Deficiency of laryngeal collagen type II in an infant with respiratory problems

K Frenzel, G Amann, B Lubec

Abstract
A dysmorphic infant is described who presented with laryngeal collapse leading to intubation and respiratory problems that were assigned clinically to the Sussman syndrome. The baby had repeated episodes of respiratory distress necessitating assisted ventilation. At 6 months old, uvulopharyngopalatotomy was done to enlarge the supraglottic airway without any benefit. Surgical reduction of the tongue and cricoid splitting did not ameliorate the respiratory distress; repeated extubation attempts failed with the baby developing stridor, respiratory distress, and episodes of cardiac arrest. At 10 months old he developed seizures and computed tomography showed diffuse cerebral atrophy consistent with hypoxic-ischaemic damage. He died at 17 months old.

In addition to persistent respiratory problems the child had dysmorphic features including short stature, short neck, coarse facial features, enlargement of the tongue, ptosis of the left eye, short limbs with thick, tapering fingers, redundant skin over the palms and feet, puffiness over the dorsum of the hand, hypoplastic toenails, and floppiness.

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Based on clinical symptoms and phenotype we diagnosed Sussman syndrome, although lack of segmentation of the thoracolumbar vertebrae, metaphyseal modelling abnormalities, and small secondary ossification centres located abnormally to the metaphyses were not found on radiography up to 6 months of age.

Investigations
The larynx, freed from adjacent connective tissue, was obtained at necropsy. It was kept frozen at −70°C until sample preparation for biochemical studies, minced to small pieces, washed in isotonic saline, homogenised in an ice bath, and acid salt soluble collagen was extracted. Homogenised material was incubated in 0.05 M acetic acid containing 0.005 M EDTA and 5 mg pepsin (Merck, Darmstadt, Germany) per 100 mg (wet weight) tissue.

The tissue was incubated for 72 hours at 4°C on a shaker. The samples were spun down in a refrigerated centrifuge at 4000 ×g, the
supernatant was filtered through Millipore filters, and the protein concentration measured by the method of Bradford using a commercially available colorimetric assay (BioRad, Laboratories, Munich, Germany).

SDS polyacrylamide gel electrophoresis was done as described previously. Protein (100 µg) was put in each well and gels were stained with a commercially available silver stain kit (BioRad Laboratories). Corresponding triplicates were unstained and transferred to nylon membranes for western blotting. Western blots were done according to a standard method. Polyclonal antibodies against collagens type I (α1(I)) and II (Institut Pasteur, Paris, France) were used.

MEASUREMENT OF LARYNX COLLAGEN
After mechanical removal of adjacent tissue, laryngeal tissue was defatted by diethylether: chloroform (1:1) and freeze dried. The dry tissue was weighed and 4-hydroxyproline was measured by the method of Woessner. The hydroxyproline content per milligram of larynx tissue was calculated and considered to reflect total collagen content. Laryngeal tissue from three age matched children was used for comparison of collagens type I and II, and for quantitative collagen studies.

Results
Western blots of extracted collagens from our patient showed an absence of the α1(II) band (fig 1A). The band assigned to collagen α1(I) was found in laryngeal tissue from our patient comparable to the three control samples (fig 1B).

Quantitative Studies
The mean (SD) 4-hydroxyproline content of the patient’s larynx was less than for the three control children: 17 (1.3) µg/mg laryngeal tissue for the patient; 49 (7.9) µg/mg for the three controls.

Discussion
This is the first report of an absent collagen type II chain in the larynx. Mechanoeelastic properties changed by the absence of this structural protein may well be compatible with impaired laryngeal function. Mechanisms leading to absent collagen chains in general have been described and range from mutations to impaired post-transcriptional modifications and impaired secretion. Our patient had normal morphology by radiography; therefore, we suggest that the larynx was the only tissue with collagen type II deficiency, at least at the time of examination.

Kratochwil et al showed that retrovirus induced insertion mutation in mov13 mice affects collagen expression in a tissue and chain specific manner, and this model could explain our findings of tissue (and chain) specificity.

Another tentative mechanism for the absence of collagen type II exclusively from the larynx could be explained by the observations of Ryan and Sandell who showed that there were different mRNA populations for collagen type II. Metsaranta et al generated transgenic mice by microinjection of a 39 kb mouse pro α1(II) collagen gene construct with a deletion of exon 7 and intron 7. This mutation was expected to disturb the assembly and processing of the homotrimeric type II collagen molecule in cartilage. The result of this genetic manipulation was a severe chondrodysplastic phenotype with short limbs, hypoplastic thorax, and abnormal craniofacial development; the affected pups died at birth from respiratory distress. We postulate that the deletion in the α1(II) collagen acts as a dominant negative mutation disrupting the assembly and secretion of type II collagen molecules. The transgenic mice had exactly the same phenotype and clinical appearance as our patient, but the authors did not investigate the larynx of the transgenic model.

The possibility that collagen type II was not expressed in the larynx during early infancy can be ruled out, as Cohen et al demonstrated that type II collagen appeared soon after birth with levels increasing rapidly during the first months. The finding of a specific structural (biochemical) defect of laryngeal tissue in a dysmorphic child stimulated us to propose this mechanoelastic–biochemical link.

2 Krawston RG, Weaver EJ, Struyk AF, et al. Genetic linkage of hereditary arthro-ophthalmopathy (Stickler syndrome)
Coagulation tests in inflicted head injury

When child abuse is suspected the finding of abnormal coagulation test results may be seized upon by the defence as evidence of pre-existing bleeding disorder. Now data from Denver, Colorado (Kent P Hymel and colleagues, Pediatrics 1997;99:371-5) suggest that inflicted head trauma may cause such abnormalities.

The records of 265 children with inflicted head injury were reviewed and adequate data were available from 147 of whom 101 had computed tomography and/or magnetic resonance imaging evidence of injury to brain substance (IBS). A mildly prolonged prothrombin time was found in 54% of patients with and 20% without IBS. Median prothrombin time (normal 11.8 +/-1.0) was 13.1 with IBS and 12.0 without. Presumptive evidence of disseminated intravascular coagulation was found in 37% of IBS and 7% of non-IBS patients. Mortality in IBS children was 32% and of those who died 94% had prolonged prothrombin time and 63% evidence of disseminated intravascular coagulopathy.

Head injury in children may cause coagulation test abnormalities and the medicolegal significance of this finding needs to be acknowledged.
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