Serum Lipoperoxidation Products in an Infant with Hemolytic-Uremic Syndrome

R. E. Brown,1 Solomon L. Aalde,2 Joseph A. Knight,3 and Barry J. Evans4

We detected increased concentrations of lipoperoxidation products, as malondialdehyde, in the serum of an infant with classic hemolytic-uremic syndrome. The concentrations declined when peritoneal dialysis was initiated and eventually returned to normal after clinical recovery. Our observation adds to the existing body of evidence that links the pathogenesis of hemolytic-uremic syndrome to peroxidative damage and to the rationale for using antioxidant agents as part of the therapy for this disease process.

Additional Keyphrases: pediatric chemistry • antioxidants in therapy

The pathogenesis of the sporadic form of hemolytic-uremic syndrome (HUS) has been linked to the presence of an inhibitor of the vessel-wall enzyme, prostacyclin synthase (EC 5.3.99.4), resulting in a deficiency of prostacyclin and therefore, increased platelet aggregation with thrombosis (1-3). The inhibitor, which is present in the plasma of such patients with HUS, has biochemical characteristics of peroxides of polyunsaturated fatty acids (1).

Here we document the presence of increased serum thio-barbituric acid reactivity for lipoperoxidation products at diagnosis in a patient with HUS. We also observed that the concentrations of these products, measured as malondialdehyde, declined coincidentally with the initiation of peritoneal dialysis and returned to normal in association with clinical recovery.

Case Report

A two-month-old white boy was referred to Cook–Fort Worth Children’s Medical Center with anemia and renal dysfunction. He had been well until two weeks before admission, when he developed an upper respiratory infection with rhinorrhea. Four days before admission he began vomiting, which increased in frequency during the next several days and was accompanied by diarrhea and anorexia. His stools became black and his urine became pink. He became increasingly pale and urinated less frequently. Pertinent physical findings included the presence of dry mucous membranes, marked pallor without significant jaundice, and tachycardia.

Laboratory studies on specimens obtained shortly after admission revealed anemia (hemoglobin concentration, 39 g/L); schistocytes and acanthocytosis in the peripheral smear; thrombocytopenia along with leukocytosis and increased serum urea nitrogen and creatinine concentrations and hyperuricemia (vide infra); hypertriglyceridemia (5.44 mmol/L; expected range for this patient’s age group, 0.22–1.69 mmol/L, probably pathologic given his prandial circumstance); and mild hyperbilirubinemia (total, 58 μmol/L; expected range for age, <28 μmol/L).

Treatments (1–2) included administration of fresh frozen-stored plasma and initiation of peritoneal dialysis on the day of admission; vitamin E administration (100 int. units every 12 h, per nasogastric tube) began on the third hospital day. There was a subsequent improvement in his clinical state, accompanied by a decline in his serum urea nitrogen, creatinine, and uric acid concentrations. Dialysis was continued for four days.

The patient recovered and was discharged on the tenth hospital day. Laboratory values at this time included hemoglobin, 105 g/L; platelet count, 315 000/μL; serum urea nitrogen, 1.1 mmol/L; and serum creatinine, 26 μmol/L. He was returned for a follow-up visit three months after discharge and was found to be in a normal state of health.

Methods

Serum lipoperoxides and Schiff-base derivatives were hydrolyzed by boiling in dilute acid (4–7). Malondialdehyde (MDA), one of the hydrolysis products, reacts with thio-barbituric acid (TBA) to form an MDA-(TBA)2 adduct. The latter was quantified by two different techniques. Aliquots hydrolyzed in dilute H2SO4 were extracted into n-butanol and the absorbance of the resulting chromogenic adduct(s) was measured in a spectrophotometer at 532 nm relative to a reference measurement at 590 nm (4). The absorbance reading was then converted to moles of MDA by using a value for molar absorptivity of 1.56 × 105 L mol⁻¹ cm⁻¹. Aliquots hydrolyzed in dilute H3PO4 were rendered protein free by precipitation with methanol and fractionated by high-performance liquid chromatography (HPLC). We spectrophotometrically quantified the MDA-(TBA)2 adduct at 532 nm by determining the concentration from the peak height with a calibration curve (6, 7).

