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Pseudohyperphosphatemia associated with high-dose liposomal amphotericin B therapy¹

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Abstract

Background— Acute increases in serum inorganic phosphorus (Pi) up to 4.75 mmol/l in the absence of hypocalcemia and tissue deposition of calcium phosphate were noted in 3 patients receiving liposomal amphotericin B (L-AMB). We investigated L-AMB as a possible cause of pseudohyperphosphatemia.

Methods— Serum samples from the index patient were analyzed for Pi content by our laboratory's primary analyzer (Synchron LX20) and by an alternate analyzer (Vitros). Clear and lipemic serum pools, and normal saline, were spiked with L-AMB and analyzed by the LX20 Pi method. Ultrafiltration studies were performed on patient and spiked sera.

Results— Increased Pi values were obtained only from the LX20 analyzer. There was a direct linear relationship between the concentration of L-AMB in the spiked samples and the LX20 Pi results, indicating a 0.9 mmol/l Pi increase for every 100 mg/l increase in L-AMB. Ultrafiltration normalized the Pi results.

Conclusion— Serum Pi results may be falsely increased in patients receiving L-AMB when measured by the LX20 analyzer. This novel cause of pseudohyperphosphatemia is due to interference of L-AMB with the method and is corrected by ultrafiltration of the specimen. Since the LX20

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analyzer is widely used by the clinical laboratories clinicians and laboratory personnel should recognize this interference in order to avoid unnecessary diagnostic procedures and interventions.

Keywords

pseudohyperphosphatemia; liposomal amphotericin B (L-AMB); Synchron LX20

1. Introduction

Liposomal amphotericin B (L-AMB) consists of amphotericin B embedded in a phospholipid bilayer of small unilamellar liposomes [1]. This lipid formulation has a favorable toxicity profile when compared to conventional amphotericin B deoxycholate [2–4] and has been approved for use at dosages ranging from 3 to 6 mg/kg/day for indications including empirical therapy of persistent febrile neutropenia, systemic aspergillus, candida, and cryptococcus infections, visceral leishmaniasis, and cryptococcal meningitis in HIV infected patients. The improved safety and tolerability of this formulation have allowed for the use of increasingly higher dosages for the treatment of refractory infections [5–8]. However, a single case in the pediatric literature reported severe hyperphosphatemia related to therapy with 25 mg/kg/day of L-AMB [9].

Hyperphosphatemia is a potentially life-threatening condition. Exceeding a calcium-phosphate product of 5.64 (mmol/l)^2 can result in tissue deposition of calcium phosphate crystals and organ dysfunction. The precipitation of calcium phosphate can also result in symptomatic hypocalcemia, manifested by cardiac arrhythmias, hypotension, and tetany. Physiologic causes of hyperphosphatemia include decreased glomerular phosphate clearance due to renal failure, release of endogenous phosphate stores as in tumor lysis syndrome and rhabdomyolysis, and increased renal tubular reabsorption of phosphate as in hypoparathyroidism [10,11]. Administration of phosphate containing products, such as certain enemas and laxatives, has also resulted in symptomatic hyperphosphatemia [12–14].

Physiologic hyperphosphatemia must be distinguished from pseudohyperphosphatemia in which measured phosphorus concentrations are increased by leakage from red blood cells during specimen processing or by interferences with the phosphorus assay [15].

Pseudohyperphosphatemia secondary to assay interference has been described in the setting of paraproteinemia, hyperbilirubinemia, and hyperlipidemia [16–19]. We report our investigation of the previously unrecognized occurrence of extreme pseudohyperphosphatemia due to therapy with high-dose liposomal amphotericin B.

2. Patients

Our index case is a 53-y-old woman (patient A) with invasive pulmonary zygomycosis who was treated with 10 mg/kg/day of L-AMB (AmBisome; Fujisawa). A serum specimen drawn on the first day of therapy revealed a serum Pi of 1.26 mmol/l (reference interval 0.81–1.55 mmol/l), serum calcium (Ca) of 2.05 mmol/l (2.05–2.50 mmol/l), and serum creatinine of 133 mmol/l (62–115 mmol/l).

