Preretinal Neovascularization Associated with Acetazolamide-Induced Systemic Acidosis in the Neonatal Rat

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PURPOSE. NH₄Cl gavage in the neonatal rat produces a metabolic acidosis-induced retinopathy which serves as a model for retinopathy of prematurity (ROP). Acetazolamide induces a metabolic acidosis via an alternative biochemical mechanism (bicarbonate loss versus hydrogen ion load). In the present study, the following hypothesis was tested: acetazolamide-induced acidosis is associated with preretinal neovascularization in the neonatal rat.

METHODS. All studies used newborn Sprague-Dawley rats raised in expanded litters of 25. Arterial blood pH was measured to determine the level of acidosis induced by intraperitoneal (IP) acetazolamide (50 or 200 mg/kg) or saline. In a separate retinopathy study, newborn rats (n = 75) were randomized to either IP acetazolamide, 50 mg/kg (low-dose), or IP saline twice daily from days 2 to 7. After 5 days of recovery, retinal vasculature was assessed using ADPase staining and light microscopy. The presence and severity (clock hours) of neovascularization were assessed by three masked observers. In an additional retinopathy study, newborn rats (n = 100) were randomized to either IP acetazolamide, 200 mg/kg (high-dose), or IP saline twice daily from days 2 to 7. After 5 days of recovery, the retinas were similarly analyzed.

RESULTS. Neovascularization occurred in 59% of rats receiving high-dose acetazolamide (200 mg/kg). High-dose acetazolamide produced a severe acidosis (pH 7.13 ± 0.06) during drug delivery. Low-dose acetazolamide (50 mg/kg) produced a pH (7.22 ± 0.07) that was intermediate between high-dose (200 mg/kg) acetazolamide (p < 0.001) and saline controls (7.42 ± 0.06, p < 0.001); however, neither low-dose acetazolamide nor saline induced preretinal neovascularization.

CONCLUSIONS. Acidosis induced by high-dose acetazolamide, independent of hyperoxemia or hypoxemia, is associated with preretinal neovascularization in the neonatal rat. Induction of neovascularization appears to depend on a critical threshold of acidosis severity. This study further supports a proposed independent role for acidosis in the pathogenesis of ROP. (Invest Ophthalmol Vis Sci. 2001;42:1066–1071)

Retropective clinical surveys and laboratory studies suggest that acidosis may be an independent risk factor in the development of retinopathy of prematurity (ROP) in human neonates. We have previously reported that systemic acidosis is associated with preretinal neovascularization in the neonatal rat, regardless of whether the origin of the systemic acidosis was excess CO₂ (i.e., a respiratory acidosis) or gavage with NH₄Cl (i.e., a metabolic acidosis due to a hydrogen ion donor). This acidosis-induced retinopathy is independent of hyperoxemia or hypoxemia. Because the concept of a purely acidosis-induced retinopathy in neonatal animals is somewhat novel, we designed a further study to determine whether or not an alternative acidosis-inducing agent would cause preretinal neovascularization in immature animals.

Acetazolamide, a carbonic anhydrase inhibitor, is known to induce systemic acidosis in both humans and animals. The biochemical mechanism of acetazolamide-induced acidosis differs from that of NH₄Cl-induced acidosis. Acetazolamide inhibits carbonic anhydrase and thereby increases the loss of bicarbonate from the kidney. In contrast, NH₄Cl ingestion produces a hydrogen ion load.

In the present study, we tested the hypothesis that acetazolamide-induced acidosis is associated with preretinal neovascularization in the neonatal rat.

METHODS

All experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Institutional Animal Care and Use Committee at our institution.

Animals

All studies used newborn Sprague-Dawley rats raised in expanded litters of 25. Pups were randomly assigned to a mother within 24 hours of birth. Expanded litters were used so that results from this study could be compared with results of previous studies in our laboratory, that is, oxygen-induced retinopathy (OIR), carbon dioxide-induced retinopathy (CDIR), and metabolic acidosis-induced retinopathy (MAIR). Mothers and litters received standard laboratory diet and water ad libitum. Light was cycled on a 12-hour-on, 12-hour-off schedule, and the room temperature was maintained at approximately 21°C.

Arterial Blood pH and Blood Gas Study

We obtained carotid arterial blood gas samples in a preliminary study to determine the level of arterial blood pH and arterial blood gases (i.e., PaO₂ and PaCO₂) induced by our study drugs. Rats were given either low-dose intraperitoneal (IP) acetazolamide (50 mg/kg), high-dose IP acetazolamide (200 mg/kg), IP saline, or gavaged NH₄Cl (10 mM/kg). Although NH₄Cl was not studied in the subsequent retinopathy experiments, arterial blood pH data were needed to compare levels with our previous study.