Other serum analytes—including urea nitrogen, creatinine, total bilirubin, triglyceride, and uric acid—were assayed in the aca III (E. I. DuPont de Nemours & Co., Wilmington, DE).

Complete blood counts were performed with the Technicon H 1 system (Technicon Instruments Corp., Tarrytown, NY). Peripheral blood smears were stained with Wright's stain and examined by conventional light microscopy.

Because there were no expected ranges for either MDA-(TBA)2 concentrations by HPLC or TBA reactivity in the serum of infants, we assembled our own "control" population. Aliquots of sera remaining from thyroid-function testing performed in our laboratory are routinely kept frozen at...
Table 1. Peripheral Blood Counts and Serum Analytes vs Clinical State in a Patient with Hemolytic–Uremic Syndrome

<table>
<thead>
<tr>
<th>Clinical state</th>
<th>Platelet count per µL</th>
<th>Leukocyte count</th>
<th>Lipid peroxidation products: MDA, µmol/L</th>
<th>Urea N, mmol/L</th>
<th>Creatinine µmol/L</th>
<th>Uric acid µmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Admission</td>
<td></td>
<td></td>
<td>MDA–(TBA)_2 adduct^a</td>
<td>TBA reactivity^b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>61 000</td>
<td>20 800^c</td>
<td>3.06</td>
<td>9.03</td>
<td>40.7</td>
<td>380</td>
</tr>
<tr>
<td>Post dialysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td>68 000</td>
<td>11 800</td>
<td>1.09</td>
<td>6.25</td>
<td>16.4</td>
<td>212</td>
</tr>
<tr>
<td>Day 4</td>
<td>50 000</td>
<td>10 100</td>
<td>1.22</td>
<td>5.96</td>
<td>10.7</td>
<td>141</td>
</tr>
<tr>
<td>Recovered</td>
<td></td>
<td></td>
<td>Three months postdischarge</td>
<td>451 000</td>
<td>9 800</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.74</td>
<td></td>
<td>274</td>
</tr>
</tbody>
</table>

^a Performed by liquid chromatography (6, 7).

^b Performed according to the method of Satoh as modified by Thayer (4).

^c Absolute lymphocyte counts in the peripheral blood, calculated from the leucocyte count and % lymphocytes, were 10 200, 7100, 3800, and 4600/µL on days 1, 3, and 4 and three months postdischarge, respectively.

Expected ranges, calculated from mean ± 2 standard deviations: platelet counts, 200 000 to 470 000/µL; leukocytes, 4700 to 14 000/µL; MDA–(TBA)_2 adduct, 0.05 to 2.16 µmol (MDA)/L; TBA reactivity, 0.71 to 4.36 µmol (MDA)/L; urea N, 1.8 to 8.2 mmol/L; creatinine, <3.0 µmol/L; uric acid, 119 to 416 µmol/L.

−70 °C for up to six months after the reports are finalized. All samples used for controls in this study came from such a pool and appeared to be nonhemolyzed. The corresponding patient population comprised of 10 male and 7 female infants ranging in age from four days to 6.7 months (mean age, 1.4 months). Only patients who were awaiting discharge from the hospital or otherwise healthy out-patients were included as "controls." None was acutely ill.

Results

Increased serum TBA reactivity for lipoperoxidation products, as assessed by standard colorimetric assay, was present at diagnosis in our patient (Table 1). This was accompanied by an increase in the concentration of the MDA–(TBA)_2 adduct after fractionation by HPLC (Table 1, Figure 1). The values for each of these decreased during periportal dialysis, with a corresponding decrease in the serum urea nitrogen and in serum creatinine and uric acid concentrations (Table 1, Figure 1). All variables, including the platelet count, had returned to normal limits either by the time of discharge or by three months after discharge.

