Acquired 5-oxoprolinuria (Pyroglutamic acidaemia) as a cause of early high anion gap metabolic acidosis in acute massive paracetamol overdose

5-oxoprolinuria is an uncommon and under-recognised cause of early high anion gap metabolic acidosis after paracetamol overdose. We reported a 30-year-old Indian woman with history of chronic alcoholism who ingested 150 g crushed paracetamol tablets for suicide 14 hours before attendance to the A&E Department. Initial arterial blood gas showed a high anion gap metabolic acidosis with respiratory compensation. Serum paracetamol level reached 5004 umol/L and a prolonged course of N-acetylcysteine was given. She was complicated by hepatotoxicity and 5-oxoprolinuria (with laboratory confirmation) which reverted after antidote administration. There were no neurological and hepatic sequelae. In case of massive overdose, pathways of drug metabolism are altered prior to the centrilobular hepatic necrosis. A metabolic intermediate of gamma-glutamyl cycle, 5-oxoproline, accumulates upon saturation of endogenous glutathione store. The specific antidote N-acetylcysteine is the only definitive treatment. Prolonged course of antidote may be required in cases of massive overdose and treatment should be individualised. (Hong Kong j.emerg. med. 2011;18:264-270)

Keywords: Acetaminophen, acidosis; drug toxicity, Glutathione, Pyrrolidonecarboxylic Acid

Introduction

Paracetamol is a widely prescribed and commercially available over-the-counter analgesic and antipyretic. According to the Hong Kong governmental registry, the number of registered pharmaceutical products containing it fell from more than 1000 in late 2010 to more than 980 in April 2011 after a recent de-registration of dextropropoxyphene since 29th December 2010.1 It is generally regarded to be safe and well tolerated in the therapeutic doses.
Animal studies showed a possible association between chronic paracetamol exposure and presence of transient acquired 5-oxoprolinuria (or pyroglutamic acidaemia), and its reversibility by restoration of glutathione store in gamma-glutamyl cycle with dietary methionine. However there had been few reports to suggest possible relations between acute massive paracetamol overdose and presence of 5-oxoprolinuria causing high anion gap metabolic acidosis in human subjects.

We would like to discuss a patient who presented with early high anion gap metabolic acidosis due to transient 5-oxoprolinuria after a massive paracetamol overdose.

**Case**

A 30-year-old Indian salesgirl was brought to the Accident and Emergency Department for confused speech and drowsiness for 3 hours. She complained of epigastric pain and vomiting after drinking 4 glasses of beer 15 hours prior to her attendance. Initially she refused to disclose any history of drug ingestion. She was a chronic heavy drinker and her past health was unremarkable.

On initial physical examination she was alert with a blood pressure at 101/58 mmHg, a pulse at 125 bpm and a respiratory rate of 20 per minute. She was afebrile. Her body weight was 43 kg. Neurological, abdominal and cardiovascular examinations were unremarkable. Chest X-ray was clear. Electrocardiogram (ECG) showed sinus tachycardia with normal QRS duration and QTc intervals. Laboratory results were summarised in Table 1. Arterial blood gas with Abbott i-STAT® showed a metabolic acidosis with respiratory compensation (pH=7.211, PCO₂=2.05 kPa, PO₂=17.7 kPa, HCO₃⁻=6.2 mmol/L, BE=-22, SaO₂=98% in room air). The calculated anion gap was 30.8 mmol/L. Serum lactate was 9.0 mmol/L. Capillary blood beta-hydroxybutyrate and random glucose were 1.6 mmol/L and 9.4 mmol/L respectively by Abbott Optiium™ Ketone. Serum salicylate and ethanol were undetectable. Creatine kinase was normal.

On repeated questioning the patient admitted ingestion of more than 300 tablets of crushed immediate-release 500 mg paracetamol (150 g in total) around 14 hours before attendance. There were no other co-ingested drugs. Toxicology screening showed an alarming serum paracetamol level at 5004 umol/L (755.6 mg/L). Activated charcoal, sodium bicarbonate and intravenous N-acetylcysteine (NAC) infusion were given prior to admission to intensive care unit (ICU).

