plasma specimen containing 2.1 mmol/L thiocyanate. The thiocyanate concentrations in the patient we studied reached 6.5 mmol/L. Therefore, we examined the effect of higher concentrations of thiocyanate on the E700 chloride electrode. Consistent with the results of Wang et al. [12], thiocyanate concentrations ≤2 mmol/L had minimal effect on the measured chloride values on the E700. Chloride values were measured as being much greater, however, when the thiocyanate concentration was increased beyond this. Plasma chloride was not routinely measured in the patient described here.

Although thiocyanate was a positive interferent in the E700 creatinine assay, it did not appear to affect the modified Jaffe assay carried out on the AU800. The main feature of the E700 creatinine assay is its utilization of a multienzyme cascade of creatinine aminohydrolase, creatine amidinohydrolase, sarcosine oxidase, and peroxidase. The effect of thiocyanate on this assay, if any, may be complex with many potential sites of action. It is not clear at present how thiocyanate increases creatinine measurement by the E700 method. Fortunately, the magnitude of the error imparted to the E700 creatinine assay was not sufficient to affect the treatment course or diagnosis in the patient described.

To our knowledge no previous report has described an inhibitory effect of cyanide on plasma ALT activity. Several reports have, however, described interaction of cyanide with pyridoxyl 5'-phosphate, a cofactor of ALT, to form 4-pyridoxic acid lactone and 4-pyridoxic acid 5'-phosphate [13, 14]. This may be one explanation for the inhibitory effect of cyanide on ALT activity measured by the AU800 ALT assay and the E700 ALT assay. Surprisingly, the AST assay, which also requires pyridoxyl 5'-phosphate as coenzyme, was only modestly affected by the presence of cyanide. The reason for this may lie in differences in the mode of interaction and in the affinities of the coenzyme for each enzyme.

This report demonstrates the possible interfering effects that may occur in blood chemistries of patients accumulating toxic amounts of cyanide and thiocyanate as a result of nitroprusside therapy. In the case described, no measurement was suspect until glucose was measured by two different methods and the result of thiocyanate and cyanide analysis highlighted a possible source of interference. The impaired renal function in this patient was an important factor in limiting the clearance of thiocyanate and cyanide. However, considering the effect of thiocyanate on the E700 assay for creatinine, diagnosis of impaired renal function on the basis of creatinine values should be made cautiously. The mechanism for the interference in the calcium electrode and the measurement of total calcium remains uncertain. Considering the magnitude of the effect of thiocyanate on the measured ionized calcium values, serious questions may be raised concerning the accuracy and value of the calcium results generated for this patient at the time of her increased thiocyanate concentrations. Moreover, during the entire course of increased thiocyanate and cyanide concentrations, there were no symptoms of toxicity other than those that could be expected from her cardiomyopathy. Because of the wide use of nitroprusside in the treatment of heart failure, it is important that the laboratory as well as the clinician be aware of these interferences and the effect they might have on diagnosis and treatment.

References