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Case Report

Unidentified anion gap metabolic acidosis

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ABSTRACT

A 35 – month – old female with nonketotic hyperglycinemia (NKH) presented to the Emergency department with severe hypoglycemia, fever, and several episodes of seizures. Due to worsening respiratory status, additional seizures and anion gap worsening metabolic acidosis the patient was transferred to the pediatric intensive care unit. The useful mnemonics for causes of high anion gap metabolic acidosis are the classic MUDPILES (representing Methanol, Uraemia, Diabetes, Paraldehyde, Iron (and Isoniazid), Lactate, Ethylene glycol, and Salicylate) and the more recently proposed GOLD MARK (Glycols [ethylene and propylene], Oxoproline, L-lactate, D-lactate, Methanol, Aspirin, Renal failure, and Ketoacidosis) as causes of the anion gap metabolic acidosis were all ruled out. Relatively stable concentrations of salicylate (approximately 10 mg/dL, 0.7 mmol/L) were noted, despite no evidence the patient received aspirin Therefore further laboratory testing was performed. A Basic-Acid-Neutral (BAN) gas chromatography mass-spectroscopy (GC–MS) Drug screen of urine was undertaken. A large benzoic acid peak was identified by spectral match, which supported the clinical history that the patient was taking sodium benzoate powder 1175 mg as a dietary supplement three times a day. However, salicylate was not identified. This patient had benzoic acid concentration in excess of 2000 μg/mL. Given that benzoic acid is a weak acid, with a pK of approximately 4 it is almost completely ionized at pH 7. Therefore, the large amount of benzoic acid was not only thought to be contributing to the patient's anion gap metabolic acidosis, but the source of the interference in the salicylate assay.

1. Case description

A 35 – month – old female with nonketotic hyperglycinemia (NKH) presented to the Emergency department with severe hypoglycemia (< 25 mg/dL), fever, and several episodes of seizures. NKH is an inborn error of glycine degradation in which large quantities of glycine accumulate in all body tissues, including the central nervous system [1]. Patients who survive infancy develop intractable seizures and profound mental retardation. Many therapeutic strategies have been tried in an effort to ameliorate the intractable seizures and relentless brain damage characteristic of this disorder, but none of these approaches has been consistently effective [1]The patient's medications at the time of admission included albuterol, diazepam, erythromycin, levetiracetam, levocarnitine, topiramate, acetaminophen, and sodium benzoate.

Benzoate is thought to conjugate with glycine to form hippurate, which would then be excreted in the urine [1]. Literature reports that sodium benzoate doses up to 750 mg/kg per day achieve a reduction in plasma glycine back down to normal ranges and a partial reduction of CSF glycine concentrations, as well as, decreased seizure frequency [1]. Unfortunately, none have seen significant improvement in developmental progress.

Due to worsening respiratory status, increased seizure frequency, along with possible new seizure type in the Emergency Department ED, and worsening metabolic acidosis (venous pH 7.22, bicarbonate 9 mmol/L, pCO2 24 mmHg), the patient was transferred to the pediatric intensive care unit (PICU) for monitoring. Gasping respirations in the setting of cough and vomiting were noted in the PICU. Initial laboratory results are shown below:

Analyte	Result	Reference interval
Na	152H	135–145 mmol/L
K	2.5 L, C	3.2–5.0 mmol/L
Cl	108H	96–106 mmol/L
Bicarbonate	5 L	22–28 mmol/L
Glucose	279H (16 mmol/L)	70–140 mg/dL
Urea	17 (6.0 mmolL)	8–24 mg/dL
Creatinine	1.7H (150 μmolL)	0.1–0.4 mg/dL
Anion gap	39H	13–21

H = High, L = Low, C = Critical value.

The increased anion gap (Na –[Cl + HCO_3]) can suggest a possible error in measurement of one of the electrolytes when the value is beyond the reference limit, or like in this case when it is accompanied by a metabolic acidosis it represents an increase in unmeasured endogenous (e.g., lactate) or exogenous (e.g., salicylates) anions [2]. Clinically useful mnemonics for causes of high anion gap metabolic acidosis are

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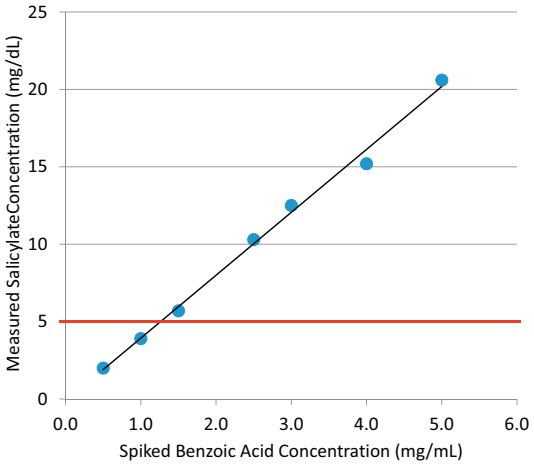

Fig. 1. Benzoic Acid was spiked into drug free serum at various concentrations (mg/mL) and compared to measured salicylate concentration (md/dL). Red line represents the LOQ of the assay.

