Effects of Hyperchloremic Acidosis on Arterial Pressure and Circulating Inflammatory Molecules in Experimental Sepsis

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Study objective: To determine the effects of hyperchloremic acidosis, induced by dilute HCl infusion, on BP and circulating inflammatory mediators in an experimental model of severe sepsis in the rat.

Design: Randomized, open-label, controlled experiment.

Setting: University research laboratory.

Participants: Twenty-four adult, male, Sprague-Dawley rats.

Intervention: Eighteen hours after inducing lethal sepsis by cecal ligation and puncture, animals were randomized and classified into three groups. In groups 2 and 3, we began an IV infusion of 0.1 N HCl to reduce the standard base excess (SBE) by 5 to 10 mEq/L and 10 to 15 mEq/L, respectively. In group 1, we infused a similar volume of lactated Ringer solution. In all groups, infusions were continued for 8 h or until the animals died.

Measurements: We measured mean arterial pressure (MAP), arterial blood gases, electrolytes, plasma nitrate/nitrite, tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-10 levels at 0 h, 3 h, 6 h, and 8 h.

Results: MAP remained stable in group 1 but decreased in groups 2 and 3 (p < 0.001), such that at 8 h MAP was much higher in group 1 (94 ± 9.2 mm Hg) [± SD] compared to either group 2 (71.6 ± 20.1 mm Hg) or group 3 (49.4 ± 33.2 mm Hg) [p = 0.01]. This change in 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 h of acidosis, plasma nitrite levels were significantly higher in group 2 animals compared to either group 1 or group 3 animals (p < 0.05). Plasma TNF-α, IL-6, or IL-10 levels were not significantly different from control animals.

Conclusion: Moderate acidosis (SBE of 5 to 10 mEq/L), induced by HCl infusion, worsened BP and increased plasma nitrate/nitrite levels but had no effect on circulating cytokines in septic rats. However, severe acidosis (SBE of 10 to 15 mEq/L), while still causing hypotension, did not affect plasma nitrate/nitrite levels.

Key words: acid-base balance; chloride; fluid resuscitation; metabolic acidosis; nitric oxide; saline; sepsis; septic shock

Abbreviations: CLP = cecal ligation and puncture; ELISA = enzyme-linked immunosorbent assay; IL = interleukin; iNOS = inducible nitric oxide synthase; MAP = mean arterial pressure; NO = nitric oxide; SBE = standard base excess; SID = strong ion difference; SIDa = strong ion difference apparent; TNF = tumor necrosis factor

Hyperchloremic metabolic acidosis is a common feature of sepsis. Often the source of the acidosis is at least partly iatrogenic, because saline solution resuscitation is often used to treat shock. Large-volume saline solution infusion produces metabolic acidosis by increasing the plasma chloride concentration relative to the plasma sodium concentration.1–6 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 hydrogen ions to pre-

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Support for this project was provided, in part, by a grant from Abbott Laboratories and by the Laerdal Foundation for Acute Medicine.

Manuscript received March 19, 2003; revision accepted June 19, 2003.

www.chestjournal.org

CHEST / 125/1 / JANUARY, 2004

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serve electrical neutrality. However, the clinical consequences of metabolic acidosis are not well understood. In a recent study, we found that 0.9% saline solution resuscitation resulted in a decreased survival time and reduced the standard base excess (SBE) by 5 to 10 mEq/L compared to a balanced colloid solution. Although survival time was inversely correlated with the increase in plasma chloride, the effects of hyperchloremic acidosis itself could not be assessed directly.

