Bilirubin Binding and Neonatal Acidosis

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Plasma of neonates with severe metabolic acidosis secondary to fetal asphyxia bound less bilirubin than that of neonates without acidosis, as determined by Sephadex gel filtration. There was a significant correlation between the amount of bilirubin adsorbed by Sephadex and the base deficit. The method used ruled out any influence of plasma pH per se on binding. Our results suggest that organic anions that accumulate in the plasma of asphyxiated acidotic neonates may compete with bilirubin for binding sites on albumin.

Additional Keyphrases: Sephadex gel filtration • neonatal jaundice • hyperbilirubinemia • bilirubin encephalopathy • kernicterus • exchange transfusion • asphyxia • base deficit

Acidosis is thought to predispose to kernicterus on the basis of observed clinico-pathological associations (1−3). The effects of acidosis may be due to decreased binding of bilirubin (BR) by albumin, and (or) to increased cellular toxicity of BR at low pH (4−6). The present study was designed to determine the effect of metabolic acidosis secondary to fetal asphyxia on BR binding by neonatal plasma, as determined by Sephadex gel filtration.

BR binding determined by this method has been shown to correlate better with the clinical outcome in the neonatal period, and especially with the appearance of kernicterus, than do plasma BR concentrations or BR/albumin molar ratios (7, 8).

Materials and Methods

Patients. Twenty-seven full-term infants (>37 completed gestational weeks) were randomly selected from consecutive births to give approximately equal numbers in each of the following clinical groups (Table 1): IA, infants born by elective cesarean section with no asphyxia (1-min Apgar score >7); IB, infants born by cesarean section for cephalopelvic disproportion after trial of labor, with mild labor stress but no asphyxia (1-min Apgar score >7); II, infants born by uncomplicated vaginal delivery with the usual labor stress and minimal asphyxia (1-min Apgar score >7); III, infants with severe asphyxia requiring resuscitation, regardless of route of delivery (1-min Apgar score <4). Infants not complying with these criteria within 1 min of delivery and those expected to have blood-group incompatibilities were excluded, as were also three infants whose plasmas were hemolytic. There were no differences among the groups with regard to average gestational age or birth weight.

Blood sampling procedure. The umbilical cord was double-clamped at delivery and umbilical venous blood was immediately drawn from between the clamps into a heparinized syringe, which was sealed and put on ice; blood gases were measured within 30 min (Table 1); 30−40 mL of blood was drawn from the umbilical vein on the placental side of the clamps into a heparinized 50-mL syringe. Plasma was separated within 30 min and kept frozen at −20 °C until examined. This freezing procedure does not impair BR binding (7).

Laboratory methods. Model jaundiced-plasma solutions with increasing concentrations of BR were prepared by dissolving crystalline BR (ICN Pharmaceuticals, Inc., Life Sciences Groups, Cleveland, OH 44128) in 0.1 mol/L NaOH and adding 0.1-mL aliquots of these solutions to 0.9-mL aliquots of plasma, followed by 0.1 mL of 0.1 mol/L HCl. The BR concentration was determined (9) in 0.2 mL of each final solution. The remaining 0.9 mL was diluted with 0.9 mL of phosphate buffer (67 mmol/L, pH 7.45) and used for the study of BR binding by a Sephadex gel filtration method (10). Small columns7 packed with 2 mL of Sephadex G-25, fine (Pharmacia, Uppsala, Sweden), were equilibrated with the phosphate buffer and 1.4-mL aliquots of each of the buffered model-jaundiced plasmas were applied to the columns. After all protein was washed out of the column with the phosphate buffer, the BR, which remained adsorbed to the Sephadex, was eluted with about 5 mL of 0.1 mol/L NaOH and collected into 2 mL of a mixture of 0.5 mol/L citric acid and 0.1 mol/L ascorbic acid to prevent oxidation of BR. The BR was extracted from this solution into 1.5 mL of CHCl3 and the absorbance of the CHCl3 extract was measured at 450 nm (λmax of BR). The absorbance values were converted into micrograms of BR recovered from the Sephadex column (1 μg of BR = 0.98 Å).

