Basic science review

Metabolic Acidosis in Patients with Sepsis: Epiphenomenon or Part of the Pathophysiology?

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ABSTRACT

Objective: To review the mechanisms of metabolic acidosis in sepsis.

Data sources: Articles and published reviews on metabolic acidosis in sepsis.

Summary of review: Sepsis affects millions of patients each year and efforts to limit mortality have been limited. It is associated with many features one of which is acidosis which may be a result of the underlying pathophysiology (e.g. respiratory failure, shock, renal failure) or may also result from the way in which we manage critically ill patients.

Lactic acidosis identifies septic patients at risk and aggressive fluid resuscitation (along with inotropes and blood in some patients) to reverse acidosis and improve venous oxygen saturation will improve mortality. However, most patients with severe sepsis or septic shock receive 0.9% saline and therefore may develop hyperchloraemic acidosis as a consequence of their resuscitation. Therefore alterations in acid-base balance are almost always in the background in the management of patients with sepsis. What is unknown is whether acidosis is in the causal pathway for organ dysfunction or whether it is simply an epiphenomenon. Changes in acid-base balance, of the type and magnitude commonly encountered in patients with sepsis, significantly alter the release of inflammatory mediators. Less significant changes in the immune response have already been implicated in influencing outcome for patients with sepsis and a reduction in acidosis in septic patients may have the same effect.

Conclusions: Understanding the effects of acid-base on the inflammatory response is relevant as all forms of metabolic acidosis appear to be associated with prolonged hospital and ICU length of stay. Since metabolic acidosis is both commonly caused and treated by clinicians, understanding of the physiologic consequences of altered blood pH is imperative. (Critical Care and Resuscitation 2004; 6: 197-203)

Key words: Metabolic acidosis, sepsis, severe sepsis, strong ion difference

Severe sepsis affects millions of patients each year killing between 29-40%. Efforts to limit mortality have been limited in both their absolute success in trials and their subsequent translation into clinical practice. Sepsis is associated with a myriad of clinical features, including acidosis.1 While acidosis may be a result of the underlying pathophysiology (e.g. respiratory failure, shock, renal failure) it may also result from the way in which we manage critically ill patients. In patients with sepsis-induced organ failure significant improvements in survival have been produced by altering the way in which organ support2,3 and resuscitation4 are carried out. Reducing tidal volumes and airway pressures in mechanically ventilated patients with acute lung injury improves survival5 but may also result in respiratory acidosis, which in turn is managed differently from

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Metabolic acidosis

Metabolic acidosis is a common problem in patients with sepsis and other forms of critical illness and is associated with poor outcome. Acidosis appears to be common in critically ill patients. However, the exact incidence and prevalence of metabolic acidosis has not been established for patients with sepsis or for critically ill patients in general. Although it remains uncertain whether or not there is a true cause-effect relationship between acidosis and adverse clinical outcomes, acidosis is a powerful marker of poor prognosis in critically ill patients.\(^8\text{-}10\) Acidosis may occur as a result of increases in arterial \(p\text{CO}_2\) (respiratory acidosis) or from a variety organic or inorganic fixed acids (metabolic acidosis). There appears to be a difference in epidemiology between patients with respiratory and metabolic acidosis, leading some investigators to hypothesise that the cause of acidosis rather than the acidosis \textit{per se} is driving the association with clinical outcomes. However, in both respiratory and metabolic acidosis, non-survivors have a lower arterial blood pH. Interestingly, in both groups, non-survivors have blood gases that were further separated from the line of pure respiratory acidosis. In other words, non-survivors had more metabolic acidosis.\(^11\)

Metabolic acidosis results from a variety of common etiologies including lactic acidosis, hyperchloraemic acidosis, renal failure and ketoacidosis. While the underlying disease process associated with each of these sub-types carries its own clinical consequences, acidosis itself might potentially contribute to, or even attenuate, the adverse effects of these conditions. Understanding the mechanisms whereby acid-base disorders occur is paramount in evaluating the clinical consequences and the therapeutic options.