Drug or saline was delivered twice daily from day 2 to day 7 of life. Blood gases were drawn at days 2, 3, 4, 5, 7, 8, and 10. Separate litters
were used for blood samples drawn a day apart (i.e., day 2 and 3, day 4 and 5, day 7 and 8, and day 10). This was done to minimize the effect of the change of litter size on animal growth.\(^1\) If only a few animals were taken each day, the last animals would have been disproportionately large and not representative of those in the retinopathy studies. The number of rats for each drug and time point ranged from 4 to 12, totaling 172 rats.

Simultaneous blood gas sampling and analysis for retinopathy was not performed because obtaining a carotid blood sample is a terminal event; survival to the conclusion of the retinopathy study (day 13) was not possible if a blood gas sample had been obtained at an earlier time point.

### Blood Gas Sampling Method

We modified the blood gas sampling method of Berkowitz.\(^10\) All pups underwent urethane IP injection (1.5 g/kg) anesthesia while the animals breathed room air. Body temperature was preserved using a warming pad set at 39°C (Deltaphase isothermal pad; Braintree Scientific, Braintree, MA). Previous pilot studies in our laboratory have shown that this maintains the rectal temperature at 35.7 ± 0.1°C, which is normal for age.\(^11\) The left carotid artery was exposed through a skin incision, and a small drop of heparin (100 USP units/ml) was shown that this maintains the rectal temperature at 35.7 ± 0.1°C (Deltaphase isothermal pad; Braintree Scientific, Braintree, MA). Previous pilot studies in our laboratory have shown that this maintains the rectal temperature at 35.7 ± 0.1°C, which is normal for age.\(^11\) The left carotid artery was exposed through a skin incision, and a small drop of heparin (100 USP units/ml) was placed in the incisional area. A small incision was made in the carotid artery, and 50 µl of arterial blood was collected in a heparinized microhematocrit capillary tube (Fisher Scientific, Pittsburgh, PA) and analyzed immediately using a blood gas analyzer (System 1306; Instrumentation Laboratory, Lexington, MA). Animals were killed after blood gas sampling.

### Retinopathy Study

In the retinopathy study, rats in 3 expanded litters of 25 were randomized, within each litter, to either IP injected acetazolamide (50 mg/kg; low-dose, \(n = 38\)) or saline (\(n = 57\)). In a separate study, rats in 4 litters of 25 rats, were randomized, within each litter, to receive either high-dose acetazolamide (200 mg/kg; \(n = 50\)) or saline (\(n = 50\)). We chose the 50-mg/kg dose of acetazolamide because this dose had been used by others\(^7\) to induce systemic acidosis in adult rats. We chose an alternative dose of 200 mg/kg, based on blood gas data presented below, to more closely match the pH of NH\(_4\)Cl-treated rats of a previous study.\(^5\)

In the present retinopathy study, acetazolamide or saline was delivered twice daily from days 2 to 7 of life. Rats were allowed to recover for 5 days. The timing of drug delivery and length of recovery were chosen to parallel our previous studies of OIR, CDIR, and MAIR,\(^3-5\) where a 7-day period of exposure to drug or gas was followed by a 5-day recovery period. It was not the purpose of the present study to explore timing issues.

On day 13 of life, all animals were killed by a lethal intramuscular injection of ketamine (160 mg/kg) and xylazine (50 mg/kg). The left eye retinal vasculature was assessed using ADPase staining and light microscopy.\(^12\) ADPase-stained retinas were graded for neovascularization (clock hours) in a masked manner by three independent observers using a standard method that has been validated in our laboratory.\(^13\) Briefly, neovascularization was defined as abnormal vascular structures arising from the normal vasculature at the junction of the vascular and avascular retina. Retinas were scored for the severity of neovascularization by counting the number of clock hours containing neovascularization.\(^3-5,13\) The final score for each retina was defined as the median score for three observers.\(^5,3-5,13\) Cross-sectional retinal histology was not performed, because scoring of abnormal neovascularization in flat-mounted ADPase-stained retinas\(^12\) has recently been validated in our laboratory for the rat,\(^13\) using serial cross sections as the previous 'gold-standard.'