A significant amount of unknown anions seemed to be contributing to the high anion gap after adjustment for albumin and lactate (30.1 minus 9.0 mmol/L=21.1 mmol/L). In view of the recent history of massive paracetamol overdose, pyroglutamic acidaemia was suspected. Presence of 5-oxoproline in plasma and urine was detected by our hospital biochemistry laboratory. Levels were analysed by gas chromatography-mass spectrometry after extraction with ethyl acetate at low pH and conversion to trimethylsilyl derivative. Urine 5-oxoproline level was found elevated and further increased from 0.23 to 1.25 mmol/mmol creatinine (normal <0.07) in eight hours, while plasma 5-oxoproline increased from 0.06 to 0.22 mmol/L (normal <0.07). It strongly proposed that a substantial amount of 5-oxoproline was present that contributed to the remarkably high anion gap.

During her ICU stay, she was complicated by shock that requiring a low-dose inotropic support, progressive liver function derangement and coagulopathy. Liver enzymes and the International Standardised Ratio (INR) peaked on Day 3. Serum ammonia increased to 54 umol/L (normal <33). Serial renal function tests and urine output were all along normal.

A prolonged course of N-acetylcysteine was administered for seven days until improvement of liver function with an undetectable serum paracetamol level. The liver function was normal at follow up after 2 weeks.

**Discussion**

Reduced glutathione (GSH) is an essential tripeptide responsible for various physiological functions, including detoxification, free radical scavenging and immunomodulation. It transports extracellular amino acids across the plasma membrane into the cell via the
gamma-glutamyl cycle, in forming gamma-glutamyl amino acid conjugates. After entrance into the cytosol this conjugate is degraded to reproduce the amino acid and a by-product 5-oxoproline (pyrrolidonecarboxylic acid, or pyroglutamic acid). The respective metabolic pathway is illustrated in Figure 1. Most reactions in the cycle are ATP-dependent. With the abundance of GSH, action of gamma-glutamyl cysteine synthetase is regulated via non-allosteric feedback inhibition. Once endogenous GSH store is depleted, intermediate metabolites follow a secondary metabolic pathway, forming a substantial amount of 5-oxoproline which accumulates in plasma and urine.

5-oxoprolinuria is an uncommon cause of high anion gap metabolic acidosis traditionally attributed to defects in gamma-glutamyl cycle such as congenital deficiencies in glutathione synthetase and 5-oxoprolinase. Recent literatures showed that acquired 5-oxoprolinuria could be more common than the hereditary form, and could have a diverse cause as outlined in Table 2.4-15

Our case is one of the first laboratory confirmed cases of 5-oxoprolinuria associated with acute massive paracetamol overdose and high anion gap metabolic acidosis in our locality. In normal circumstances,

Table 1. Laboratory results

<table>
<thead>
<tr>
<th>Days (Hours post overdose)</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Day 1 (Admission, 15h)</td>
</tr>
<tr>
<td>pH (7.35-7.45)</td>
<td>7.21</td>
</tr>
<tr>
<td>Serum bicarbonate (22-26 mmol/L)</td>
<td>6.2</td>
</tr>
<tr>
<td>Corrected Anion Gap (8-12 mmol/L)</td>
<td>30.05</td>
</tr>
<tr>
<td>Paracetamol* (umol/L)</td>
<td>5004</td>
</tr>
<tr>
<td>Plasma 5-oxoproline (&lt;0.07 mmol/L)</td>
<td>0.06</td>
</tr>
<tr>
<td>Urine 5-oxoproline (&lt;0.07 mmol/mmol creatinine)</td>
<td>0.23</td>
</tr>
<tr>
<td>Total Bilirubin (&lt;20 umol/L)</td>
<td>6</td>
</tr>
<tr>
<td>Alkaline Phosphatase (34-104 U/L)</td>
<td>50</td>
</tr>
<tr>
<td>Alanine Transaminase (5-31 U/L)</td>
<td>97</td>
</tr>
<tr>
<td>Aspartate Transaminase (12-28 U/L)</td>
<td>129</td>
</tr>
<tr>
<td>International Standardised Ratio</td>
<td>1.2</td>
</tr>
<tr>
<td>Serum creatinine (45-82 umol/L)</td>
<td>77</td>
</tr>
<tr>
<td>Estimated creatinine clearance by Cockcroft formula (100-110 ml/min)</td>
<td>62.7</td>
</tr>
</tbody>
</table>