Hyperchloraemic metabolic acidosis

Although common causes of metabolic acidosis such as lactic acidosis and renal failure may be unavoidable, often the source of metabolic acidosis is at least partly iatrogenic. This is because saline resuscitation is often used to treat shock. Large volume saline infusion produces metabolic acidosis by increasing the plasma \(\text{Cl}^-\) concentration relative to the plasma \(\text{Na}^+\) concentration.\(^12\text{-}15\) The result is a reduction in the strong ion difference (SID), the difference between positive and negative charged electrolytes, which in turn produces an increase in free \(\text{H}^+\) ions to preserve electrical neutrality.\(^16\) The clinical effects of these changes have been documented over the past several years. Comparing starch preparations in saline versus a balanced electrolyte solution for elderly abdominal surgery patients, Wilkes and coworkers recently described a worsening of acid-base balance and more adverse events with the saline-based fluid.\(^17\) Similar findings have been reported with 0.9% saline compared with lactated Ringers solution.\(^18\) Indeed, the side effect profile of saline-induced acidosis, nausea, vomiting, abdominal pain, headache, thirst, hyperventilation and delayed urination is identical to what occurs with ammonium chloride administration.\(^19\)\(^20\) Thus, while there is little evidence that treating metabolic acidosis improves clinical outcomes,\(^21\) there is evidence that iatrogenic metabolic acidosis may be harmful. To the extent that acidosis is avoidable and/or treatable, a better understanding of the impact of acidosis on a variety of systems is needed to better balance potential risks and costs with benefits.

Saline resuscitation produces hyperchloraemic acidosis in a predictable fashion.\(^12\) By use of a mathematical model based on a physical-chemical acid-base analysis we have accurately predicted the serum \(\text{Cl}^-\) concentration and resulting arterial blood pH changes in healthy dogs given large volumes of intravenous 0.9% saline.\(^12\) By applying this model to dogs given bolus intravenous lipopolysaccharide (LPS), 1 mg/kg, and subsequent large-volume saline resuscitation (100
mL/kg over 3 hr) we have quantified the effects on acid-base balance. The total acid load was calculated from the change in standard base excess (SBE) attributable to each source. In LPS-treated animals mean arterial pH decreased from 7.32 to 7.11, P < 0.01; pCO2 and lactate were unchanged. Saline accounted for 38% of the total acid load. Although serum Na+ did not change, serum Cl− increased (127.7 ± 5.1 mmol/L vs. 137.0 ± 6.1 mmol/L, P = 0.016).

From these experiments we concluded that saline resuscitation alone accounts for more than a third of the acidosis seen in this canine model of acute endotoxemia, whereas lactate accounts for less than 10%. Furthermore, a large amount of the unexplained acid load in this model appears to be attributable to differential Na+ and Cl− shifts presumably from extracellular to vascular or intracellular to extra-cellular spaces. This study and work from other groups has lead to the hypothesis that Cl− (both exogenous and endogenous) plays an important role in the development of acidosis.

Compared with starch in a balanced electrolyte solution, resuscitation with saline resulted in hyper-chloroaemic metabolic acidosis and worse short term survival in rats. Normal saline (0.9%) (NS) was compared with other fluids in the resuscitation of Sprague-Dawley rats given bolus intravenous LPS on short-term survival and acid-base balance. An inverse relationship between the change in serum CI and survival time in these animals was demonstrated. We studied 60 rats for 12 hr after intravenous infusion of LPS (20 mg/kg). We volume resuscitated to maintain a MAP > 60 mm Hg using either NS or 6% hetastarch in a balanced electrolyte solution (bHS) or lactated Ringer’s (LR). Mean survival time among animals treated with NS or LR was 45% less compared with bHS-treated animals: 391 ± 151 min and 362 ± 94 min vs. 567 ± 140 min, p < 0.0001. Overall survival (at 12 hr) was 0% with NS or LR vs. 20% with bHS, p = 0.05. After resuscitation with NS, arterial SBE and plasma apparent strong ion difference (SIDa) were both significantly lower (-19.3 ± 5.2 vs. -12.1 ± 5.7 p < 0.001 and 23.0 ± 6.2 vs. 30.3 ± 2.9, p < 0.0001, respectively) and plasma Cl− was significantly higher (123 ± 7 vs. 115 ± 3 mmol/L, p < 0.0001) compared to bHS. Resuscitation with LR resulted in a SBE, and CI between that of NS and bHS (-15.4 ± 3.1, and 117 ± 3 respectively). These data showed that compared with bHS, volume resuscitation with NS was associated with more metabolic acidosis and shorter survival in this experimental animal model of septic shock.