Discussion

There was increased TBA reactivity for lipid peroxidation products (wide infra), along with azotemia, hyperuricemia, and thrombocytopenia, at the time this patient was diagnosed with HUS. The urea nitrogen, creatinine, and uric acid concentrations in serum declined in parallel with the TBA reactivity after peritoneal dialysis was begun, and values for all variables returned to normal with clinical recovery. Azotemia per se is probably not responsible for increasing the concentration of circulating lipid peroxidation products, given the observation (Knight JA et al., submitted for publication) that MDA concentrations in plasma of 13 renal-transplant patients were within the normal reference interval despite increased urea nitrogen and creatinine values.

Although other substances undoubtedly are contributing to the increased TBA reactivity observed at diagnosis using the standard colorimetric assay, the demonstration of a corresponding singular peak for MDA–(TBA)_2 with an increased vertical amplitude in the HPLC chromatogram indicates that malondialdehyde is a significant component (6, 7). There are several possible sources of the MDA in this assay system. Acid hydrolysis could have generated MDA from hydroperoxides (lipoperoxides) or from Schiff-base derivatives of MDA (6, 7). In vivo, excessive hydroperoxide and MDA production may reflect the presence of a pro-oxidant/antioxidant imbalance (8). This is consistent with the observation that there is decreased antioxidant protection in some patients with HUS, because of low concentrations of vitamin E in their plasma and low erythrocyte superoxide dismutase (EC 1.15.1.1) activity (1, 2, 9). Moreover, in our patient, the hematological finding, on admission, of leukocytosis with 49% lymphocytes suggests a possible source of pro-oxidants: because human T-lymphocytes produce lipoxygenase (EC 1.13.11.12) products (10) whose biochemical intermediaries include 15-hydroperoxyarachidonic acid (8, 10), there is the potential for an excess of pro-oxidants in a patient with relatively increased numbers of circulating lymphocytes. It is perhaps noteworthy that the absolute lymphocyte count decreased progressively, coincidental with his clinical recovery in the hospital (Table 1).

A pro-oxidant/antioxidant imbalance with lipid peroxidation could explain, at least in part, several of the biochemi-
cal aberrations and pathophysiological phenomena in HUS. For example, lipid peroxides, as previously reported (2, 3), can inhibit prostacyclin synthesis and therefore could account for the presence of such an inhibitor in plasma in HUS with characteristics of peroxides of polyunsaturated fatty acids (1). Additionally, lipid peroxides through the process of autoxidation could explain both the depression of phosphatidylylethanolamine content and the presence of lipid peroxides in erythrocyte membranes of patients with HUS (2, 8, 9).

Hypertriglyceridemia in HUS (9) might also be consequent to the inhibition of endothelial lipoprotein lipase (EC 3.1.1.34) (11) by lipid peroxides. Pathogenetically, lipid peroxides could contribute to platelet aggregation with thrombosis in HUS by: (a) producing endothelial cell injury; (b) decreasing the synthesis of prostacyclin, creating a pro-aggregatory/anti-aggregatory imbalance; and (c) activating phospholipase A2 (EC 3.1.1.4) in platelets, leading to the production of pro-aggregatory prostaglandins and thromboxane A2 (1, 3, 8, 12, 13).

Finally, we hope that this observation can be extended to look at possible correlations between serum TBA reactivity and the severity of the disease process in future patients with HUS. In the meantime, however, we believe that our observation of increased serum TBA reactivity adds to the growing body of evidence linking the pathogenesis of HUS to lipid peroxidation; and from a therapeutic standpoint, it supports the use of vitamin E a la Powell et al. (2) as a treatment for hemolytic–uremic syndrome in infants and children.

References