*Reference Range:
Therapeutic = about 100
Toxic = >1000 (4 hours post-dose) or >260 (12 hours post-dose)
paracetamol is predominantly detoxified via glucuronidation and sulfation, or directly excreted in urine. Less than 5% is metabolized via N-hydroxylation and rearrangement by hepatic cytochrome P450, forming a toxic intermediate NAPQI (N-acetyl-p-benzoquinone-imine) which is subsequently conjugated with sulfadryl group of GSH, producing mercapturic acid and other non-toxic metabolites for renal excretion. In case of massive paracetamol overdose, early saturation of glucuronidation and sulfation pathways rapidly shunts the metabolism to the cytochrome P450 pathway and results in increased production of NAPQI. It in turn inhibits mitochondrial aerobic respiration and thus production of lactic acid from anaerobic respiration. Exhaustion of endogenous GSH leads to a self-sufficient alternative pathway in gamma-glutamyl cycle, resulting in substantial accumulation of 5-oxoproline in plasma and urine. The intended proposition of NAC administration is to restore endogenous GSH with an exogenous L-cysteine supply as a precursor. The feedback inhibition is re-established by GSH against gamma-glutamyl cysteine synthetase, thus a decrease in gamma-glutamyl cysteine for 5-oxoproline synthesis.

**Figure 1.** Gamma-glutamyl Cycle and its role in transcellular amino acid transport. Secondary metabolic pathway with glutathione depletion is denoted with dashed lines.
Significant Type B2 lactic acidosis and 5-oxoprolinuria co-existed in this case as evidenced by the high serum lactate of 9.0 mmol/L and elevated plasma and urine 5-oxoproline. Both contributed to the high anion gap metabolic acidosis. Interestingly, despite a history of confused speech prior to admission, this patient with severe acidaemia and hyperlactataemia did not progress to an altered sensorium or even coma in her clinical course.16

Early high anion gap metabolic acidosis associated with massive paracetamol overdose should be distinguished from metabolic acidosis secondary to structural liver damage. It is defined as onset within 24 hours post-ingestion and an anion gap higher than 12 mmol/L. Ketosis was present as revealed by capillary blood beta-hydroxybutyrate level of 1.6 mmol/L. However, plasma 2-hydroxybutyric acid, 3-hydroxybutyric acid and acetoacetic acid were not measured individually in laboratory. These could contribute to elevated anion gap apart from lactic acid. Apart from hyperlactataemia secondary to suppression of aerobic respiration by the mechanism as mentioned above, high plasma 5-oxoproline level upon exhaustion of GSH store is also proposed to contribute for the early high anion gap metabolic acidosis. A retrospective review of 74 patients17 showed that 41% patients with isolated paracetamol ingestion had a high anion gap on presentation. There was an association between serum lactate and paracetamol level. As such, blood pH, HCO₃⁻, and lactate level should be checked to look for early acid base disturbance for patients who have ingested a significant overdose of paracetamol.