Metabolic acidosis might reduce survival from sepsis through a variety of mechanisms. First, acidosis has been associated with haemodynamic instability, although the association is not always consistent and the underlying mechanisms are uncertain. Pedoto and colleagues have recently shown that metabolic acidosis may increase inducible nitric oxide synthase (iNOS) expression in animals and this could exacerbate vasodilation and shock. They have also demonstrated that hyperchloroaemic acidosis increases lung and intestinal injury in healthy rats. In order to control for other effects of large-volume resuscitation (e.g. cell swelling) we increased serum Cl− concentration by infusing a dilute HCl solution into Sprague-Dawley rats with CLP-induced sepsis. We showed that hyperchloroaemic acidosis induced changes in MAP and NO release in these septic animals. Eighteen hours after inducing lethal sepsis by caecal ligation and puncture (CLP), we randomised 24 rats and divided them into 3 groups. In groups 2 and 3 we began an 8-hour intravenous infusion of 0.1N HCl to reduce the SBE by 5 - 10 and 10 - 15 mEq/L, respectively. We measured mean arterial pressure (MAP), arterial blood gases, electrolytes, plasma nitrate/nitrite levels at 0, 3, 6 and 8 hr. The MAP remained stable in group 1 but decreased in groups 2 and 3 (p < 0.001) such that at 8 hr the MAP was much higher in group 1 (94 ± 9.2 mmHg) compared to either group 2 (71.6 ± 20.1 mmHg) or group 3 (49.4 ± 33.2 mmHg), p = 0.01. This change in the MAP correlated with the increase in plasma Cl− (R² = 0.50, p < 0.0001) and less well with the decrease in pH (R² = 0.24, p < 0.001). After 6 hr of acidosis, plasma nitrite levels were significantly higher in group 2 animals compared with either group 1 or group 3 animals, p < 0.05. We concluded that moderate acidosis, induced by HCl infusion, worsened blood pressure and increased plasma nitrate/nitrite levels in septic rats. Our results are in general agreement with reports by Pedoto and coworkers that demonstrated that metabolic acidosis increased inducible nitric oxide synthase (iNOS) leading to vasodilation and shock in healthy rats. Studies of non-stimulated resident peritoneal macrophages and LPS-stimulated RAW 264.7 cells have shown increased NO formation at moderately reduced pH (7.0 - 7.2). However, more severely acidic pH reduces NO formation and there is an apparent dissociation between the pH effects on iNOS mRNA, protein and final NO release. Thus, HCl seems to affect inflammatory mediators differently at different stages in their synthesis and release.

Effects of acidosis on inflammatory mediator release

If acidosis alters the release of NO, might it also affect the release of other inflammatory mediators? There are now several studies documenting the effects of decreased pH on the synthesis and release of inflammatory mediators, especially tumour necrosis...
factor (TNF) and interleukin 6 (IL-6). Most of these studies have been conducted in resident macrophages or macrophage-like cell lines and have found conflicting results. However, studies using HCl have consistently shown pro-inflammatory effects at the level of nuclear factor kappa-B (NF-κB) DNA-binding or TNF synthesis, provided pH was not less than 6.0.\textsuperscript{28,30,31} Although TNF secretion was reduced even at pH as high as 7.0,\textsuperscript{30-32} Little is known about the effects of HCl on other cytokines or on the kinetics of pH mediated effects.

Lactic acid has been studied in an even more limited way than HCl. Lactic acid (pH 6.75) has been shown in one study\textsuperscript{33} to result in increased TNF release in LPS-stimulated peritoneal macrophages. This finding is surprising in light of the growing evidence of a protective effect of lactic acid in neuronal injury.\textsuperscript{34-36} Several studies have sought to explore the effect of dialysis solutions on the immune response.\textsuperscript{37,38} These acidic, lactate-based, solutions have been shown to decrease various aspects of the immune response including TNF synthesis and release.\textsuperscript{37,38} Doudevani also demonstrated a decrease in LPS-induced NF-κB DNA-binding in human blood-derived macrophages when incubated with dialysis solution.\textsuperscript{38} Although these solutions are also hyper-osmolar and have excessive glucose concentrations, variables known to influence immune function,\textsuperscript{37,39} they provide additional evidence of a potential anti-inflammatory role of lactate and highlight potential differences between various acids and their effects on the immune response.