Metabolic acidosis might reduce survival from sepsis through a variety of mechanisms. First, acidosis has been associated with hemodynamic instability, though the association is not always consistent and the mechanisms uncertain. Pedoto and colleagues have shown that metabolic acidosis may increase inducible nitric oxide synthase (iNOS), and this may lead to vasodilation and shock. Second, acidosis might lead to an alteration in the expression of inflammatory cytokines. Jensen and colleagues exposed macrophages to lactic acid (to a pH of 6.75) and found that tumor necrosis factor (TNF-α) secretion was increased secondary to increased gene transcription. In addition, in the intact organism, acidosis might lead to changes in the inflammatory response through its effect on catecholamine synthesis. Conversely, metabolic acidosis might produce beneficial effects on oxygen delivery or metabolism, and reversing metabolic acidosis with sodium bicarbonate has not been shown to be beneficial in most studies. Thus, we sought to determine the effects of hyperchloremic acidosis on BP and on circulating inflammatory mediators.

**Materials and Methods**

**Surgical Preparation**

Following approval by the Animal Care and Use Committee of the University of Pittsburgh Medical Center, we anesthetized 24 adult, male, Sprague-Dawley rats with pentobarbital sodium (40 mg/kg intraperitoneal). We performed a midline laparotomy, exteriorized the cecum, and placed a ligature inferior to the ileocecal valve using 4–0 silk. We then punctured the cecum three times using a sterile 18-gauge needle, placing one puncture site on each of the three antimesenteric surfaces. We returned the cecum to the abdominal cavity, and closed with a 2–0 suture. We then administered a subcutaneous bolus of saline solution (50 mL/kg) as fluid resuscitation and returned the animals to their cages and allowed food and water *ad libitum*. Eighteen hours after cecal ligation and puncture (CLP), we re-anesthetized animals and intubated them with a beveled 16-gauge angiocatheter and ventilated them with room air using a rodent ventilator (Harvard Apparatus; Holliston, MA) at a tidal volume of 10 mL/kg and a frequency sufficient to maintain an arterial PCO₂ between 35 mm Hg and 45 mm Hg. We isolated the right carotid artery and the left femoral vein by dissection and cannulated each with 1.27-mm polyethylene-90 tubing. The tubing was formed into a catheter by inserting a beveled 20-gauge needle into one end. The femoral vein catheter was advanced into the inferior vena cava. We flushed each cannula with 1 mL of heparinized saline solution (2,000 U/L). We placed a three-way stopcock on each catheter so that blood sampling could be achieved using the carotid arterial catheter and the venous catheter could be used for fluid administration.

**Experimental Protocol**

Before the administration of HCl or lactated Ringer solution, we maintained the animals in a steady state as defined by stable BP, and arterial blood gas values for at least 30 min. Arterial pressure was measured continuously and recorded in real-time on a strip-chart recorder (Gould; Cleveland, OH). Eighteen hours after CLP, we randomized and classified the animals into three groups. In groups 2 and 3, we began an IV infusion of 0.1 N HCl via the femoral vein, to reduce the SBE by 5 to 10 mEq/L and 10 to 15 mEq/L, respectively. We sampled the arterial blood for blood gas measurements (0.2- mL samples) every 1 to 2 h as dictated by the variation in arterial pH between sampling. We adjusted the rate of HCl infusion as needed to achieve the target SBE. In group 1, we infused a similar volume of lactated Ringer solution. In all groups, infusions were continued for 8 h or until the animals died. We administered additional pentobarbital sodium (5 mg/kg intraperitoneal) when needed to maintain anesthesia for the entire protocol.

**Measurements and Calculations**

Mean arterial pressure (MAP) was recorded continuously. Blood work including blood gas analysis, whole blood lactate, and electrolytes necessary to calculate SID, plasma nitrate/nitrite, TNF-α, interleukin (IL)-6, and IL-10 levels, were obtained at baseline (time 0) and at 3 h, 6 h, and 8 h. SBE was calculated as previously described. The apparent SID (SIDa) was calculated from arterial plasma as follows:

\[
\text{SIDa} = \text{Na}^+ + \text{K}^+ + \text{Mg}^{2+} + \text{Ca}^{2+} - \text{Cl}^- - \text{lactate}
\]

where all are expressed in milliequivalents per liter.