Albumin concentration in the original plasma samples was estimated by an acid-alcohol precipitation method (11). BR/albumin molar ratios (BR/alb) up to about 1.8 were achieved in model-jaundiced plasma by addition of BR (8.5 mg of BR per gram of albumin = 1:1 molar ratio). But because the original plasma specimens varied in both BR and albumin concentrations, the standard additions of BR did not give identical molar ratios in the final solutions applied to the Sephadex columns. Therefore, the amount of BR adsorbed by the Sephadex column from each individual model jaundiced plasma sample and the corresponding BR/alb ratio were fed into a computer for estimation of the best-fitting quadratic polynomial (y = a + bx + cx2). The computer curve obtained for the plasma from a given infant served for the calculation of the amount of BR theoretically adsorbed by Sephadex from this plasma at the arbitrarily chosen BR/alb ratios of 0.5, 0.8, 1.0, 1.3, and 1.5. These calculated values were then used to compare BR binding by the plasma of infants in the various clinical groups and for relating the amount of BR adsorbed by Sephadex to the plasma base deficit. Statistical significance was determined by Student’s t-test.

Results

The amount of BR adsorbed by Sephadex increased with increasing BR/alb ratio in plasma from each of the four clinical groups (Table 2). The plasma of infants with severe asphyxia

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7 "Kernlute," was graciously supplied by Ames-Vissum, Ltd., Jerusalem, Israel.
Table 1. Clinical and Acid–Base Data (Means ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Delivery</th>
<th>No. neonates</th>
<th>Male:female ratio</th>
<th>Labor stress</th>
<th>Asphyxia</th>
<th>General anesthesia</th>
<th>PCO₂ (mmHg)</th>
<th>pH</th>
<th>Base deficit, meq/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>I A</td>
<td>Elective cesarean section</td>
<td>7</td>
<td>4:3</td>
<td>–</td>
<td>–</td>
<td>7</td>
<td>45 ± 5</td>
<td>7.29 ± 0.03</td>
<td>6.1 ± 1.6</td>
</tr>
<tr>
<td>B</td>
<td>Cesarean section for cephalo-pelvic disproportion</td>
<td>8</td>
<td>4:4</td>
<td>+</td>
<td>–</td>
<td>8</td>
<td>42 ± 9</td>
<td>7.30 ± 0.07</td>
<td>6.1 ± 2.1</td>
</tr>
<tr>
<td>II</td>
<td>Uncomplicated vaginal delivery</td>
<td>7</td>
<td>2:5</td>
<td>++</td>
<td>±</td>
<td>0</td>
<td>32 ± 3</td>
<td>7.31 ± 0.04</td>
<td>9.0 ± 1.0</td>
</tr>
<tr>
<td>III</td>
<td>Severe asphyxia requiring resuscitation</td>
<td>5</td>
<td>5:0</td>
<td>+</td>
<td>+++</td>
<td>4</td>
<td>53 ± 18</td>
<td>7.10 ± 0.13</td>
<td>16.9 ± 6.9</td>
</tr>
</tbody>
</table>

* Agents used included thiopentone, scoline, and pancuronium bromide.  
* One infant delivered vaginally, four by cesarean section.

Table 2. Relationship between Bilirubin Binding in Neonatal Plasma and Base Deficit (Means ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. neonates</th>
<th>Albumin concn, g/L</th>
<th>BR adsorbed by Sephadex a at BR/alb molar ratio of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>I A</td>
<td>7</td>
<td>32.6</td>
<td>0.29, 0.90, 1.90, 4.27, 6.43</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>±2.9</td>
<td>±0.17, ±0.21, ±0.32, ±0.57, ±0.80</td>
</tr>
<tr>
<td>I B</td>
<td>7</td>
<td>35.1</td>
<td>0.26, 0.96, 2.05, 4.73, 7.19</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>±3.3</td>
<td>±0.18, ±0.34, ±0.70, ±1.36, ±1.90</td>
</tr>
<tr>
<td>II</td>
<td>7</td>
<td>35.4</td>
<td>0.38, 1.44, 2.66, 5.22, 7.38</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>±5.0</td>
<td>±0.29, ±0.54, ±0.78, ±1.07, ±1.41</td>
</tr>
<tr>
<td>III</td>
<td>5</td>
<td>±3.4</td>
<td>±0.21, ±0.58, ±0.85, ±0.88, ±0.89</td>
</tr>
<tr>
<td>p (Group I A and B, vs Group III)</td>
<td>&lt;0.05</td>
<td>N.S. &lt;0.005 &lt;0.01 &lt;0.005 &lt;0.005</td>
<td></td>
</tr>
</tbody>
</table>

* Calculated from best-fitting quadratic polynomial (see Methods); values are µg of BR recovered from Sephadex columns.  
* Correlation between means of BR adsorbed and means of base deficits (Table 1) for each of the 27 plasma samples, at each BR/alb ratio.  
* Probability for each correlation coefficient.