There are a few limitations affecting case analysis in our study and a few assumptions have been made. It was assumed that patient had no inborn error of metabolism as she did not have any features of acidosis, haemolytic anaemia and neurological symptoms suggestive of hereditary 5-oxoprolinuria in her pre-morbid state. There was no test done on glutathione synthetase and 5-oxoprolinase activities from patient tissues e.g. erythrocytes or skin fibroblasts (as done in case series by Pitt6,7), or formal confirmation of the absence of propionyl CoA carboxylase deficiency.18 This patient was subsequently pregnant one year after the event. However no repeat test of plasma and urine 5-oxoproline was done during her pregnancy to demonstrate whether she had a tendency to develop 5-oxoprolinuria during pregnancy.
There were no concurrent analysis including blood gas, renal and liver function tests, lactate, ketones, and 5-oxoproline on specimens collected at the same time for precise calculation of anion gap and its contributing factors. There was no subsequent biochemical proof of disappearance of 5-oxoproline either in blood or in urine after administration of intravenous NAC. It was not certain whether plasma and urine 5-oxoprolinuria showed a downtrend after administration of antidote in our case. However it was demonstrated in a case series that urine excretion of 5-oxoproline dropped significantly after drug withdrawal for patients taking therapeutic dose of paracetamol. It was evident the corrected anion gap was rapidly brought down from an initial value of 30.1 mmol/L to a near-normal 16.0 on the third day of ICU stay with maintenance dose of NAC, so was the acidosis. Yet it is interesting to note that the plasma and urine 5-oxoproline levels continued a brief rise after 8 hours (0.06 to 0.22 mmol/L, 0.23 to 1.25 mmol/mmol creatinine respectively) after NAC treatment. It is postulated that it takes time for the NAC to replenish the endogenous GSH store and then revert the 5-oxoprolinameia. Correction in anion gap seemed to precede that of pyroglutamic acidaemia; probably attributed by the early administration of intravenous sodium bicarbonate to correct acidemia, fluid resuscitation and good haemodynamic support.

Our patient carried several risk factors. She was a chronic alcoholic and explicitly took a massive dose of paracetamol for suicide. Her presentation and administration of antidote were delayed to provide ample time for 5-oxoproline synthesis.

The indication of the specific antidote NAC relies on risk stratification. It was administered early to our patient because serum paracetamol level lied well above the high risk-line (300 line) suggestive of high risk hepatotoxicity in the Rumack-Matthew Normogram.

Recently there is a gradual shift of paradigm from traditional fixed-period protocol to patient-tailored NAC administration especially in massive overdose. Betten et al19 performed a prospective observational study after a shortened course of oral NAC therapy. It proposed that instead of a strict adherence to a time-based regimen, treatment should be goal-directed. The author’s clinical bottom-line to terminate oral NAC therapy included (1) a minimum 20-hour therapy, (2) a zero or near-zero serum paracetamol level lower than 10 ug/ml, (3) normal or at least a remarkable improvement of serum ATL/AST levels, (4) an INR lower than 1.3, and (5) clinical well-being. It potentially carries a financial implication in terms of shorter hospital stay and more appropriate resource allocation. On the other hand, a case report by Smith et al20 exemplified that a protracted course of NAC therapy might be necessary in massive overdose. Rebound might occur in serum paracetamol level and liver transaminases to even higher levels because of altered absorption kinetics and liver toxicity. In our case a prolonged course of NAC was given until there was clinical and biochemical recovery of liver toxicity as well as restoration of acid base balance.

**Conclusion**

High anion gap metabolic acidosis secondary to massive paracetamol overdose may have been under-recognised due to lack of awareness of its occurrence. It can be contributed by lactic acidosis alone or together with the presence of pyroglutamic acidaemia. Clinicians are advised to be vigilant of organic acid accumulation as an important consequence of paracetamol overdose. Arterial or venous blood gas and anion gap would be helpful in picking up the diagnosis and guiding management. On the other hand, pyroglutamic acidaemia secondary to drug overdose should be considered as one of the less common causes of high anion gap metabolic acidosis when the patient’s history is unreliable and the common differential diagnosis have been exhausted. This case also illustrates that organic acid accumulation does not necessarily occur only among patients with inborn error of metabolism. Further studies are required for the causal relationship between the accumulation of 5-oxoproline and the severity of paracetamol overdose; for patients with and without underlying risk factors.
References