We have conducted a series of experiments in LPS-stimulated RAW 264.7 murine macrophage-like cells in which we have decreased the pH of the medium using different acids. Remarkably, dramatically different patterns of inflammatory mediator expression occurred with different acids despite normalisation to the same pH. In our first set of experiments\textsuperscript{40} we acidified the cell culture medium using HCl and stimulated the cells with 10 ng/mL of LPS (\textit{E. coli} 0111:B4) for 24 hours. Acidic medium itself barely affected the release of inflammatory mediators, including NO, IL-6 and IL-10. However, compared with a pH 7.4, acidosis (pH 7.0) was associated with a significantly increased NO release in response to LPS stimulation. Interestingly, under more extreme acidic conditions (pH 6.5), NO release decreased in response to LPS and was again similar to pH 7.4 (Table 1). At pH 6.5 both IL-6 and IL-10 release was significantly reduced compared with either pH 7.0 or 7.4. However, IL-10 release was reduced far greater than IL-6 and thus the ratio of IL-6 to IL-10 increased significantly from 5.1 at pH 7.4 to 55.1 at pH 6.5. These results suggest a pro-inflammatory effect of HCl, consistent with the existing literature on the effects of HCl on TNF synthesis.\textsuperscript{38,30,31}

**Table 1. Affects of lactic acid vs. HCl on LPS-stimulated RAW 264.7 cells**

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Adapted from Kellum et al.\textsuperscript{40}

To clarify the mechanism by which HCl influenced the release of cytokines from LPS-stimulated cells, we measured NF-κB DNA binding using electrophoretic mobility shift assay (EMSA) after exposure to different concentrations of HCl.\textsuperscript{40} Again, acidosis (pH 7.0) significantly increased LPS-induced NF-κB activation, compared with a pH 7.4, whereas more extreme acidosis (pH 6.5) actually attenuated NF-κB activation. Thus, different degrees of hyperchloreaemic acidosis have differing effects on inflammatory mediator release as well as NF-κB activation. Overall, the effects of HCl appear to be pro-inflammatory. These results agree with studies in resident peritoneal macrophages in which Bellocq and colleagues found that these cells produced more NO when incubated in medium at pH 7.0 compared to pH 7.4 and this effect was associated with up-regulation of iNOS mRNA as well as the activation of NF-κB.\textsuperscript{28}

By contrast, our data using lactic acid demonstrates that this acid is anti-inflammatory to RAW 264.7 cells as assessed by decreased cytokine expression and NF-κB activation.\textsuperscript{40} In these experiments increasing concentrations of lactic acid (0 - 30 mM) caused increasing acidification of the media; and trypan blue exclusion and LDH release demonstrated that lactic acid did not reduce cell viability. However, lactic acid inhibited LPS-induced NF-κB DNA binding. Lactic acid also significantly decreased LPS-induced NO, IL-6, and IL-10 expression, both RNA and protein, in a dose-dependent manner. These effects may be partially mediated through NFκB since DNA-binding of this transcription factor is generally consistent with the effects on NO and IL-6 (table 1). However, extracellular acids also have effects on IL-10, which is outside the
NFκB pathway. What is apparent is that the effects of extracellular acids are not limited to the effects on pH since different acids produce different effects despite similar pH. Whether different effects can be explained by differences in pH are, as yet, unknown, although the patterns of response (table 1) do not make this likely.

Management of metabolic acidosis

Despite the association between metabolic acidosis and adverse clinical outcomes and evidence of injury from metabolic acidosis in animals, clinical evidence that treating metabolic acidosis is beneficial has not been forthcoming. In their systematic review, Forsythe and Schmidt could not find evidence to support the routine use of sodium bicarbonate (NaHCO₃) in the treatment of lactic acidosis. A similar absence of evidence has characterised the literature on diabetic ketoacidosis where no proof exists that treating acidosis improves clinical outcomes and prevailing opinion is against treatment, at least when the arterial pH is > 7.0. However, many of the patients in studies of ketoacidosis have been young, otherwise healthy patients with type I diabetes mellitus and such patients may bare little resemblance to patients with sepsis. Furthermore, low-level ketosis is a physiologic condition and one might envision that endogenous defense mechanisms have evolved for this kind of acidosis. In a similar way, even severe lactic acidosis commonly occurs as a result exercise and it would seem unlikely that short-term lactic acidosis would be harmful. However these conditions may be quite distinct from hypercholaemia or organic acidoses of renal failure or even prolonged lactic acidosis. Indeed, chronic acidemia, even when mild (pH < 7.35), may produce a variety of adverse effects including metabolic bone disease and protein catabolism.

