

Review

Unmeasured anions in metabolic acidosis: unravelling the mysteryLui G Forni^{1,2}, William McKinnon³ and Philip J Hilton³¹Department of Critical Care, Worthing Hospital, Worthing, West Sussex BN11 2DH, UK²Brighton and Sussex Medical School, University of Sussex, Brighton, East Sussex BN1 9PX, UK³Renal Laboratory, St Thomas' Hospital, London SE1 7EH, UKCorresponding author: Lui G Forni, lui.forni@wash.nhs.uk

Published: 12 July 2006

This article is online at <http://ccforum.com/content/10/4/220>

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Critical Care 2006, **10**:220 (doi:10.1186/cc4954)**Abstract**

In the critically ill, metabolic acidosis is a common observation and, in clinical practice, the cause of this derangement is often multifactorial. Various measures are often employed to try and characterise the aetiology of metabolic acidosis, the most popular of which is the anion gap. The purpose of the anion gap can be perceived as a means by which the physician is alerted to the presence of unmeasured anions in plasma that contribute to the observed acidosis. In many cases, the causative ion may be easily identified, such as lactate, but often the causative ion(s) remain unidentified, even after exclusion of the 'classic' causes. We describe here the various attempts in the literature that have been made to address this observation and highlight recent studies that reveal potential sources of such hitherto unmeasured anions.

Introduction

Metabolic acidosis remains a common problem in acute medicine and is frequently encountered on the intensive care unit (ICU) [1-3]. Although many 'classic' causes of metabolic acidosis are known, including diabetic ketoacidosis, lactic acidosis and the ingestion of acid-generating poisons, the origin is often multifactorial and, indeed, often cannot be ascribed solely to such 'classic' causes or a single causative anion. In such cases, the source of the acidosis remains unidentified or unmeasured. For example, given that hydroxybutyrate is seldom measured, diabetic ketoacidosis is, strictly speaking, an example of acidosis associated with large quantities of an unmeasured anion, although in practice its concentration is regularly inferred. Similarly, it is only in the past 15 years or so that prompt and repeatable measurement of arterial blood lactate has become commonplace. Prior to this, lactic acidosis could also reasonably be considered to represent the presence of an unmeasured anion.

One of the earliest tools for addressing the potential aetiology of metabolic acidosis is that of the anion gap, which even in its simplest form helps to characterise many cases of metabolic acidosis. This measure has undergone various

refinements over the years but one of its purposes is to alert the physician to the presence of unmeasured ions in plasma [4-7]. Those studying critically ill patients with metabolic acidosis have been aware that such a simple categorisation is often an inadequate description of the metabolic state of these patients. In lactic acidosis, for example, there is often a significant discrepancy between the blood lactate concentration and the base deficit and, more tellingly, when calculations are made during bicarbonate-based haemofiltration, it is apparent that significant quantities of acid other than lactic acid are being titrated by the administered bicarbonate. This has given rise to the concept of the 'unmeasured anions' as an important component of human metabolic acidosis. Sometimes these appear to be quantitatively significantly more important than lactic acid itself. But what is the nature of these unmeasured anions? We discuss the evidence to date coupled with recent work from our laboratory that may go some way in elucidating the nature of these anions.

Identifying unmeasured anions

The presence of unmeasured anions contributing to metabolic acidosis has been recognised for some time and as early as 1963 Waters and colleagues, whilst discussing lactic acidosis, hypothesised that under certain conditions disturbances in acid-base balance may be "characterised by the accumulation of an organic acid other than lactate" [8]. Furthermore, studies from Cohen's group in London described a case where hydroxybutyrate contributed significantly to an observed metabolic acidosis of a non-diabetic patient [9]. The same group also demonstrated an elevation in succinate levels in both hypoxic patients and perfused hypoxic canine livers [10]. They proposed that disturbances in the oxidation of succinate to oxaloacetate could account for this. Interest in this area was rekindled by studies on critically ill patients in which elevations in anion gap could not be accounted for solely by increased lactate levels [11,12]. Further work

ICU = intensive care unit.

examining the concentrations of other hitherto unmeasured ions such as urate and phosphate as well as plasma proteins could not account for the observed anion gap [13,14]. To try to elucidate these species further, several workers have employed animal models.