the classic MUDPILES and the more recently proposed GOLD MARK [2]. To rule out some of the eight underlying mechanisms additional laboratory testing was ordered and is shown below:

Analyte	Result	Reference interval
Ethanol	Not detected	Not Detected
Methanol	Not detected	Not detected
Acetone	Not detected	Not detected
Isopropanol	Not detected	Not detected
Ethylene glycol	Not detected	Not detected
Lactate	3.9	0.60–2.30 mmol/L
D-lactate	Not detected	Not detected
Beta-Hydroxybutyrate	0.3	< 0.4 mmol/L
Pyruvic acid	0.9	0.7–1.4 mg/dL
Acetaminophen	33 (218 μmol/L)	< 50 μg/mL
Ammonia	31 (18.2 μmol/L)	< 50 μg/dL
Salicylate	10 (0.7 mmol/L)	Toxic > 50 mg/dL

To rule out other inborn errors of metabolism and any metabolic derangements that may be the cause of the metabolic acidosis additional studies were performed. A plasma acylcarnitine analysis, which enables the diagnosis of many disorders of fatty acid oxidation and several organic acidurias was performed. The results were not indicative of a specific disorder. Additionally, a plasma amino acid profile that included the following amino acids: taurine, threonine, serine, asparagine, glutamic acid, glutamine, proline, alanine, citrulline, alpha-amino-n-butyric acid, valine, cysteine, methionine, isoleucine, leucine, tyrosine, phenylalanine, beta-alanine, ornithine, lysine, histidine, argininosuccinic acid, allo-isoleucine, arginine, phosphoserine, phosphoethanolamine, hydroxyproline, glycine, aspartic acid, ethanolamine, sarcosine, 1-methylhistidine, 3-methylhistidine, carnosine, anserine, homocitruline, alpha-aminoadipic acid, gamma-amino-n-butyric acid, beta-aminoisobutyric acid, hydroxylysine, cystathionine, and tryptophan were essentially normal.

None of the above findings alone or in combination were thought to be the cause of the profound metabolic acidosis in this patient However, serial measurements of salicylate were performed because the patient was not prescribed it and said they didn't consume any. Relatively stable concentrations of approximately 10 mg/dL (0.7 mmol/L) were noted; despite no evidence the patient received ASA. Therefore further laboratory testing was performed. A Basic-Acid-Neutral (BAN) gas chromatography mass-spectroscopy (GC–MS) Drug screen of urine was undertaken. The sample confirmed the presence of levetiracetam, topiramate and acetaminophen which had been previously measured and found to be consistent with therapeutic dosing. However, a large benzoic acid peak was also identified by spectral match. This data supported the clinical history that the patient was taking sodium benzoate powder 1175 mg (67 mg/kg) as a dietary supplement three times a day. It should also be noted that the BAN did not identify salicylate which was previously reported at concentrations well above the assay's detection limit.

The salicylate was originally measured using a homogeneous enzyme immunoassay technique [3]. Of the prescribed medications the patient was taking, the only ones included on the list of analytes tested for interference on the immunoassay package insert were benzoic acid and acetaminophen. No significant interference was found for either compound when tested up to 1000 μg/mL [3]. Since the patient had an acetaminophen concentration of 33 μg/mL, this was excluded as a possible interference.

Benzoate was subsequently quantitated in several of the serum samples where salicylate was also identified, and the concentrations ranged from 2310 to 3260 μg/mL. When benzoic acid was added to drug free serum samples at concentrations ranging from 0.5–5.0 mg/mL and measured using the immunoassay for salicylate there was a linear response ($r^2 = 0.995$, Fig. 1). Additionally, when benzoic acid was added to drug free serum samples at similar concentrations to those found in the patient (2500–3000 μg/mL) it gave similar salicylate immunoassay results (10.3–12.5 mg/dL). Lastly, when the patient was not receiving benzoic acid, the salicylate immunoassay results were negative.

While the salicylate immunoassay package insert indicated that benzoic acid should not interfere up to 1000 μg/mL, this patient had concentrations in excess of 2000 μg/mL. Therefore, clinical chemists and physicians must recognize that higher concentrations may cause interference and completely investigate erroneous salicylate all potential sources of interferences and confusion when unusual clinical situations or conditions are present.

2. Conclusion

This patient had benzoate concentration in excess of 2000 μg/mL. Given that benzoic acid is a weak acid, with a pK of approximately 4 it is almost completely ionized at pH 7. Metabolic acidosis has been previously reported in an infant who received 1 g/kg/day orally of sodium benzoate in the management of seizures in NKH ([4]. The metabolic acidosis can be due to the inhibition of the oxidative enzymes in the tricarboxylic acid cycle, similar to the action of salicylate through inhibition of primarily alpha-ketoglutarate dehydrogenase and succinic acid dehydrogenase, which results in accumulation of lactic acid [5]. Therefore, the large amount of benzoic acid was not only thought to be contributing to the patient's anion gap metabolic acidosis but the source of the interference in the salicylate assay.

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