Glycosaminoglycan in the Blood and Renal Tissue of a Patient with Nephroblastomatosis

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ABSTRACT

Bilateral nephroblastomatosis was diagnosed in a 15-month-old white female. Prior to surgery, multiple peripheral blood smears (Wrights’ stain) revealed an azurophilic staining extracellular material. When serum was added to a three percent acetic acid solution, a floccular, fibrous precipitate formed at the meniscus of the tube. Serum protein electrophoresis on cellulose acetate support media resulted in a distorted pattern which corrected to a normal pattern upon treatment with hyaluronidase. These peripheral blood abnormalities disappeared following a left nephrectomy. Quantitative chemical analysis of diseased renal tissue yielded 81 μg of readily extracted glycosaminoglycan (GAG) per gram of tissue. The importance of abnormal glycosaminoglycan production in patients with malignant disease is discussed both in terms of clinical importance and possible roles of cell exudates.

Introduction

An association between elevated plasma levels of glycosaminoglycans (GAG) and particular neoplasms has been reported in neuroblastoma, reticulum cell sarcoma, and nephroblastoma. This report describes studies on the first documented case of nephroblastomatosis to be associated with elevated GAG production.

Case History

R.R., a 15-month-old white female, presented with a large asymptomatic abdominal mass. Ultrasound and CT studies revealed bilateral nephromegaly. Laboratory studies on admission showed a hematocrit of 32 percent; a white blood cell count of 15,200 per mm³ with 20 percent segmented neutrophils, 66 percent lymphocytes, 12 percent monocytes, one percent eosinophils, one percent basophils, a platelet count of 364,000 per mm³, and a hemoglobin of 108 g per L. A urine screen revealed a hazy yellow fluid (pH 5.0) with a specific gravity of 1.022 which was negative for protein, glucose, ketones, bilirubin,
blood and urobilinogen. Coagulation studies demonstrated both a slightly prolonged prothrombin time of 13 seconds (reference range: 9 to 12), and a significantly prolonged partial thromboplastin time of 68 seconds (reference range: 23 to 39) with a normal serum fibrinogen concentration of 1900 ng per L (reference range: 1500 to 4000). The only abnormal serum chemistry tests were: total protein 55 g per L (reference range: 60 to 80), albumin 31 g per L (reference range: 35 to 50) and phosphorus 54 mg per L (reference range: 25 to 45).

A peripheral blood smear stained with Wright's stain revealed an azurophilic staining extracellular material (figure 1).

Surgical exploration was undertaken four days post-admission.

Exploration of the abdomen revealed no ascitic fluid. Both lobes of the liver were smooth. There was a left-sided retroperitoneal mass that was not fixed. The mass appeared to be lobulated but there was no distinct normal kidney adjacent to the mass. The mass measured 6 × 16 × 10 cm. The right kidney was also lobulated and had many smaller lobules present, especially in the upper pole which was estimated to be three to four times normal size. The operating physician decided to remove the left kidney and do a wedge biopsy of the inferior pole of the right kidney.

Sections of both kidneys revealed a diffuse superficial proliferation of immature metanephric blastema. The neoplasm was composed to small basophilic oval or fusiform cells with scanty, indistinct cytoplasm. The abnormal cells grew in irregular tubular structures or in vague blastema-like cell aggregates (figure 2). No significant anaplastic change, atypical mitosis or sarcomatous pattern was noted. The neoplasm, on gross inspection, was sharply demarcated, with a pushing-type margin, from the adjacent normal renal parenchyma. The fetal-type immature glomeruli and some sclerotic glomeruli were present in the normal kidney. Mitotic figures averaged one to 0.5 per high-power field. Rare 'tumor' fragments in small vessels were noted. Hamartomatous renal parenchyma changes were not seen. The pathological diagnosis was bilateral nephroblastomatosis without involvement of any of four hilar lymph nodes and with no evidence of extra-renal spread or renal vein involvement. Following surgery, the patient was placed on chemotherapy consisting of actinomycin D and vincristine. The right kidney responded to chemotherapy, returning to normal size. Coagulation parameters returned to normal following surgery and treatment.