Animal studies

Some of the earliest studies that attempted to identify the nature of the unmeasured anions were performed in animal models. In 1990, Rackow and colleagues [15] assessed the contribution of such species to the anion gap observed in rats following caecal perforation. Compared to controls, the septic animals demonstrated a metabolic acidosis with an increase in plasma lactate and decrease in bicarbonate concentrations. Only 15% of the anion gap observed could be explained by lactate. The concentrations of pyruvate, β -hydroxybutyrate, acetoacetate, citrate as well as some amino acids were determined. No differences in these anions could be detected between the study group and sham animals. However, no detail as to the handling of the samples was provided. These studies followed earlier work by Gossett and colleagues [16] on critically ill horses with increased anion gap acidosis. Again, the unexplained anion gap could not be accounted for by pyruvate, β -hydroxybutyrate, acetoacetate, phosphate or albumin.

In other studies on diarrhoeic calves, the observed anion gap was explained in part, but not completely, by the accumulation of D-lactate [17]. To date, animal studies have, therefore, provided little information as to the nature of the unmeasured anions. Further animal work, employing a canine model of sepsis, demonstrated that the liver released anions into the circulation at a rate of 0.12 mEq/minute [18]. This study also observed that the gut became a 'consumer' of anions following development of endotoxaemia. Other canine models have proposed that, in lactic acidosis, impaired extraction of lactate by the liver coupled with increased splanchnic production of lactate contributed to the generation of the metabolic acidosis. Studies with humans, however, do not support this view [19].

Studies on ICU patients

Pyroglutamic acidaemia

Pyroglutamic acidaemia is an inherited disorder presenting in infancy due to a deficiency of either 5-oxoprolinase or glutathione synthetase. Several case reports have described this phenomenon occurring in adults, causing an elevated anion gap acidosis often in association with drug administration [20]. An early study of ICU patients described four patients in whom pyroglutamic acid levels were noted to be elevated [21]. The authors suggested that patients with this condition be screened for obvious precipitants. However, a further study examined pyroglutamic acid levels in 23 ICU patients with metabolic acidosis and an unexplained increase in ion gap. They found no correlation between the ion gap and pyroglutamic acid levels and concluded that, in their

population, pyroglutamic acid could not account for the unmeasured anions [22].

Krebs cycle intermediates

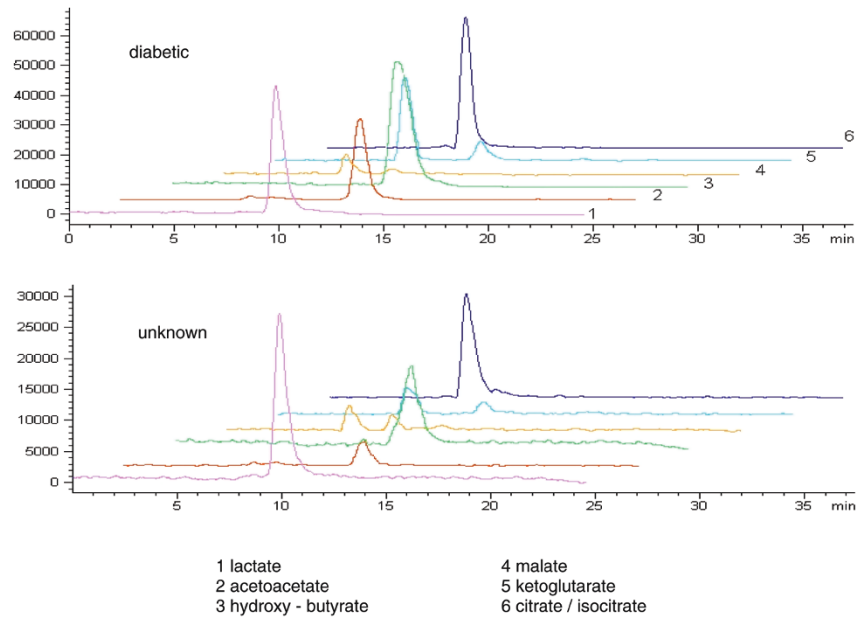
We recently attempted to identify the missing anions, arguing that being negatively charged, they should reveal themselves on negative ion mass spectrometry and should be at least partially separable by ion exchange chromatography. There was no predetermined view as to the likely nature of the anions. Plasma from patients with various forms of metabolic acidosis was examined. The patients were acidotic with an average arterial pH of 7.18 (± 0.11) and a base deficit of 13.4 mmol/l (± 4.7) [23].