**NO⁻ Production Assay**

Total nitrite was measured using cadmium-mediated reduction of NO₃⁻ to NO₂⁻ followed by the Griess reagent. To reduce NO₃⁻ to NO₂⁻ in plasma, cadmium filings (0.4 to 0.7 g per tube; Fluka Chemicals; Milwaukee, WI) were loaded into 1.5-mL microcentrifuge tubes. The filings were washed twice with 1.0 mL of deionized water, twice with 1.0 mL of 0.1 mol/L HCl, and twice with 0.1 mol/L NH₄OH. Ten microliters of 30% (weight/volume) ZnSO₄ was added to 200 μL of culture supernatant or plasma, vortexed, incubated at room temperature for 15 min, and centrifuged at 14,000g for 5 min. The resulting supernatants were added to the cadmium-containing microcentrifuge tube and incubated at room temperature overnight with constant mixing. The samples were transferred to fresh microcentrifuge tubes and centrifuged again. The samples were subsequently measured for NO₂⁻ content by the Griess reagent. The plates were read using a MRX microplate reader (Dynex Technologies; Chantilly, VA) at 550 nm. NaNO₂ was used to generate a standard curve.

**Cytokine Enzyme-Linked Immunosorbent Assays**

The cytokine enzyme-linked immunosorbent assays (ELISAs) were performed as recommended by the manufacturer. ELISA
kits for TNF-α, IL-6, and IL-10 were obtained from R&D Systems (Minneapolis, MN). The rat TNF-α ELISA measured levels of TNF-α < 5 pg/mL. A reference curve was obtained by plotting the TNF-α concentration of several dilutions of standard protein vs absorbance. The other ELISA kits were capable of measuring levels of < 5 pg/mL and < 10 pg/mL for IL-6 and IL-10, respectively.

Statistical Analysis

Our primary analysis was between the two experimental groups (HCl) and the control (lactated Ringer) group and was based on the MAP for surviving animals over time. Secondary analyses included plasma nitrate/nitrite and cytokines. Mean differences between and within groups were analyzed by analysis of variance adjusted for multiple comparisons (Student-Newman-Keuls) for all pairwise comparisons. Associations were analyzed using the Pearson correlation coefficient. A power calculation was based on an 80% power to detect a 30% difference in MAP. A sample size of 24 was sufficient to achieve this power. Error values given in the text are SDs. Statistical analysis was performed using MedCalc (version 4.2; Mariakerke, Belgium) and Stata (version 6.0; College Station, TX) software; \( p < 0.05 \) was considered statistically significant.

Results

Two animals died before completing the 8-h protocol, one in group 1 and the other in group 3; both died just prior to 7 h. We administered a mean volume of 0.1 N HCl of 10.2 mL to group 2 animals and 13.1 mL to group 3 animals; control animals received 9.1 mL of lactated Ringer solution (\( p = \text{not significant} \)).

Acid-base variables at each time point are summarized in Table 1. After 8 h, SBE was significantly lower in HCl-treated animals (-10.0 ± 3.4 and -18.8 ± 4.6 for groups 2 and 3, respectively) compared to control animals (-3.0 ± 1.5) [\( p < 0.0001 \) ]. MAP among the three groups over time is shown in Figure 1. There was a dose-dependent effect of hyperchloremic acidosis on MAP. While MAP was stable in group 1 animals, group 2 had a significant decrease in MAP by 6 h (\( p < 0.001 \)), and group 3 animals were significantly more hypotensive by 3 h (\( p < 0.001 \)). At 8 h, MAP was significantly higher in group 1 (94 ± 9.2 mm Hg) compared to either group 2 (71.6 ± 20.1 mm Hg) or group 3 (49.4 ± 33.2 mm Hg) [\( p = 0.01 \)].

Plasma TNF-α, IL-6, or IL-10 levels were not significantly different in either acidosis group compared to control animals. Acidosis appears to have increased the variability of cytokine response without significantly altering the overall pattern. However, unlike the cytokine response, plasma nitrite levels were significantly different in group 2 animals. Higher levels were seen in group 2 animals (26.4 ± 9.1 μmol/L) compared to either group 1 (19.3 ± 5.6 μmol/L) or group 3 animals (14.5 ± 3.9 μmol/L) [\( p < 0.05 \); Fig 2].