Figure 1. Peripheral blood smear (Wrights' stain) with arrows indicating precipitated extracellular material.

Materials and Methods

Hematology studies were conducted by continuous flow analysis (Hemalog-890).* An SMA-12 was used for serum chemistry studies.* Coagulation results were generated using a Lancer Coagulizer.† Electrophoretic analysis was completed on cellulose acetate‡ and agarose support media.§ Testicular hyaluronidase (EC 3.2.1.35) was purchased commercially.||

Analysis of the extracted GAG was determined colorimetrically by the carbazole procedure of Bitter and Muir.† A frozen specimen of the diseased left kidney was minced and ground at room temperature for one hour in pH 7.4 buffer (100 mmol per L Tris containing 400 mg

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* Technicon Instruments Corp., Tarrytown, NY 10591.
† Lancer-Division of Sherwood Medical St. Louis, MO 63103.
‡ Helena Laboratories, Beaumont, TX 77704.
§ Panagel, Worthington Diagnostics, Freehold, NJ 07728.
|| Wyeth Labs., Philadelphia, PA 19101.
per L disodium EDTA, total volume 35 ml). Following this procedure, the mixture was centrifuged at 2800 g for 30 minutes (3°C). The pink supernatant was filtered and lyophilized. Prior to digestion, the extract was redissolved in water (5 ml) and 1.0 ml of a solution of calcium chloride (8.8 g per L) and sodium azide (2.0 g per L) was added. Digestion was achieved with the addition of 6 mg of pronase followed by an 18 hour incubation at 55°C. The solution was again filtered (Miracloth) and to the clean digest were added 30 ml of chilled absolute ethanol. The solution was stored overnight (−5°C) and a white gel-like precipitate was collected. The precipitate was dissolved in 4.0 ml of water and GAG was analyzed using D-glucuronolactone as a standard.

**Results**

Following the observation of abnormal extracellular material in the Wrights' stained blood smear (figure 1), serum was added to three percent acetic acid. A resulting fibrous precipitate formed as had been previously described in cases of nephroblastoma.\textsuperscript{1,14,16,17}

Electrophoretic fractionation of serum proteins on a cellulose acetate support medium showed a distortion of all the observed bands, suggesting that a serum factor was interfering with the mobility and separation of proteins (figure 3). A

**Figure 2.** Microscopic section of the left kidney. Hematoxylin and eosin stain.
similar effect has been reported in cases of neuroblastoma\textsuperscript{4} and nephroblastoma.\textsuperscript{1,17} Electrophoretic separation on agarose demonstrated a smear between the alpha 2 and beta bands but otherwise a normal pattern (figure 3). Treatment of the serum with hyaluronidase (overnight) corrected the distorted cellulose acetate pattern.

Tissue from the removed left kidney, upon analysis, yielded 81 μg of readily extractable GAG residue per gram of tissue. This result is presented in table I with other renal tissue samples which have been analyzed.

Discussion

Nephroblastomatosis (persistent renal blastema) is the abnormal accumulation of primitive metanephric epithelium which can present as a confluent mass replacing the entire renal cortex. The occurrence of nephroblastomatosis, as a clinical entity, has been extensively documented.\textsuperscript{5,9,10} The precipitation of mucopolysaccharides in either dilute acid or ethanol (Wright's stain is an ethanolic stain) is a well documented occurrence. This precipitation is a strong qualitative indicator of elevated mucopolysaccharide levels. The disruption of the cellulose acetate electrophoretic pattern which was observed by us has been duplicated by the addition of hyaluronic acid to specimens.\textsuperscript{17} In our particular case, the disappearance of the electrophoretic abnormality upon incubation of the sample with hyaluronidase strongly implicates hyaluronic acid as the disrupting agent. It is germane to call attention to the agarose pattern. As can be observed in figure 3, this pattern, although unusual, is not as much altered as the cellulose acetate pattern. Wu et al.\textsuperscript{17} upon agarose electrophoresis of a nephroblastoma patient, concluded that hyaluronic acid migrated near the albumin fraction and speculated that hyaluronic acid might form salt bridges with albumin. There is no evidence of this in our particular case, but this could be due to the difference in electrophoretic methods.