There has been a long-standing debate in the literature over whether administration of sodium bicarbonate can help reverse shock, possibly through improving the function of exogenous and endogenous catecholamines. This debate has not been resolved but the weight of existing evidence does not support the use of sodium bicarbonate. Animal experiments using hypoxic lactic acidosis have yielded conflicting results. Two clinical trials have been conducted to determine the effectiveness of NaHCO₃ therapy in reversing acidosis and improving haemodynamics in patients with lactic acidosis. The results of both studies were the same; NaHCO₃ neither improved nor worsened systemic haemodynamics despite improving arterial pH. There was also no evidence that NaHCO₃ treatment worsened tissue hypoxia. Adding to this debate is the evidence that metabolic acidosis might produce beneficial effects on oxygen delivery or metabolism and that correcting it might therefore be harmful. Indeed some authors have argued that some forms of acidosis such as respiratory or lactic acidosis may even have beneficial effects. In support of this point of view are data from experimental models of ischemia/reperfusion injury in animals suggesting that correcting respiratory acidosis with NaHCO₃ may worsen inflammation and organ injury. Although experimental evidence is emerging to suggest an effect of acidosis on the immune response, it has been difficult to establish a clear link between metabolic acidosis in humans and alterations in immune function. Data from clinical studies have been extremely limited and confounded by numerous other physiologic and pharmacologic variables known influence the immune response. For example studies of ketoacidosis fail to disentangle the effects of glucose and insulin. Similarly, patients with chronic renal failure are also exposed to the effects of uremic toxins, nutritional deficiencies, plasma volume and tonicity changes and treatments such as haemodialysis. Finally, lactic acidosis, while being a common clinical condition, frequently occurs as a result of other “immunologically” active conditions such as shock and sepsis. Even exercise presumably has effects on the immune response mediated by catecholamines and perhaps other hormonal responses. Clinical studies of acid infusion (e.g. HCl) have not generally focused on the immune response and therefore it is difficult know what effects correcting hypercholaemic acidosis would produce. Thus, it is impossible to discern from the existing clinical or basic science literature whether acidosis should be treated and if so under what conditions and using which therapies. This lack of evidence has lead to ambiguity in clinical recommendations in textbooks and review articles and further highlights the need for basic research in this area.

Conclusion

Understanding the effects of acid-base on the inflammatory response is highly relevant to clinical medicine for a variety of reasons. First, current deficiencies in our understanding of the effects of acidosis on a wide range of cellular processes have lead to controversy in way in which patients are managed in a variety of clinical settings. Most clinicians tend to ignore the effects of exogenous CI on blood pH, yet many will treat even mild forms of acidemia. In addition, all forms of metabolic acidosis appear to be associated with prolonged hospital and ICU length of stay. Since metabolic acidosis is both commonly caused and treated by clinicians, understanding of the physiologic consequences of altered blood pH is imperative.

Second, our ability to alter acid-base balance as a
tool to manipulate cellular processes is dependent on an improved understanding of the relationship between blood pH and the synthesis and release of inflammatory molecules. Investigators continue to seek for means to modulate the inflammatory response as primary therapy for sepsis and related conditions. These efforts have been focused not only on reducing pro-inflammatory mediators in an effort to reduce tissue injury but also on the converse, augmenting the inflammatory response to infection. These interests also extend into other fields including autoimmune disease and cancer therapy.

Third, even when it is not practical or desirable to manipulate blood pH as a primary means of altering the inflammatory response, an understanding of how pH affects this response is necessary to 1) interpret data from studies of immunomodulation; 2) avoid unintended immunomodulation in clinical and laboratory settings; 3) explore the capacity of pH to improve the effectiveness of existing treatments. Finally understanding how blood pH is involved in the regulation of inflammation by intracellular signaling pathways or other mechanisms might ultimately lead to other strategies for immunomodulation.

REFERENCES