The decrease in MAP seen in our study was associated with the decrease in pH (\( R^2 = 0.24, p < 0.001 \)). MAP weakly correlated with nitrite levels (\( R^2 = 0.09, p = 0.01 \)), and this correlation was strengthened by excluding samples with pH < 7.0 (\( R^2 = 0.17, p < 0.001 \)). However, change in MAP correlated best with the increase in plasma Cl⁻ (\( R^2 = 0.50, p < 0.0001 \)) [Fig 3].

Discussion

The primary finding of our study is that hyperchloremic acidosis, induced by HCl infusion, significantly

### Table 1—Acid-Base Variables for Each Group, by Time*

<table>
<thead>
<tr>
<th>Group/Time, h</th>
<th>pH</th>
<th>PCO₂</th>
<th>Cl⁻</th>
<th>Lactate</th>
<th>SBE</th>
<th>SIDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>7.43 ± 0.05</td>
<td>36.7 ± 4.7</td>
<td>106.6 ± 1.7</td>
<td>1.7 ± 0.7</td>
<td>0.3 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7.35 ± 0.12</td>
<td>40.6 ± 13.1</td>
<td>109.0 ± 4.8</td>
<td>4.0 ± 2.7†</td>
<td>-3.5 ± 5.0†</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>7.37 ± 0.1</td>
<td>41.6 ± 12.6</td>
<td>110.7 ± 3.6†</td>
<td>2.6 ± 1.2</td>
<td>-2.1 ± 1.9†</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>7.33 ± 0.04†</td>
<td>43.0 ± 8.4</td>
<td>113.3 ± 2.1†</td>
<td>1.9 ± 0.8</td>
<td>-3.0 ± 1.5†</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>7.44 ± 0.09</td>
<td>35.6 ± 8.7</td>
<td>108.0 ± 2.6</td>
<td>2.1 ± 0.7</td>
<td>1.2 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7.33 ± 0.05†</td>
<td>40.8 ± 6.8</td>
<td>113.0 ± 1.1†</td>
<td>2.4 ± 0.8</td>
<td>-4.0 ± 1.8†</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>7.29 ± 0.04†</td>
<td>37.7 ± 8.3</td>
<td>118.4 ± 5.8†</td>
<td>2.8 ± 1.4</td>
<td>-8.1 ± 3.8†</td>
</tr>
<tr>
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<td>8</td>
<td>7.28 ± 0.07†</td>
<td>33.8 ± 5.3</td>
<td>117.9 ± 2.4†</td>
<td>3.8 ± 2.3</td>
<td>-10.0 ± 3.4†</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>7.41 ± 0.09</td>
<td>42.3 ± 10.2</td>
<td>106.9 ± 2.8</td>
<td>1.8 ± 1.1</td>
<td>1.8 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7.33 ± 0.07†</td>
<td>38.3 ± 8.3</td>
<td>113.0 ± 3.9†</td>
<td>2.6 ± 1.0</td>
<td>-5.3 ± 2.2†</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>7.24 ± 0.05†</td>
<td>34.2 ± 10.0</td>
<td>118.5 ± 7.1†</td>
<td>3.3 ± 1.6</td>
<td>-11.9 ± 3.0†</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>7.03 ± 0.19†</td>
<td>38.1 ± 8.4</td>
<td>122.1 ± 7.4†</td>
<td>5.4 ± 3.0</td>
<td>-18.8 ± 4.6†</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SD.
†\( p < 0.05 \) vs baseline within each group.
‡\( p < 0.05 \) vs control.
reduced the MAP in normotensive, septic animals. Moderate acidosis (decrease in arterial SBE by 5 to 10 mEq/L) was associated with increased plasma nitrate/nitrite levels, but, surprisingly, more severe acidosis (decrease in arterial SBE by 10 to 15 mEq/L) was associated with changes in nitrate/nitrite levels in response to sepsis that closely resembled that of nonacidemic control animals. Our results are in agreement with a recent report by Pedoto and coworkers,\(^1\), who demonstrated that metabolic acidosis increased iNOS leading to vasodilation and shock in healthy rats. Our study extends these findings by examining the effects of acidosis in nonshock, septic animals and by exploring more severe acidosis. By using rat peritoneal macrophages, Bellocq and colleagues\(^2\) found that these cells produced more nitrite when incubated in medium at pH 7.0 than that at pH 7.4, and this effect was associated with up-regulation of iNOS messenger RNA as well as the activation of nuclear factor-κB. We have recently obtained similar results over this pH range using murine alveolar macrophages. However, we also observed that severe acidosis (pH 6.5) did not enhance nitrite production.\(^3\) This paradox has not been satisfactorily explained. However, some authors suggest that the optimal pH for iNOS is near 7.0. Intracellular pH is normally around 7.3; therefore, addition of acid would lower intracellular pH toward the optimal value, thus increasing iNOS activity and nitric oxide (NO) production. Further addition of acid would cause intracellular pH to fall below the optimal value leading to decrease in NO production.\(^1\) Additional studies are necessary to test this hypothesis.

