Original article

Extracellular matrix changes in congenital hypertrophic pyloric stenosis

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Abstract. In pyloric stenosis, investigations of the histological pathology have concentrated on neuromuscular changes. These have shown a decrease in the innervation of the hypertrophied circular muscle and variable changes in the myenteric plexus. This study shows that the extracellular matrix (ECM) molecules have characteristic patterns in pyloric stenosis: chondroitin sulfate was markedly increased, with smaller increases in fibronectin and laminin. The increase in chondroitin sulfate was present in patients who were only several weeks old, with a short history, and in whom there was minimal circular muscle hypertrophy, and therefore was an early feature. It is not possible to ascribe aetiological significance to the increase in ECM molecules; nevertheless, the increase of chondroitin sulfate is the most likely explanation for the characteristic “cartilaginous” quality of the pyloric swelling.

Key words: Pyloric stenosis – Extracellular matrix molecules – Chondroitin sulfate – Laminin – Fibronectin

Introduction

Previous studies of the altered histology in pyloric stenosis specimens have shown a decrease in the innervation of the hypertrophied circular muscle [11, 19, 20, 22] with most studies showing some changes in the myenteric plexus [1, 2, 6, 7, 13, 15]. There has been considerable controversy over the interpretation of these changes in the ganglia, some authors suggesting they represent neuronal degeneration [1, 2, 13] while others propose immaturity [6, 7] or selective loss of Dogiel type I neurons [15]. Another recent interpretation of the range of histological patterns is a peripheral withdrawal of neural elements from their contacts with the circular muscle fibres, with resultant increased neuropeptide staining in the swollen nerve fibres situated in the connective-tissue septa as well as in the neuronal perikarya [5, 22]. The initiating event and early pathogenesis before clinical presentation remains unknown.

Previous studies have often commented on the increase in connective tissue, particularly of the septa that run between the circular muscle bundles [1, 2], but there has been no detailed investigation of any of the constituents. Recent studies have shown that the extracellular matrix (ECM) molecules are important in neural-cell migration and interaction with receptors not only during embryonic development, but also in postnatal wound healing [9, 10, 17, 23, 25]. These molecules may thus play a role in the pathogenesis of pyloric stenosis. This study examined several ECM molecules in the connective tissue of specimens removed at the time of pyloromyotomy. Chondroitin sulfate, fibronectin, and laminin were chosen as they are well-recognised ECM molecules and antibodies were available.

Materials and methods

Thirty specimens from pyloric stenosis patients were removed at the time of operation by making a second longitudinal incision approximately 2–3 mm in width. The specimen included tissue from near the pyloroduodenal junction up onto the gastric antrum. A suture was placed at the proximal end so the specimen could be orientated. These specimens were pinned and fixed in 10% formalin for 48 h, washed in phosphate-buffered saline (PBS) twice for 24-h periods, and stored in PBS/30% sucrose for at least 48 h before sectioning. The age range was 3 to 15 weeks.

Control specimens were obtained within 36 h of death from age-matched infants dying of sudden infant death syndrome (SIDS) and from several neonates. Previous studies have shown that, given early cooling, immunohistochemical staining is unchanged for at least 48 h after death [4, 22]. These control specimens were fixed in 10% formalin for 30 min and then opened along the greater curvature after which they were pinned to balsa wood and fixed for a further 3 days in 10% formalin. Longitudinal sections 5 mm wide and 2 cm long were cut from the pylorus and proximal duodenum and processed as above. A second control series comprised 6 pyloric specimens removed at the time of organ donation and immediately fixed. These secondary controls were important to ensure that ECM molecules were not altered quickly after death.
Fig. 1. Extracellular matrix molecules in the circular muscle of the human pylorus (all photographs × 25). Chondroitin sulfate (A–C): A Normal controls show minimal staining. B Pyloric stenosis: large increase in degree and distribution of staining. Arrows indicate the bright staining in the connective-tissue septa. In addition, there is increased staining around the muscle fibres (area between the arrows). C Neonatal obstruction control shows mild increase in staining relative to the normal control but this is markedly less than that found in pyloric stenosis. Fibronectin (D–F): D Normal control: clear demarcation of individual muscle fibres with little staining in the connective tissue septa. E Pyloric stenosis: moderate increase in staining but still restricted to around individual muscle fibres. Connective-tissue septa (dark areas) show no increase in staining. F Neonatal obstruction control: degree of staining is greater than in normal control tissue but less than in pyloric stenosis. Laminin (G–I): G Normal control: staining only around muscle fibres. H Pyloric stenosis: staining still restricted to muscle bundles but there are flecks of intense staining (arrows) associated with some muscle fibres. I Neonatal obstruction control: degree of staining similar to normal controls.

To see if the changes in ECM molecules could be the nonspecific result of bowel hypertrophy, a further control series consisted of specimens removed from the proximal thickened intestine of neonates with duodenal and ileal atresia. Histological processing was as above.

The pyloric and control tissues were comounted in tissue tech; 10 μm frozen sections were cut and placed onto polylysine-treated glass slides. Sections that were to be stained for chondroitin sulfate were treated with St. Marie fixation [18] to prevent later washing out of this ECM molecule during processing. Sections to be stained for laminin required pre-treatment with 0.5% trypsin for 30 min at room temperature [8]. The slides were air-dried for 2 h and then washed in distilled water and treated with non-immune sheep serum for 30 min (this step was omitted in sections for fibronectin staining as there is fibronectin in the sheep serum). Primary antibodies were applied overnight at room temperature. The chondroitin sulfate (courtesy B Geiger) was used at 1:10, the fibronectin and laminin (Bethesda Research Laboratories) at 1:400 dilution. After three washes with PBS the second fluorescein-labelled antibody (goat antirabbit FITC, CSL 1:80) was applied for 1 h at room temperature. Slides were then washed in PBS, cover-slipped, sealed with nail polish, viewed using a Wild-Lietz Fluorovert, and photographed using Kodak TX400 film.

Specimens for electron microscopy were fixed in Karnovsky’s solution for 48 h, changed to MOPPS, and routinely processed. Sections were viewed on a Phillips EM 400 microscope.

Results

There was no difference between control tissue fixed immediately at the time of organ donation and tissue removed 36 h after death from SIDS. Therefore, there was no post-mortem leaching out of the ECM molecules.

In pyloric stenosis the most prominent feature was the dramatic increase in chondroitin sulfate staining (Fig. 1A–C). The increase involved all layers and extended up onto the gastric antrum. The heaviest staining was in the pyloric region. In control tissue there was minimal staining (Fig. 1A). In pyloric stenosis the increase in chondroitin sulfate involved not only the connective tissue septa between circular muscle bundles, but there was also dense staining among the circular muscle fibres (Fig. 1B). The increase in staining was present in several specimens from patients who had a very short history and who at the time of operation had early thickening of approximately 3 mm restricted to the lesser curvature area. In these same specimens immunoreactivity to glial, neural, and neuropeptide markers was already decreased [22]. In specimens from infants who had presented at about 10–15 weeks there was still increased chondroitin sulfate, but the staining ap-