Although the amount of GAG extracted and analyzed from the present patient is higher than one normal renal tissue sample, some Wilms' tumor patients were less elevated (table I). This discrepancy may relate both to the nature of individual Wilms' tumors and to analytical uncertainties of measurement. Unfortunately, serum samples were not available for GAG analysis prior to nephrectomy. It is of interest to note that fetal kidney samples contain higher levels of hyaluronate than normal tissue and significant amounts of chondroitin sulfate. The similarity of GAG patterns for fetal kidney and Wilms' tumor tissue may indicate a metabolic reversion of Wilms' tumor cells to a fetal state.
The observed prolongation of the partial thromboplastin time may be related to a reported decrease in Factor VIII activity in a similar patient. The mechanism by which Factor VIII might be affected is unknown.

This case report is of particular interest from various perspectives. First, the association of GAG production with nephroblastomatosis is important clinically. In this aspect, GAG may assume the role of a monitoring tool for affected patients. Although the incidence of overt GAG production in nephroblastomatosis patients is totally unknown, it is reasonable to surmise that once a patient has experienced both the disease and elevated GAG production, disease recurrence may proceed in tandem with a rise in serum GAG levels. Therefore, as in the case of classical tumor markers, the appearance of elevated GAG levels in serum may signal disease recurrence. Although more experimental work would be required, the surveillance of these patients may require no more than the examination of a peripheral blood smear (Wright's stain) for extracellular material. At this point it is prudent to reiterate the warning of Powars et al. Mucopolysaccharides are quite soluble in hydrophilic fixatives, such as formalin. Therefore, studies of "routinely" processed tissues are pointless. Fresh tissue must be fixed rapidly, avoiding hydrophilic materials.

In addition to the clinical perspective gained from this case, this report possibly adds support to the supposition that nephroblastomatosis is a precursor of nephroblastoma (Wilms' tumor). The precursor relationship between these two disease entities remains a point of contention. However, the similarity of findings in this report to those of other documented cases of GAG production associated with nephroblastoma does implicate a similarity of tumor cellular function. Hopwood and Dorfman have shown that nephroblastoma tumor cells grown in tissue culture produce much higher levels of GAG than normal renal tissue. Additionally, Hopwood demonstrated that GAG production by viral transformed skin fibroblasts also increases greatly. Documentation that nephroblastomatosis cells actually produce the GAG levels observed in this study does not exist, but the similarity between this case and the previously documented nephroblastoma studies does imply a congenersous mechanism.

Reports of tumor cell production of GAG tempt speculation on the reason for such metabolic activity. Reports exist that extend growth promoting properties, cell aggregation properties, and cell mobility effects to hyaluronate. Such reports clearly suggest a cell-mediated modulatory effect of tumor cell exudates and underscore the need to study further cases, such as the one presented here.

References


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### TABLE I

Renal Tissue Levels of Fractionated Glycosaminoglycan (µg per g tissue)*

<table>
<thead>
<tr>
<th>Tissue Specimen</th>
<th>Hyaluronate</th>
<th>Chondroitin Sulfate</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present patient:</td>
<td></td>
<td></td>
<td>81</td>
</tr>
<tr>
<td>R.B.</td>
<td>N.A</td>
<td>N.A</td>
<td></td>
</tr>
<tr>
<td>Wilms' tumor:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O.O.</td>
<td>85</td>
<td>35</td>
<td>120</td>
</tr>
<tr>
<td>M.B.</td>
<td>31</td>
<td>20</td>
<td>51</td>
</tr>
<tr>
<td>J.B.</td>
<td>28</td>
<td>20</td>
<td>48</td>
</tr>
<tr>
<td>A.P.</td>
<td>28</td>
<td>17</td>
<td>45</td>
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<tr>
<td>Fetal kidney:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>B-53</td>
<td>88</td>
<td>55</td>
<td>143</td>
</tr>
<tr>
<td>B-47</td>
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<td>B-45</td>
<td>70</td>
<td>44</td>
<td>114</td>
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<tr>
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<td></td>
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<tr>
<td>O.O.</td>
<td>39</td>
<td>0</td>
<td>39</td>
</tr>
</tbody>
</table>


†Result not available.