### Statistical Analysis

In the blood gas study, we analyzed the data from days 2 to 8 (the period of drug delivery) using two-factor ANOVA methods (drug and time) to compare blood pH, \(\text{PaCO}_2\), \(\text{PaO}_2\), and \(\text{HCO}_3^-\) among groups. Where differences were found between drugs, individual \(t\) tests comparing means were performed with Bonferroni corrections for multiple comparisons. A mean value for each study group (high-dose acetazolamide, low-dose acetazolamide, NH\(_4\)Cl, or saline) was calculated by taking all pH values for the pool of study animals from day 2 to day 8 and dividing the sum by the total number of measurements. It should be noted that the day 10 data were not included in the mean, because the analysis was intended to represent the level of acidosis during drug delivery, and the drug was stopped at day 8. This mean value, along with all other parametric values in the study, is reported as mean ± SD. For analysis of retinal data, comparisons between experimental groups were made using two-tailed \(t\) tests (retinal area) and Fisher’s exact tests (incidence of neovascularization, rat survival), with Bonferroni corrections for multiple comparisons. Bonferroni-corrected \(P\) values < 0.05 were considered statistically significant.

### RESULTS

#### Arterial Blood pH and Blood Gas Study

In the arterial blood gas study, we found that the level of acidosis during drug delivery (days 2–8) in rats receiving low-dose acetazolamide (50 mg/kg) was less severe than that of rats receiving NH\(_4\)Cl (10 mM/kg; mean pH 7.22 ± 0.07 vs. 7.11 ± 0.06, \(n = 38\) and 40, \(P < 0.001\); Table 1, Fig. 1). However, the mean arterial blood pH (days 2–8) in rats receiving high-dose acetazolamide (200 mg/kg; \(7.13 ± 0.06, n = 50\)) was similar to that of the rats receiving NH\(_4\)Cl. The minimum pH values during days 2 to 8 for high-dose acetazolamide and NH\(_4\)Cl rats were 7.06 and 7.03, respectively; both were at day 4 (Fig. 1). The pH values from saline controls (\(n = 44\)) were similar to room air values previously reported from our laboratory (i.e., pH 7.57).\(^4\)

<table>
<thead>
<tr>
<th></th>
<th>Saline ((n = 44))</th>
<th>Low-Dose Acetazolamide (50 mg/kg) ((n = 38))</th>
<th>High-Dose Acetazolamide (200 mg/kg) ((n = 50))</th>
<th>NH(_4)Cl (10 mM/kg) ((n = 40))</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.42 ± 0.06†‡</td>
<td>7.22 ± 0.07†‡</td>
<td>7.13 ± 0.06†‡</td>
<td>7.11 ± 0.06†‡</td>
</tr>
<tr>
<td>(\text{PaO}_2) (mmHg)</td>
<td>95 ± 8†‡</td>
<td>110 ± 16†‡</td>
<td>111 ± 12†‡</td>
<td>120 ± 17†‡</td>
</tr>
<tr>
<td>(\text{PaCO}_2) (mmHg)</td>
<td>39.2 ± 4.6†‡</td>
<td>45.9 ± 8.1*</td>
<td>45.9 ± 5.7*</td>
<td>35.1 ± 6.1†‡</td>
</tr>
<tr>
<td>(\text{HCO}_3^-) (mEq/L)</td>
<td>25.4 ± 2.8†‡</td>
<td>18.8 ± 3.2†‡</td>
<td>15.6 ± 2.1†‡</td>
<td>11.5 ± 2.7†‡</td>
</tr>
</tbody>
</table>

Values are means ± SD; \(n = \) no. of rats.

* Significantly different from saline (\(P < 0.05\)).
† Significantly different from low-dose acetazolamide (\(P < 0.05\)).
‡ Significantly different from high-dose acetazolamide (\(P < 0.05\)).
FIGURE 1. Arterial blood pH values (mean ± SD) obtained with IP acetazolamide, 50 or 200 mg/kg, or saline or gavaged NH₄Cl, 10 mM/kg.

Retinopathy Study

Low-Dose Acetazolamide (50 mg/kg). Of the 75 rats from 3 litters, 24 (63%) of 38 acidoic rats and 29 (78%) of 37 saline control rats survived to the conclusion of the study (day 13). No preretinal neovascularization was observed in either the low-dose acetazolamide rats or the saline control rats (Table 2).