Figure 1 shows an ion exchange chromatogram/negative ion mass spectrum of a plasma extract from a patient with metabolic acidosis of unknown aetiology. This shows peaks of relatively low mass that fitted those of known Krebs cycle components. Standards of these anions proved to have identical retention times to the plasma-derived peaks. Interestingly, no ions attributable to other substances could be seen apart from urate, which was also seen in control samples. For comparison, we present the spectrum obtained from a patient with diabetic ketoacidosis where the large peaks attributable to acetoacetate and β -hydroxybutyrate are clearly seen [24].

These preliminary results led us to examine the anions of the Krebs cycle using enzyme assay (we also measured D-lactate). Table 1 simplifies our results and, as can be seen, plasma from patients with diabetic ketoacidosis showed significant increases relative to the control values in α -ketoglutarate, malate and D-lactate levels. However, citrate and succinate concentrations were not elevated. In lactic acidosis, increased concentrations of citrate, isocitrate, α -ketoglutarate, succinate, malate and D-lactate were observed. In patients with an acidosis of unknown origin (acidosis disproportionate to the blood lactate concentration), elevations in the concentrations of isocitrate, α -ketoglutarate, succinate, malate and D-lactate were seen. This observation that plasma concentrations of acids usually associated with the Krebs tricarboxylic acid cycle are significantly increased in patients with lactic acidosis as well as those with 'unexplained acidosis' with normal or near normal blood lactate concentrations may go some way to addressing the 'imbalance' in the anion or strong ion gap.

In the main, these anions are effectively fully ionised at the measured pH but, unlike lactate, they are not all monobasic, with tribasic acids (citric and isocitric) contributing three protons, whilst the dibasic acids (α -ketoglutaric, malic and succinic) add two protons to the solution on ionisation. Our study showed that, on average, the contribution to the observed anion gap by such anions was regularly in excess of 3 mEq/l and, in some cases, over 5 mEq/l. Therefore, the role of these anions in generating the anion gap is of much

Figure 1



Ion exchange chromatogram/negative ion mass spectra of plasma from a patient with diabetic ketoacidosis (top) and a patient with acidosis of unknown aetiology (bottom). Liquid chromatography/electrospray ionisation mass spectrometry was performed on a Hewlett-Packard Series 1100 liquid chromatography system directly coupled to a Series 1100 Mass Spectrometer fitted with electrospray ionisation and operating in 'negative ion' mode (Agilent Technologies UK Ltd, Wokingham, Berkshire, UK). The extracted ion currents are shown.

Table 1

Relative changes observed in Kreb's cycle intermediates and D-Lactate in patients with differing causes of acidosis

Acid	DKA	LA	AUO	NAG
Citrate	-	? ^a	-	-
Isocitrate	+	+++	+++	-
α-Ketoglutarate	+++	+++	+++	-
Succinate	-	+++	+	-
Malate	+++	+++	+++	-
D-lactate	+++	+++	+++	+++

Dashes represent no significant difference from controls; a plus sign represents $p < 0.02$; three plus signs represent $p < 0.001$. ^aThis result may be unreliable since four of the patients in this group had received an infusion of heparin (containing citrate as an anticoagulant) prior to the blood sample being obtained. AUO, acidosis of unknown origin; DKA, diabetic ketoacidosis; LA lactic acidosis; NAG, normal anion gap acidosis.

greater significance than is apparent from their molarity. We would stress that in data such as these, at least as much attention should be given to the extreme values as to the means.

From our preliminary work it became clear that rapid separation of the plasma from red cells and also from its proteins through centrifugation and ultrafiltration of the samples together with prompt assay was vital. Even at -20°C we observed steady degradation of the measured anions. The most extreme example of the instability of these metabolic

intermediates is oxaloacetate, whose half-life in aqueous solutions is so short that it is effectively unmeasurable [25].

D-lactate

Although we observed modest elevations in D-lactate concentration in both diabetic and non-diabetic acidosis, this never reached levels in these groups that would impact significantly on the acid-base status of the patients. However, in the patients with a normal anion gap acidosis, the level of D-lactate was significantly raised. D-lactate is normally present at nanomolar concentrations through the metabolism

of methylglyoxal, although millimolar concentrations can be observed through excess gastrointestinal metabolism and elevated levels of D-lactate have been observed in critically ill patients with intestinal ischaemia [26]. Interestingly, plasma D-lactate levels have been proposed as an early potential predictor of reduced 28 day ICU mortality [27] and has been suggested as a tool for assessing colonic ischaemia in post operative patients [28]. In rat models, however, D-lactate has not been confirmed as a reliable marker of gut ischaemia [29]. However, what is clear is that D-lactate may contribute to metabolic acidosis and, in some cases, may contribute significantly to the unmeasured anions.