After day 8 of L-AMB therapy, the patient's serum Pi started to increase without significant change in her serum Ca or creatinine (Fig. 1.). Despite the institution of the oral phosphate binder Sevelamer, a low phosphate diet, and intravenous fluids, her reported serum Pi concentration remained increased and reached 4.75 mmol/l on day 16 of therapy. Her serum Ca at this time was 2.24 mmol/l and her serum creatinine was 141 mmol/l. This apparent severe hyperphosphatemia continued in the absence of clinical signs or symptoms of calcium

phosphate crystal deposition. Further workup included evaluation of her serum concentrations of 1,25-dihydroxyvitamin D was <24 pmol/l (53–161 pmol/l), 25-hydroxyvitamin D 40 nmol/l (62–200 nmol/l), parathyroid hormone 48.8 ng/l (6–40 ng/l), parathyroid related peptide < 0.2 pmol/l (0.0–1.9) pmol/l), total protein 51 g/l (60–76 g/l), and triglycerides 7.02 mmol/l. A 24-h urine collection revealed a creatinine clearance of 39 ml/min (90–125 ml/min), and a Pi excretion of 12.9 mmol/d (12.9–42.0 mmol/d).

The time course of the rise in serum Pi suggested a relationship to the L-AMB so the medication was discontinued. Over the next 48 h the patient's reported serum Pi decreased sharply to 1.23 mmol/l at which time the L-AMB was restarted at a lower dose of 7.5 mg/kg/day. Immediately thereafter the reported serum Pi began to rise again, reaching a peak of 3.62 mmol/l 2 days later. She was maintained on liposomal amphotericin B for another 24 days with persistently increased reported serum Pi concentrations. She suffered no sequelae of hyperphosphatemia. She was ultimately switched from liposomal amphotericin B to an investigational medication due to worsening renal insufficiency. Her reported serum Pi returned to normal after L-AMB was discontinued. She had resolution of all radiologic abnormalities 4 months after the onset of the infection, and she continues to do well after 1 y follow-up. Subsequently, 2 additional patients (patients B and C) were noted to have acute increases in reported serum Pi without clinical manifestations of hyperphosphatemia. Patient B was status post allogeneic stem cell transplant and patient C had chronic granulomatous disease. They did not have renal failure, paraproteinemia, or other potential causes that could explain the observed hyperphosphatemia. However, both patients were treated for invasive fungal infection with high-dose L-AMB (7.5 mg/kg and 10 mg/kg, respectively).

3. Methods

All serum samples were analyzed on Synchron LX20 Clinical System (Beckman Coulter Inc., Brea, CA). Pi results above the alert limits (>3.23 mmol/l) were verified by repeat analysis with a second LX20 instrument. In addition, 4 serum samples from patient A were analyzed outside our laboratory on a Vitros Chemistry System (Ortho-Clinical Diagnostics, Inc., Raritan, NJ).

Clear and lipemic (triglycerides = 7.1 mmol/l) serum pools, and normal saline, were spiked with L-AMB (4 g/l) to achieve final L-AMB concentrations of up to 200 mg/l. The spiked samples were then analyzed on LX20. The L-AMB present in patient specimens and in spiked specimens was removed by ultrafiltration (Minicon 30 filter, 25 min at 10,000 rotations/min) and retested on LX20.

Serum L-AMB concentration (C_{L-AMB}) for patient A on days 13, 16, 18 and 21 was estimated from the calculated Pi amount that was overestimated by LX20 ($Pi_{LX20-Vitros}$) and the regression slope ($S = Pi/L-AMB$) obtained for the spiked serum pool using equation $C_{L-AMB} = Pi_{LX20-Vitros}/S$, where Pi_{LX20} was 3.52, 4.75, 1.81 and 3.62 mmol/l, and Pi_{Vitros} was 1.78, 1.65, 1.07 and 1.68 mmol/l, respectively.