High-Dose Acetazolamide (200 mg/kg). Of the 100 rats in 4 litters, 29 (58%) of 50 rats receiving high-dose acetazolamide (200 mg/kg), and 44 (88%) of 50 saline control rats survived. Data from surviving rats were used for analysis. Pre-retinal neovascularization similar to ROP occurred in 17 (59%) of 29 rats receiving high-dose acetazolamide vs. 0% of saline controls (P = 0.001; Table 2). In affected rats, the median severity of neovascularization was 1 clock hour, with a range of up to 2 clock hours.

Rats receiving high-dose acetazolamide demonstrated growth retardation compared with saline controls at day 8 (intermediate weight: 7.9 ± 1.3 vs. 11.1 ± 1.4 g, P < 0.001) and at day 13 (final weight: 15.0 ± 2.2 vs. 18.2 ± 2.2 g, P < 0.001; Table 2). Growth retardation was more severe in the high-dose than in the low-dose acetazolamide rats (Table 2).

Ratios of the vascularized to total retinal areas were smaller in rats receiving high-dose acetazolamide compared with saline controls (94% ± 5% vs. 98% ± 1%, P < 0.001; Table 2). Low-dose acetazolamide rats had vascularized areas similar to those of saline controls. It should be noted that “vascularized retinal area” refers to the entire superficial retinal vasculature, normal and abnormal, from the optic nerve to the periphery. The vascularized retinal area is the converse of the peripheral avascular zone in immature retinas.

Table 2. Retinopathy Study: Acetazolamide Versus Saline

<table>
<thead>
<tr>
<th></th>
<th>Saline Controls (From Both Studies)</th>
<th>Low-Dose Acetazolamide (50 mg/kg)</th>
<th>High-Dose Acetazolamide (200 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of surviving pups*</td>
<td>73 (84)</td>
<td>24 (63)%</td>
<td>29 (58)%</td>
</tr>
<tr>
<td>No. of pups with neovascularization*</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>17 (59)%</td>
</tr>
<tr>
<td>Total retinal area (mm²)</td>
<td>32.8 ± 2.3</td>
<td>33.1 ± 1.3</td>
<td>30.5 ± 2.5</td>
</tr>
<tr>
<td>Vascularized retinal area (mm²)</td>
<td>32.2 ± 2.3</td>
<td>32.4 ± 1.3</td>
<td>28.4 ± 3.2</td>
</tr>
<tr>
<td>Ratio of vascularized to total retinal area (%)</td>
<td>98 ± 1</td>
<td>98 ± 1</td>
<td>94 ± 5</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>6.6 ± 0.4</td>
<td>6.6 ± 0.4</td>
<td>6.5 ± 0.4</td>
</tr>
<tr>
<td>Intermediate weight (day 8) (g)</td>
<td>11.1 ± 1.4</td>
<td>9.5 ± 1.3</td>
<td>7.9 ± 1.3</td>
</tr>
<tr>
<td>Final weight (day 13) (g)</td>
<td>18.2 ± 2.2</td>
<td>15.5 ± 2.6</td>
<td>13.0 ± 2.2</td>
</tr>
</tbody>
</table>

* Values in parentheses are percentages.
† Significantly different from saline (P < 0.05); ‡Significantly different between low- and high-dose acetazolamide (P < 0.05).
§ Values are means ± SD.

DISCUSSION

In the present study, we report that acetazolamide (a drug causing metabolic acidosis primarily by bicarbonate loss) is associated with preretal neovascularization in the neonatal rat. Our results support our hypothesis that systemic acidosis per se is a risk factor for retinopathy in immature retinas.

The precise mechanism for acidosis-induced retinopathy remains speculative at this time. We have previously suggested that, in the pathogenesis of ROP, a variety of initiating factors may damage the developing retinal vasculature, leading to a neovascular response. On the basis of results of the present study and previous studies, we propose that one such initiating factor is systemic acidosis. We have previously reported that retinopathy similar to ROP is associated with acidosis induced by exposure to 10% CO₂ (i.e., a respiratory acidosis) and gavage with NH₄Cl (i.e., a metabolic acidosis due to increased hydrogen ion load). The present study also identified acetazolamide-induced acidosis as an initiating factor. Acetazolamide-induced retinopathy was morphologically similar to NH₄Cl-induced retinopathy, carbon dioxide-induced retinopathy, and clinical ROP. Perhaps all these initiating factors produce their effect by inducing a similar “damaging” insult to the developing vasculature. Future studies might address this concept, in part by investigating possible ultrastructural changes that might precede the development of neovascularization.

It was not the purpose of the present study to elucidate the mechanism of acidosis-induced retinopathy but rather to establish whether systemic acidosis per se is sufficient to induce preretinal neovascularization in an immature retina.