Hydroxybutyrate

Another anion that does not fit neatly into this concept of Krebs cycle acidaemia is hydroxybutyrate in non-diabetics. We detected this anion in concentrations up to 4 mEq/l and, as such, it could be a significant contributor to the unmeasured anions. We presumed that this was effectively a marker for the metabolic changes of 'starvation' in the patients in whom it was demonstrated, in agreement with earlier studies [9].

Discussion

Many studies have highlighted the presence of unmeasured anions in critically ill patients with metabolic acidosis, although few have been successful in addressing their chemical nature. The prognostic significance of unmeasured anions is also a source of debate but recent studies seem to suggest some predictive ability [30,31]. Certainly, the study from Dondorp and colleagues [30] supports this view, although the area under receiver operator curve for strong ion gap toward mortality was just 0.73. However, all other predictors also had values <0.8. Interestingly, recent studies on the primary patho-physiological events of malarial infection in animals revealed up-regulation of transcription of genes that control host glycolysis [32]. One may speculate that the unmeasured anions noted in severe malaria may, therefore, be related to intermediary metabolism, in keeping with our studies. Other workers have demonstrated the presence of organic acids commonly associated with intermediary metabolism under various conditions. Tricarboxylic acids have been detected in human urine [33] and various organic acids detected in the haemofiltrate of patients with acute renal failure where the presence of elevated citrate levels was loosely associated with a worse prognosis [34]. Furthermore, citrate, malate and cis-aconitate have been detected in patients with metabolic acidosis ascribed to salicylate poisoning [35].

The results obtained from our work suggest that the role of anions principally associated with the Krebs cycle in the generation of the anion gap in 'classic' lactic acidosis may be greater than previously thought and that these anions may also have a significant role in the generation of the anion gap in patients with acidosis of unknown cause. Their concentra-

tions did not differ significantly from control values in patients with normal anion gap acidosis.

The likely source for the generation of these observed anions is a matter of speculation and we have no direct evidence for the site of production. Clearly, the mitochondria are one possible source and the process could reflect mitochondrial dysfunction, a concept that is currently an area of research in critical care. It seems unlikely that the acidaemia *per se* is responsible for the generation of increased levels of Krebs intermediates given the normal values found in patients with normal anion gap acidosis. It may reflect a physiological response to a limitation in available oxygen supply and recent work from our group has demonstrated increased levels of Krebs cycle intermediates in normal subjects following severe exercise [35].

The Krebs cycle functions not only as a 'catalytic' process in intermediary metabolism but also as a source of substrates for other metabolic pathways. For example, during protein synthesis, α -ketoglutarate and oxaloacetate are removed from the cycle to become aminated to glutamate and aspartate (cataplerosis). This inevitably results in anaplerotic reactions, ensuring continued function by replenishing tricarboxylic acid intermediates. In gluconeogenesis, oxaloacetate is converted to phosphoenolpyruvate and is lost to the Krebs cycle. Lipogenesis requires the transfer of citrate from the mitochondria to the cytosol as that is the site at which the synthetic process occurs. In disease, the opposite is true; anaplerotic reactions (those that generate rather than consume Krebs cycle keto-acids) are likely to predominate. Excess protein catabolism in particular will give rise to the component amino acids. These approximately neutral compounds are rapidly transaminated and/or deaminated to form oxaloacetic acid, α -ketoglutaric and succinyl CoA (effectively succinic acid), thereby potentially providing an excess of acidic Krebs cycle components. There are few data available from the critically ill on these processes. However, under other conditions of stress, such as prolonged starvation or extreme exercise [36], the levels of tricarboxylic acid levels have been measured and it has been shown that glutamine, for example, undergoes deamination (an anaplerotic process) to form α -ketoglutarate, which enters the Krebs cycle and is sequentially converted to malate, which then leaves the mitochondria. Malate is oxidized in the cytosol to oxaloacetate, which is in turn converted to phosphoenolpyruvate.

Conclusion

The phenomenon of unexplained metabolic acidosis is well recognised, as is the generation of 'unexplained' anions. Little is known as to the nature of these species, although recent studies suggest that anions usually associated with the Krebs cycle may contribute to the observed anion or 'strong-ion' gap. Although these observations go no way to explaining their genesis, they may provide the first glimpse of the

underlying derangement in the metabolic acidosis associated with 'unmeasured anions'.

Competing interests

The authors declare that they have no competing interests.

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