Our results are relevant to the clinical arena for a number of reasons. First, solutions that contain superphysiologic concentrations of chloride (i.e., 0.9% saline solution) are routinely used for volume resuscitation in patients with sepsis. When administered rapidly and in large quantities, these solutions induce hyperchloremic metabolic acidosis,\(^2\)–\(^6\) the magnitude of which is similar to the targets we chose in this investigation.\(^8\) Although the chemical mechanisms for these effects have been well described,\(^1\),\(^7\),\(^24\) there is still controversy as to whether these effects are deleterious to patients.\(^15\),\(^25\) However, iatrogenic acidosis that occurs from saline solution resuscitation appears to be harmful.\(^8\),\(^26\)

In our previous study using an endotoxic shock model in the rat, we demonstrated that saline solution resuscitation was associated with a significantly shorter survival time compared to a more physiologic fluid containing starch in a balanced electrolyte solution.\(^8\) Furthermore, survival time was correlated with the decrease in pH and negatively correlated with the increase in serum Cl\(^--\) following initial resuscitation. The decrease in pH appeared to have

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**Figure 1.** MAP for each group (group mean ± SE) over time is shown. Solid line and diamonds correspond to group 1 (control), dotted line and squares correspond to group 2 (SBE of −5 to −10 mEq/L), and broken line and triangles correspond to group 3 (SBE of −10 to −15 mEq/L). \(*\) Differences between groups were significant at 8 h (p < 0.05 by analysis of variance). MAP did not change in group 1, while group 2 was significantly different from baseline after 6 h and group 3 was significantly different after 3 h.

**Figure 2.** Plasma nitrite concentrations for each group over time. Group 1 animals are shown in hatched bars, group 2 (SBE of −5 to −10 mEq/L) corresponds to black bars, and empty bars represent group 3 (SBE of −10 to −15 mEq/L). \(*\) p < 0.05 between groups.

**Figure 3.** Relationship between the change in MAP and the change in plasma chloride concentration compared to baseline (R\(^2\) = 0.50, p < 0.0001).
been brought on by changes in Cl\textsuperscript{−}, lactate, and PaCO\textsubscript{2}. However, lactate values were not different between groups, and changes in PaCO\textsubscript{2} were not correlated with survival time. Thus, hyperchloremic acidosis, rather than acidosis in general, was strongly associated with early mortality in these endotoxemic animals. Indeed some authors have argued that respiratory\textsuperscript{27} and lactic\textsuperscript{14} acidosis may have less harmful, even beneficial effects.