Vascular endothelial growth factor (VEGF) has been implicated in the pathogenesis of OIR. However, it is premature to speculate on a possible role for VEGF in the pathogenesis of acidosis-induced preretinal neovascularization. Although we have some preliminary evidence that acidosis results in down-regulation of VEGF mRNA with a possible subsequent rebound on recovery, further studies to address changes in VEGF expression are underway.

Although the mechanism of induction of neovascularization by systemic acidosis alone is currently unknown, it appears to be independent of hyperoxia or hypoxia. We observed a slight increase in PaO₂ in both acetazolamide and NH₄Cl-treated rats compared with saline controls (Table 1). Although these differences of 15 to 25 mm Hg were statistically significant, they may be biologically less important. There are a variety of possible explanations for the slightly raised PaO₂ in the rats with mixed systemic acidosis. These factors have been discussed by us in previous studies and include increased respiratory rate, changes in pulmonary artery pressure, and the distribution of gas and blood flow within the lung. Further...
investigation of these factors is warranted but beyond the scope of the present study. Small increases in PaO2 observed in the present study (i.e., to 111 ± 12 mm Hg) contrast to the PaO2 levels (371 ± 29 mm Hg) in our OIR model. Furthermore, in our CO2 studies, exposure to 10% inspired CO2 resulted in both raised PaCO2 and slightly raised PaO2 (153 ± 4 mm Hg) and was associated with preretinal neovascularization. In those studies, when we lowered inspired O2 to account for the raised PaO2 ("pure hypercarbia," PaCO2, 72 ± 4 mm Hg; PaO2, 93 ± 8 mm Hg), we still observed preretinal neovascularization associated with hypercarbia, independent of PaO2 changes. In view of these data, we believe that the effect of slightly raised PaO2 observed in the present study is negligible. Nevertheless, it is possible that acidosis-related increased oxygen delivery might occur at the local tissue level, independent of PaO2. Tsacopoulos and David reported that respiratory acidosis associated with hypercarbia results in retinal vasodilation. Local acidosis may have a similar effect on retinal oxygenation response, causing an increased delivery of oxygen to the developing vasculature.

Our previous studies of OIR have lead us to propose a synergistic role for growth retardation in the pathogenesis of ROP. In the present study, it is noteworthy that none of the saline-treated control animals raised in litters of 25 developed neovascularization. This supports the suggestion that modest growth retardation per se may be insufficient to induce preretinal neovascularization. Nevertheless, further studies of more severe pure growth retardation are needed before we can be sure that growth retardation per se is truly insufficient to induce preretinal neovascularization. In both the present and previous studies, we found exacerbated growth retardation in neonatal rats with systemic acidosis. At the overall litter level, our present study supports the suggestion that the severity of growth retardation is related to the severity of acidosis; growth retardation was more severe in the high-dose acetazolamide rats than in low-dose rats, which in turn, was more severe than in controls. It is not possible from our current data to separate the effect of severity of acidosis versus severity of growth retardation on retinopathy. Nevertheless, it is possible that acidosis may exacerbate growth retardation and that deprivation of a critical nutrient, antioxidant, or other element may contribute to the pathogenesis of preretinal neovascularization. Further studies controlling weight, survival, and level of acidosis are needed to address these issues. It is also tempting to speculate on an interaction between growth retardation, Insulin-like growth factor 1 and neovascularization, but such speculation may be premature.

In all our studies of acidosis-induced retinopathy, we found a lower survival rate in acidotic than the control rats. It is difficult to control for this difference in survival rate. Nevertheless, if a discrepancy in survival rate does create a bias, it is likely that such a bias would be toward failing to find neovascularization in the acidotic rats. In the present study, the primary question was “does preretinal neovascularization occur in acetazolamide-induced systemic acidosis?” Because we found the majority of retinas demonstrated neovascularization, a potential bias toward missing neovascularization is less important. If future studies compare incidence and severity be-

FIGURE 2. ADPase-stained retinas showing normal vasculature and neovascularization in the retinopathy study using high-dose acetazolamide (200 mg/kg). (A) Normal edge of vasculature showing fine, single-layer vasculature structure. (B, C) Retinas from rats receiving high-dose acetazolamide (200 mg/kg) demonstrating abnormal neovascularization (arrows) including tufts (B) and sheets (C) of vascular cells (magnification, ×550).
between experimental groups, they should account for any differences in survival rates between groups.

