Transient 5-oxoprolinuria (pyroglutamic aciduria) with systemic acidosis in an adult receiving antibiotic therapy

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5-Oxoprolinuria is a recognized condition with increased urinary excretion of 5-oxoproline and is associated with a variety of inborn metabolic defects involving the series of enzyme-linked reactions known as the γ-glutamyl cycle. We report the unusual case of a 35-year-old woman who initially presented with staphylococcal pneumonia but went on to develop a transient high anion gap metabolic acidosis. The development and subsequent complete recovery from this acidosis were subsequently shown to be related in time to the intravenous administration of the antibiotics flucloxacillin and netilmicin. Analysis of the patient’s urine for organic acids revealed massively increased excretions of 5-oxoproline at the peak of her acidosis. We suggest that this patient developed a transient disturbance in the γ-glutamyl cycle involving the 5-oxoprolinase step, which resulted in accumulation of 5-oxoproline that caused a severe high anion gap metabolic acidosis. The administered antibiotics remain as possible causative agents.

5-Oxoprolinuria (pyroglutamic aciduria) is a condition characterized by increased production and urinary excretion of 5-oxoproline. Its presence is associated with inborn errors in the synthesis and metabolism of glutathione, a tripeptide found in most tissues that is essential for a variety of detoxification, antioxidant, and stabilizing functions. These inborn errors consist of defects in the γ-glutamyl cycle, a series of enzyme-linked reactions involved in the synthesis, metabolism, and transcellular transport of glutathione (Fig. 1). Such rare defects or deficiencies have been described for most of the enzymes involved in the cycle, but those involving glutathione synthetase and 5-oxoprolinase are of particular clinical interest. An inherited deficiency of either of these two enzymes results in an increased production and plasma concentration of 5-oxoproline that is reflected as an increase in urinary excretion (5-oxoprolinuria). These conditions have been demonstrated previously to be inherited in an autosomal recessive manner [1, 2].

An inherited defect in the enzyme glutathione synthetase was first described in a 19-year-old man in 1970 [3]. Since then at least 26 cases have been reported. As seen in Fig. 1, glutathione synthetase is involved in the conversion of γ-glutamyl cysteine to glutathione. A block here, therefore, leads to reduced amounts of glutathione production and marked accumulation of γ-glutamyl cysteine, not only because its conversion to glutathione is blocked, but also because its production is increased because of the lifting of the negative feedback that glutathione concentrations have on the enzyme γ-glutamyl cysteine synthetase. This accumulation of γ-glutamyl cysteine does not, however, persist, because it is converted via a less favorable alternative pathway by the enzyme γ-glutamyl cyclotransferase back to 5-oxoproline, which is only slowly utilized forward through the cycle by the rate-limiting enzyme 5-oxoprolinase; hence, marked accumulation of 5-oxoproline persists. Clinically, this disorder manifests as a direct result of increased amounts of 5-oxoproline and a deficiency of glutathione. Increased 5-oxoproline causes a severe high anion gap metabolic acidosis, usually presenting at birth, and is a major cause of early mortality; the glutathione deficiency, then, is linked to the development of hemolytic anemia, defective leukocyte function, and a variety of progressive neurologi-
ical sequelae ranging from mental retardation to motor disturbances that develop variably over the years, if indeed the affected individuals survive the neonatal period.

An inborn defect in the enzyme 5-oxoprolinase was first described in 1981 in two brothers, ages 11 and 16 years [2]. To date, five cases in total have been described since 1981. As seen in Fig. 1, 5-oxoprolinase is involved in the decyclization conversion of 5-oxoproline to l-glutamate. An enzymatic block here, therefore, leads to accumulation of 5-oxoproline, which is reflected as an increase in plasma concentration and urinary excretion (5-oxoprolinuria). Glutathione concentrations are not affected, however, because substrate for synthesis of glutathione is available from other sources. The only main abnormality evident in affected individuals is increased concentrations of 5-oxoproline, which are not so great as those found in cases of glutathione synthetase deficiency and characteristically do not result in major metabolic acidosis. Similarly, the clinical sequelae attributable to glutathione deficiency are not present. Affected individuals, therefore, appear normal.

Besides the documented cases involving true inborn errors of glutathione synthesis resulting in 5-oxoprolinuria, some cases of apparently induced states of 5-oxoprolinuria without any evidence of inherited metabolic defects in the γ-glutamyl cycle have also been reported. The latter cases have been associated with a variety of conditions and disorders: urea cycle disorders [4]; propionic acidemia [5]; hawkinsinuria [6]; Stevens–Johnson syndrome and severe burns [7]; homocystinuria [8]; prematurity [9]; glycine deficiency [10]; patients on artificial diets [11]; and drug treatment with acetaminophen (paracetamol) [12] and vigabatrin [13].