4. Results

Spiking clear and lipemic serum pools, as well as normal saline, with increasing amounts of L-AMB resulted in proportional increases in LX20 Pi results (Fig. 2.). The relation between L-AMB concentration and Pi result was comparable for the clear and the lipemic pools (slope 0.009) indicating an average of 0.9 mmol/l increase in measured Pi for every 100 mg/l increase in the L-AMB. The regression slope obtained with spiked normal saline was lower (0.007). The effect of ultrafiltration on LX20 Pi results of patient sera and of spiked clear serum pool

specimens is summarized in Tables 1 and 2. For all specimens that contained the drug ultrafiltration eliminated the overestimation of Pi concentration.

For patient A the calculated $Pi_{LX20-Vitros}$ and the estimated C_{L-AMB} were as follows: 1.74 mmol/l and 193.3 mg/l on day 13, 3.10 mmol/l and 344.4 mg/l on day 16, 0.74 mmol/l and 82.2 mg/l on day 19, 1.94 mmol/l and 215.6 mg/l on day 21.

5. Discussion

We observed acute, asymptomatic elevations in measured serum Pi to as high as 4.75 mmol/l in 3 patients receiving high-dose L-AMB. This prompted us to consider the possibility that this medication was interfering with the LX20 Pi assay used in our laboratory. Our results indicated that serum specimens containing L-AMB yield abnormally high results only when analyzed with the LX20 instrument.

To prove that this discrepancy is due to interference of L-AMB with the LX20 analytical method, we performed spiking studies to achieve L-AMB concentrations similar to those seen in the sera of patients receiving high-dose L-AMB therapy [8,20,21]. Spiking was done with normal clear serum pool in order to mimic normal physiologic conditions, with normal saline in order to remove the possibility of alternate interfering substances, and with lipemic serum pool to verify that endogenous hyperlipidemia does not interfere with the LX20 Pi method. There was a direct linear relation between L-AMB concentration and the Pi result for both serum pools and saline. In addition, the regression slope for the clear and lipemic specimens was comparable indicating that lipemia does not interfere with the Pi method. Furthermore, removal of L-AMB by ultrafiltration of the spiked and patient's sera eliminated the overestimation of Pi. Based on these results a 100 mg/l increase in serum L-AMB concentration would cause a 0.9 mmol/l increase in serum Pi result when measured by LX20 analyzer.

Our index case, patient A, did have laboratory evidence of true hyperphosphatemia. This included increased parathyroid hormone and suppressed vitamin D. We suspect that she did indeed have mild hyperphosphatemia, due to her baseline chronic renal insufficiency, with superimposed pseudohyperphosphatemia due to the presence of L-AMB in her serum. This is supported by the mildly elevated Pi values we obtained when her sera were retested on the Vitros analyzer.

Pharmacokinetic studies have demonstrated that mean area under the concentration-time curve from 0 to 24 h (AUC_{24}) and maximum concentration of drug in plasma (C_{max}) increased with sequentially higher daily doses of L-AMB and reached the upper limit at 10 mg/kg/day [8, 20,21]. Furthermore, both AUC_{24} and C_{max} increased with the duration (days 1, 7, and up to 24 days) of drug therapy. The gradual increase in the LX20 Pi results that we observed for patient A receiving high dose L-AMB therapy is indicative of gradual increase in the serum L-AMB concentration. Thus, the increase in the estimated C_{L-AMB} from 193.3 (day 13) to 344.4 (day 16) mg/l while on 10 mg/kg/day dosage, and from 82.2 (day 18) to 215.6 (day 21) mg/l while on 7.5 mg/kg/day dosage, is consistent with the reported pharmacokinetics of the drug. In addition, on the first day of the 7.5 mg/kg/day dosage (day 18) the estimated C_{L-AMB} is in good agreement with the reported C_{max} (mean $\pm$ SD = 75.9 $\pm$ 58.4 mg/l) [8].