Our results suggest that there may be a threshold level of acidosis necessary to induce retinopathy. Neovascularization with acetazolamide was only observed in neonatal rats in which the severity of systemic acidosis was comparable to our previous NH4Cl study and previous CO2 study. In both previous studies and the present study, the level of acidosis associated with preretinal neovascularization was approximately pH 7.1. Rats receiving low-dose acetazolamide (50 mg/kg), with less severe acidosis (mean pH 7.22), did not develop any neovascularization. Therefore, the level of acidosis induced by acetazolamide, NH4Cl, or CO2, may be critical to the induction of retinopathy. Unfortunately, it is not currently feasible to perform blood gas measurements on the same rats that develop retinopathy. Carotid blood sampling was a terminal event, so we cannot currently provide data on individual rat arterial blood pH and severity of neovascularization. Future technological advances would be necessary to define the relationship of pH and retinopathy at the level of the individual rat.

The incidence and severity of neovascularization in high-dose acetazolamide rats was comparable to that in our previous NH4Cl study. NH4Cl-induced acidosis resulted in an incidence of neovascularization not statistically different from the incidence in the current high-dose acetazolamide (200 mg/kg) study (36% vs 59%, P = 0.09, Fisher’s exact test). The median severity in affected NH4Cl rats was 2 clock hours and a range of up to 5 clock hours compared with a median of 1 clock hour in affected acetazolamide rats and a range up to 2 clock hours. The incidence of neovascularization in our previous CO2 study was somewhat less than both acetazolamide and NH4Cl, with an incidence of 14% in the “pure hypercarbia” group and a severity range up to 3 clock hours. It is entirely possible that blood pH, though important, is not the sole determinant of neovascularization in these models. Nevertheless, our present study was not designed to test the relative effects of various acidosis-inducing agents on the retinal vasculature but instead to determine whether systemic acidosis per se consistently induced preretinal neovascularization in neonatal animals. It is also possible that we have underestimated the true incidence of preretinal neovascularization, because it may have occurred more frequently in those pups that died. It has been impossible to adequately analyze the retinas of pups that die during these studies, because of postmortem tissue autolysis.

Although we are not suggesting that systemic acidosis is the single major etiologic factor in the pathogenesis of ROP, we are proposing that systemic acidosis may be an important clinically relevant cofactor or exacerbating factor. Mild neovascularization induced by acidosis alone may become a component of severe vision-threatening neovascularization in the context of a premature human neonate exposed to multiple simultaneous risk factors. Based on the results of the present animal study, it is premature to extrapolate and predict the exact levels of pH that might be harmful to the developing vasculature of human neonates. Nevertheless, it is noteworthy that the sickest infants who are at highest risk for ROP may experience episodes of severe acidosis, for example, pH < 7.15. Further studies in our laboratory will address the issues of critical level of pH and the critical duration of acidosis needed for induction of retinopathy.

In our present laboratory study, acetazolamide induced an acidosis that was primarily “metabolic” but had a “respiratory” (raised CO2) component (Table 1). This combination of metabolic and respiratory acidosis is very relevant to the clinical treatment of preterm human neonates. These patients may experience respiratory acidosis resulting from periods of apnea and bradycardia and metabolic acidosis from bradycardia and sepsis. Hence, the sickest infants, most likely to develop ROP, are at increased risk for developing a mixed respiratory and metabolic acidosis. On the basis of our data, consideration should be given to a reevaluation of acid–base management in premature infants.

Currently, in some neonatal intensive care units, efforts are made to wean preterm infants from mechanical ventilation as soon as is possible to avoid the technique’s adverse side effects (e.g., barotrauma). However, early weaning may predispose neonates to periods of carbon dioxide retention and respiratory acidosis (i.e., “permissive hypercapnia”). Further, in this same patient population, there may be a reluctance to treat metabolic acidosis because of concern for therapy-related side effects (e.g., intraventricular hemorrhage associated with bicarbonate use). When these issues are considered collectively, we hypothesize that treatment patterns aimed at lessening or avoiding systemic and cerebral injury in the preterm neonate may inadvertently contribute to an increased incidence and severity of ROP. This hypothesis can only be tested adequately in high-risk human neonates and might lead to a future clinical trial that would examine the question: does rigorous acid-base control reduce the incidence and severity of ROP? However, until such studies are completed, we can gain a better understanding of the importance of acidosis in retinopathy by performing animal studies to investigate the extent and timing of acidosis required to induce preretinal neovascularization in immature retinas.

References