Pedoto and colleagues have demonstrated that hyperchloremic acidosis increases lung\textsuperscript{11} and intestinal injury\textsuperscript{23} in healthy rats. Comparing starch preparations in saline solution vs balanced electrolyte solution for elderly GI surgery patients, Wilkes and coworkers\textsuperscript{26} recently described worse acid-base balance and more adverse events with the saline-based fluid. Similar findings have been reported with 0.9% saline solution compared to lactated Ringer solution.\textsuperscript{28} Indeed, the side effect profile of saline solution-induced acidosis, nausea, vomiting, abdominal pain, headache, thirst, hyperventilation, and delayed urination is identical to what occurs with ammonium chloride administration.\textsuperscript{29,30} Thus, while there is little evidence that treating metabolic acidosis improves clinical outcomes,\textsuperscript{10,15} there is mounting evidence that iatrogenic metabolic acidosis is harmful.

The present study was designed to explore the mechanisms whereby short-term survival might be reduced by hyperchloremic acidosis. Specifically, we sought to test the hypothesis that hyperchloremic acidosis induced by HCl infusion would result in hypotension and increased NO and inflammatory cytokine release in an animal model of CLP-induced sepsis. Our results indicate that a more complex relationship between acidosis, hypotension, and inflammation is likely. Acidosis caused a decrease BP in a dose-dependent fashion. The effect of metabolic acidosis on hemodynamics is varied and complex. Acidemia has been shown to stimulate vasopressin, adrenocorticotrophic hormone, and aldosterone in experimental animal models\textsuperscript{31–33} and may therefore increase MAP. However, in agreement with previous studies,\textsuperscript{11,21} we found instead that acidosis resulted in hypotension. NO release may have contributed to hypotension during moderate acidosis. However, NO release does not appear to be the mechanism responsible for hypotension during more severe acidosis. We have seen this same pattern of NO release in our studies of RAW cells incubated at various pH and stimulated with lipopolysaccharide.\textsuperscript{23} These results are consistent with the work of Bellocq et al.,\textsuperscript{22} although the range of acidosis studied was narrower in their investigation. It is possible that the differential effects of acidosis on NO release may in part explain the disparate findings in the literature regarding the effects of acidosis and the results of its treatment.\textsuperscript{15} For example, if NO release is increased as the pH improves from severe to moderate acidosis, hypotension could be worsened. Regrettably, our methods do not permit us to test this hypothesis directly.

Our study has several other limitations. First, our study does not address the issue that some of the deleterious effects could be from hyperchloremia per se (without acidosis). Given our study design, it is difficult to delineate the effects of acidosis vs hyperchloremia. Indeed, it is clear that not all acidosis is the same and, for example, respiratory acidosis may have completely different effects compared to metabolic acidosis.\textsuperscript{27} It is entirely possible that different types of metabolic acidosis also have different effects. Second, our study does not address the physiologic mechanisms responsible for the decrease in MAP seen with hyperchloremic acidosis (ie, decreased cardiac output vs decreased systemic vascular resistance). Our finding that NO was increased was only in the moderate acidosis group, yet severely acidic animals were most hypotensive. Importantly, the targeted SBE was reached later in group 3 than in other two groups, and also the SBE at 3 h was not different between groups 2 and 3. Finally, despite evidence suggesting a plausible link between acidosis and inflammatory mediator release,\textsuperscript{12,13} we could not demonstrate a consistent relationship between these variables. This may have been due, in part, to the confounding effects of a variety stimuli on cytokine release.\textsuperscript{34} For example, hypotension itself is known to influence cytokine release.\textsuperscript{35} Thus, experiments using isolated inflammatory cells, rather than intact animals, may be better suited to explore this relationship.

**CONCLUSION**

Hyperchloremic metabolic acidosis worsens hemodynamic variables in this animal model of CLP-induced sepsis. The mechanism(s) responsible for hypotension in this setting are unclear. However, they are likely complex and may involve increased NO release when acidosis is moderate and other mechanisms when more severe.

ACKNOWLEDGMENT: The authors thank Jeff Schmigel, BS, for technical assistance.

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