The method for measuring serum Pi is based on the reaction of phosphate anions with acidified (sulfuric acid) ammonium molybdate to form a yellow molybdenum-phosphate complex that absorbs light at 340 nm. The majority of clinical analyzers, including those used in this study, determine the Pi concentration by directly measuring the absorbance of this complex with ultraviolet spectrophotometry. However, the reaction conditions of the assay, such as the concentration of molybdate and acidity of the reagent, vary among the different analyzers. If the pH of the reaction is extremely low, it may cause hydrolysis of organic phosphate

compounds that are normally present in serum (i.e., lipids) or are released from red blood cells during hemolysis. Since the LX20 assay is carried out at a pH<1 we suspect the interference of L-AMB with the LX20 method is due to hydrolysis of the organic phosphate contained in the lipid bilayer of the liposomes. In comparison, the Vitros Pi assay is carried out at pH of 4.2. Furthermore, the Vitros analyzer employs dry slide technology where the isotropically porous spreading layer could act as a filter preventing the L-AMB from reaching the molybdate layer.

After we investigated this problem an occurrence of interference in the LX20 Pi method was reported [22] for 2 patients that were treated with L-AMB. However, the possible cause of this interference was suggested to be due to a turbidity caused by biodegradation of the liposomal vehicle that could lead to light scattering or precipitation.

There are other drug products, mostly the anti-cancer drugs such as cytarabin, daunorubicin, doxorubicin and tretinoin, that are encapsulated in the liposomal bilayer. However, these drugs are given at a relatively much lower dosage and at longer time intervals (for example, the recommended dose for liposomal doxorubicin is 20 mg/m² once in 3 weeks, or 45 mg/m² once in 4 weeks, or 40–80 mg/m² once every 3–4 weeks) that their effect on the LX20 Pi result should be clinically insignificant.

Approximately 20% of clinical laboratories in the U.S. use a LX20 analyzer. In fact, a LX20 analyzer was used by the institution that previously reported severe hyperphosphatemia in a pediatric patient treated with high dose (25 mg/kg/day) of L-AMB [9]. As that patient was not reported to have suffered any sequelae of hyperphosphatemia, this may also have been a case of pseudohyperphosphatemia related to erroneous overestimation of Pi by the LX20 analyzer. Clinicians and laboratory personnel should recognize this interference in order to avoid unnecessary diagnostic procedures and interventions.

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Abbreviations

AUC₂₄	mean area under the concentration-time curve from 0 to 24 hours
C_{max}	maximum concentration of drug in plasma
L-AMB	liposomal amphotericin B
Pi	inorganic phosphorus

Fig. 1. Changes in the serum concentrations of inorganic phosphorus (Pi), Ca, and creatinine in patient A between day 9 and 24 of liposomal amphotericin B (L-AMB) therapy

Symbols: open circles, LX20 results; solid circles, Vitros results; heavy solid horizontal line shows time interval during which L-AMB administration was discontinued; dashed horizontal lines show reference interval. The LX20 and Vitros results on days 13, 16, 18, 21 are as follows: $Pi_{LX20} = 3.52, 4.75, 1.81, 3.62$ mmol/l; $Pi_{Vitros} = 1.78, 1.65, 1.07$ and 1.68 mmol/l. L-AMB dose: 10 mg/kg/day on day 1 through 16; 7.5 mg/kg/day on day 18 through 24.

Fig. 2.
The relation between liposomal amphotericin B (L-AMB) concentration and LX20 inorganic phosphorus (Pi) result for clear and lipemic serum pools and a normal saline.

Table 1

Effect of ultrafiltration (UF) on serum Pi results

Patient	LX20 Pi (mmol/l)	
	Before UF	After UF
A	2.42	0.90
A	2.13	1.39
B	1.74	1.39
C	2.97	1.81
C	2.71	1.62

Table 2

Effect of ultrafiltration (UF) on Pi results of serum pool spiked with liposomal amphotericin B (L-AMB)

L-AMB (mg/l)	LX20 (Pi mmol/l)	
	Before UF	After UF
0	1.16	1.36
60	1.68	1.32
120	2.23	1.36
180	3.04	1.26