We highlight the case of a 35-year-old woman who presented initially with an uncomplicated staphylococcal pneumonia but who went on to develop a transient but severe high anion gap metabolic acidosis associated with markedly increased urinary excretion of 5-oxoproline but with no clinical evidence of any of the features of the inherited disorders of glutathione synthesis. The onset of the acidosis and subsequent complete recovery were subsequently shown to be related in time to the administration of intravenous antibiotics.

**Case History**

A 35-year-old woman was admitted to the acute medical unit of a large city center hospital with a 2-week history of a chest infection that was previously unsuccessfully treated at home with oral ciprofloxacin. There was no past medical history of note, although a recent marriage breakup and alcohol abuse were a problem. On admission the patient was alert and orientated, with a mild pyrexia, slight breathlessness, and a productive cough. Clinical signs of a lower lobe consolidation of right lung was evident, which was confirmed on x-ray showing a cavitated right lower lobe abscess. Results for urea and electrolytes, arterial blood gases, and liver function tests...
were all normal, with C-reactive protein increased at 230 mg/L (reference range, <10 mg/L). Sputum culture grew staphylococcus; therefore, the patient was commenced on intravenous flucloxacillin, 12 g/day, and periodic netilmicin, 150–300 mg/day.

Over the first 2 weeks of admission, the patient’s clinical condition ameliorated with improvement in chest x-ray appearance and a decreasing concentration of C-reactive protein. On day 19 of admission, however, the patient was noticed to have become drowsy, confused, and disorientated and was hyperventilating. Arterial blood gases and electrolyte analysis showed a severe metabolic acidosis (pH, 7.17; [H+], 68 nmol/L; Pco2, 1.8 kPa; Po2, 13.9 kPa; and HCO3–, 5.1 mmol/L) with a calculated anion gap of 29 mmol/L (Na+ – Cl– and total CO2). She deteriorated further over the next 6 h, and with a Glasgow Coma Scale of 3, she was transferred to the intensive care unit on day 20 of her admission.

Investigation for the more common causes of metabolic acidosis proved fruitless with only a mildly increased serum lactate, no ketones or alcohols present, and no evidence of renal pathology with appropriately acidic urine. On day 21, on the basis of advice that the antibiotics the patient was receiving could be contributing to the metabolic and clinical findings (K.G.M.M. Alberti, personal communication), it was decided to stop the antibiotic therapy. Remarkably, over the next 2 days, the patient’s acidosis began to spontaneously resolve. Clinically, she improved and was well enough to be transferred back to the general wards on day 29. The patient was finally discharged on day 42, completely recovered with no clinical sequelae, and all measurements normal.

Subsequent analysis of the patient’s urine for organic acids by gas chromatography–mass spectrometry demonstrated a markedly increased excretion of 5-oxoproline of ~7300 mg/g creatinine (normal range, <70 mg/g creatinine) at the peak of her acidosis on day 21 of her admission. By day 23, these concentrations had fallen to ~5800 mg/g creatinine before returning to trace amounts by the time of her discharge from the hospital on day 42. In contrast over these periods, the concentrations of glutathione in the patient’s whole blood, plasma, and erythrocytes were within the reference interval. Similarly, no abnormalities were found in other urinary organic acids or plasma and urine amino acid concentrations. After discharge, the patient subsequently defaulted from further hospital review.

The transient 5-oxoprolinuria with normal glutathione concentration appeared to be the only obvious cause of the severe high anion gap metabolic acidosis in this patient. The development and subsequent recovery from the acidosis can be monitored by looking at the plasma bicarbonate profile over the period of hospital admission as shown in Fig. 2. The administration of antibiotics in this patient appears to be related in time to the development and recovery from acidosis and hence, presumably, the 5-oxoprolinuria.

The procedures followed in the investigation and treatment of this patient were in accordance with the ethical standards of the Victoria Infirmary NHS Trust, Glasgow, UK, and are also in line with the Helsinki Declaration of 1975 (revised 1983).

**Materials and Methods**

Routine analytes were measured with a Hitachi 717 automated analyzer (Boehringer-Mannheim Diagnostics). Blood gas measurements were made with a Ciba-Corning 278 blood gas analyzer (Ciba-Corning Diagnostics).