(III; mean base deficit 16.9 meq/L) bound less BR than that of infants without asphyxia, born by cesarean section, either elective or for cephalopelvic disproportion (Group I, A and B; mean base deficit 61). This was indicated by the greater amounts of BR adsorbed by Sephadex—at all BR/alb ratios examined—from plasma samples of severely asphyxiated infants (Table 2). In the presence of the mild acidosis resulting from the labor stress of uncomplicated vaginal delivery (Group II; mean base deficit 9.0), BR binding was intermediate between that of Groups I and III. There was a significant correlation between the base deficit of individual plasma samples and the amount of BR adsorbed by Sephadex from these samples at BR/alb molar ratios of 0.8, 1.0, and 1.3 (Table 2).

Comparison of BR binding by the plasma of males and of females from the combined data of those born without asphyxia (Group I, A and B) revealed no sex-related differences.

Exposure of infants to meperidine (administered to the mother) was similar in all groups, and no trend was observed when comparisons were made between exposed and unexposed subjects. The data did not permit analysis of the effect of individual anesthetic agents used in operative deliveries. However, there was no significant difference in BR binding between plasmas of infants born during general anesthesia for cesarean section (Group I, A and B) and those born by vaginal delivery without exposure to general anesthesia (Group II). The plasma of two infants exposed to furosemide for more than two weeks before birth (maternal dosage 80 mg/day) showed unexceptional BR binding.

Discussion

This study demonstrates impaired BR binding by the plasma of full-term infants with metabolic acidosis due to asphyxia, which very significantly correlates with base deficit. This is in accord with clinical observations and animal studies, which strongly suggest an association between acidosis and predisposition to kernicterus (1-3, 12, 13). The latter may be due to a direct effect of pH on BR-albumin interactions and (or) to altered cerebral BR uptake (6). Because the pH of the plasma samples we studied was adjusted to 7.45 before testing, the impaired BR binding by the plasma of the acidic infants cannot be attributed to a direct effect of pH per se. Neither could increased PCO₂ have affected BR binding directly, as the plasma samples had equilibrated with the atmospheric CO₂ and the PCO₂ of the plasma applied to the Sephadex column was therefore <7 mmHg.

The most probable explanation for the observed changes in BR binding is the presence of increased concentrations of substances that compete with BR for binding sites on albumin in the plasma of asphyxiated infants. Free fatty acids, the concentrations of which are increased in neonatal asphyxia, adversely affect BR binding (14-16). No other endogenous substances associated with neonatal asphyxia have been incriminated as interfering with the binding of BR by albumin (17-18). However, the role of certain organic ions for which the concentrations are increased in the plasma of asphyxiated infants—such as lactic and pyruvic acids, ketones, and corticosteroids—has not been evaluated. Our preliminary results indicate that lactate concentrations such as those seen in asphyxia do not interfere with BR binding to albumin (to be published).

The results of the present study reinforce the concept of increased vulnerability of the acidic infant to BR encephalopathy: in the acidotic infant the concentration of unbound BR would be increased, thus increasing the risk of its transfer.
into the brain. Correction of acidosis by administration of sodium bicarbonate would therefore appear to be rational therapy to reduce the risk of brain damage. Indeed, this treatment improved in vitro BR binding by serum from acidotic infants (19). However, because administration of sodium bicarbonate in intact animals appeared to increase predisposition to kernicterus (20), further investigation is needed to establish the efficacy of exogenous base in preventing BR encephalopathy in acidotic infants.

The problem of hyperbilirubinemia in acidotic neonates may also be attacked by more liberal indications for exchange transfusion than those currently used. The time-honored indication—serum BR in excess of 20 mg/dL in full-term infants—was established empirically on the basis of prospective long-term follow up of many hyperbilirubinemic infants (21, 22). However, this statistically based criterion is not applicable in the case of acidotic infants. The use of a BR-binding test as an additional criterion for exchange transfusion has been generally supported by experience from several laboratories (23), but its clinical value has yet to be fully established by more prospective studies with long-term followup (23, 24). Our findings suggest that in evaluating the clinical state of jaundiced infants, determination of BR binding (in addition to the usual clinical and laboratory criteria) would be of particular importance in those with acidosis.

References