Gas chromatography–mass spectrometry was used to measure 5-oxoproline. The gas chromatography system used was a Hewlett-Packard 5890 series II fitted with 7673A autosampler, HP-1 capillary column (25 m × 0.2 mm × 0.33-μm film thickness; Hewlett-Packard), and HP5971A mass-selective detector. Helium gas flow rate

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**Fig. 2.** Relation in time of plasma bicarbonate to antibiotic administration.
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was 0.6 mL/min (head pressure, 114 kPa). Split injections (ratio, 100:1) were made with a 1-μL sample. A one-step temperature program was run from 70 to 290 °C at 7 °C/min after an initial time of 0.5 min. The mass spectrometer in electron ionization mode, connected directly to the capillary column outlet, was operated at 70 eV. Data acquisition was carried out in the scan mode from m/z 58 to 550, with dwell time of 100 ms. The method of extraction and preparation of urine samples for gas chromatography–mass spectrometry and the method for qualitatively and quantitatively identifying 5-oxoproline were based on those described by Tanaka et al. [14, 15]. The response factor to the internal standard, isopentanoic acid, was used to approximate the 5-oxoproline peak as identified by comparison with published spectra.

Amino acid measurements, including measurement of glutathione in urine, plasma, and whole-blood hemolysate were made with a Biotronic LC5001 amino acid analyzer (Eppendorf-Netheler-Hinz, division of Biotronic) and a Trivector TRIO computing integrator (Trivector Technical Services). A glass separation column (3.2 × 385 mm) was used with BTC2710 10-μm separation exchange resin (Eppendorf-Netheler-Hinz) and lithium citrate separation buffer (flow rate, 0.30 mL/min). Separation temperatures were set at 32 °C for 44 min, 34 °C for 28 min, and 60 °C for 31 min. For colorimetric peak detection at 570 and 440 nm, 500 μmol/L aminoethyl-1-cysteine hydrochloride in 37 mmol/L lithium/76 mmol/L citrate buffer at pH 2.2 was used as the internal standard. Plasma, urine, and whole-blood hemolysate were pretreated with crystalline 5-sulfoisalicylic acid as a deproteinization step.

Discussion

This case is similar to a previous case of transient 5-oxoprolinuria reported in 1989 [16], where the source of the metabolite accumulation was suggested to be attributable to an acquired deficiency of glutathione synthetase or 5-oxoprolinase activity, gastrointestinal absorption of 5-oxoproline from an unidentified dietary source, or some combination of these factors. In our patient, there was no evidence of a possible dietary source of 5-oxoproline, and the excretion of 5-oxoproline found was greatly in excess of values reported in previous cases of dietary-induced 5-oxoprolinuria [11]. It appears likely, therefore, that the 5-oxoprolinuria demonstrated in our patient was attributable to a metabolic defect at some stage in the γ-glutamyl cycle and that this resulted in the biochemical and clinical findings. Indeed, the combination of increased urinary excretion of 5-oxoproline and normal whole blood, plasma, and erythrocyte glutathione concentrations suggests that this was not a result of a block at the more common stage of glutathione synthetase but more likely at the site of 5-oxoprolinase. The definitive proof of such a disturbance in the γ-glutamyl cycle would obviously be to measure the activity of the aforementioned enzymes, especially 5-oxoprolinase.

What is the possible mechanism behind these metabolic findings? There is the transient nature of the abnormality to consider. One could suggest that a transient influence by an exogenous or endogenous agent on the natural enzyme structure or function was responsible or a similar transient change in γ-glutamyl cycle reaction conditions, and hence, kinetics. The striking relationship between the development and regression of both clinical and biochemical findings and the intravenous administration of the antibiotics flucloxacillin and netilmicin makes them prime candidates for the exogenous agents. We have been unable to find evidence of either antibiotic being previously implicated in such a metabolic disturbance. Again, definitive proof of this would require direct measurement of enzyme activity and 5-oxoproline concentrations in the absence and presence of these antibiotics or their metabolites. Did this patient have a normal γ-glutamyl cycle in the first place? The return to normal amounts of all measurements implies that a major defect was not present. It remains possible, however, that an underlying abnormality was present that under usual circumstances had no major biochemical or clinical sequelae but only became “active” under the conditions present.

We suggest that this case represents transient 5-oxoprolinuria as a result of a transient inhibition in the γ-glutamyl cycle at the stage of the enzyme 5-oxoprolinase, resulting in an accumulation of 5-oxoproline and a resulting severe high anion gap metabolic acidosis. In addition, we further hypothesize that such a disturbance in the γ-glutamyl cycle was a direct result of the action of flucloxacillin, netilmicin, or the combination of both on the activity of 5-oxoprolinase. Accordingly, in cases of unexplained high anion gap metabolic acidoses, it is worth considering the possible role of any intravenous antibiotics in the etiology of the condition. Additional studies of enzyme activity in these cases would greatly help to further define a potential cause–effect relationship.

References

B. Hawkins


