Hyaluronan in the nuchal skin of chromosomally abnormal fetuses

S. Böhältldt1, C.S. von Kaisenberg1,2,3, K. Wewetzer1,4, B. Christ1,4, K.H. Nicolaides2 and B. Brand-Saberi1,5

1Department of Anatomy, University of Freiburg, Germany, 2Harris Birthright Research Centre for Fetal Medicine, King’s College Hospital, London, UK, 3Department of Obstetrics and Gynaecology, University Hospital, Kiel and 4Centre of Anatomy, Medical School Hannover, Germany

Nuchal skin oedema at 10–14 weeks gestation, observed by ultrasonography as increased nuchal translucency (NT), is found in ~70% of fetuses with trisomies 21, 18 and 13 as well as those with Turner’s syndrome. This study investigates the possibility that one mechanism for increased translucency is an altered composition of the skin with a higher concentration of hyaluronan; large amounts of hyaluronan can lead to excessive hydration of the extracellular matrix. We isolated the hyaluronic acid binding region (HABR) from aggrecan in the extracellular matrix of hyaline cartilage and used it in a biotinylated form in combination with a fluorescent probe as a marker for hyaluronan. Immunohistochemistry was then used to examine the nuchal skin of chromosomally abnormal and normal fetuses, obtained after termination of pregnancy. In fetuses with trisomy 21 there was a substantial increase in hyaluronan, whereas in trisomies 18 and 13 and Turner’s syndrome the amount was similar to that in chromosomally normal controls. This finding suggests that hyaluronan may be implicated in the pathogenesis of increased NT in fetuses with trisomy 21, but the common phenotypic expression of increased translucency in different chromosomal abnormalities may be the consequence of other mechanisms.

Key words: extracellular matrix/hyaluronan/nuchal translucency/trisomies/Turner’s syndrome

Introduction

Trisomy 21 and all other major chromosomal abnormalities are associated with interstitial oedema in the neck region which can be visualized by ultrasound examination at 10–14 weeks gestation as increased fetal nuchal translucency (NT) thickness (Nicolaides et al., 1992). Fetal NT thickness has now been combined with maternal age to provide the most effective method of early screening for chromosomal abnormalities (Snijders et al., 1998). Increased NT is also observed in a high proportion of fetuses with cardiac defects, and a wide range of other major structural abnormalities and genetic syndromes (Souka et al., 1998).

The heterogeneity in conditions associated with increased NT suggests that there may not be a single underlying mechanism for the interstitial oedema in the fetal neck. Possible mechanisms include (i) altered composition of the interstitial connective tissue (Brand-Saberi et al., 1994a,b; von Kaisenberg, 1998a,b), (ii) abnormal or delayed development of the lymphatic system (von Kaisenberg et al., 1999a), (iii) cardiac failure in association with abnormalities of the heart and great arteries (Hyett et al., 1996, 1997; von Kaisenberg et al., 1997, 1998c), and (iv) venous congestion in the head and neck in association with the constriction of the fetal body in amnion rupture sequence, superior mediastinal compression found in diaphragmatic hernia and the narrow chest in skeletal dysplasia, or failure of lymphatic drainage due to impaired fetal movements in various neuromuscular disorders (Souka et al., 1998).

In terms of the possible role of the altered composition of the interstitial connective tissue, ultramorphological studies in nuchal skin of fetuses with trisomy 21 showed a large amount of fine flocculate precipitate around bundles of collagen fibrils (Brand-Saberi et al., 1994a). The amount of precipitate was much lower in the skin of trisomy 18 fetuses (Brand-Saberi et al., 1994b), whereas in trisomy 13 there were regions with very little precipitate alternating with regions of high concentration (von Kaisenberg et al., 1998b). Furthermore, in the skin of trisomy 21 fetuses there is overexpression of collagen type VI (von Kaisenberg et al., 1998a). Collagen type VI binds to hyaluronan (McDevitt et al., 1991). Hyaluronan-based extracellular matrices have open structures that entrap large amounts of solvent and may therefore play an important role in the pathogenesis of nuchal oedema. This study investigates the possibility that one mechanism for increased translucency is an altered composition of the skin with abundance of hyaluronan using a highly specific technique for the detection of this molecule.

Materials and methods

Fetal tissues

Surgical termination of pregnancy was performed at the request of the parents for medical or psychosocial reasons. Nuchal skin from fetuses with trisomy 21 (n = 4), trisomy 18 (n = 4), trisomy 13 (n = 3), Turner’s syndrome (n = 2), and normal controls (n = 2) at 11–18 weeks gestation was examined. Written informed consent was obtained, the study was approved by the hospital ethical committee and tissue collection was made in accordance with the Polkinghorne guidelines on the research use of fetal tissue (Polkinghorne, 1989). The specificity of the hyaluronan binding region was examined...
histochemically using a chicken model with known amount and distribution of hyaluronan.

Preparation of the biotinylated hyaluronan binding region

The hyaluronan binding region (HABR) was isolated from bovine cartilage by combination of hydroxyapatite chromatography and gel filtration under associative and dissociative conditions as previously described (Tammi et al., 1994). Briefly, proteoglycans were extracted from cartilage obtained from a local butchery, using 4 mol/l guanidinium chloride in 0.05 mol/l sodium acetate (pH 5.8). After stirring overnight at 4°C, the solution was cleared by centrifugation and filtration. Subsequently, 200 mg hyaluronan (Healon, Pharmacia, Freiburg, Germany) per 100 g cartilage was added. Subsequently, dialysis against water was done overnight at 4°C. After trypsin digestion, the material was applied to a hydroxyapatite column (BioRad, Munich, Germany) equilibrated with 0.003 mol/l sodium phosphate buffer (pH 6.8). After washing, bound material was eluted with 0.01 mol/l sodium phosphate buffer (pH 7.0). The fractions were frozen at −80°C or further processed.

The eluted fraction was concentrated by filtration using an Amicon filtration chamber with a Diaflo PM30 membrane (Amicon, Beverly, MA, USA). Gel filtration over Sephacryl S1000 (Pharmacia) was done in 0.5 mol/l sodium acetate buffer (pH 7.0). Fractions near the exclusion volume were pooled, dialysed against water and lyophilized. Biotinylation of the hyaluronic acid–hyaluronan binding region complex using N-hydroxysuccinimide (NHS)–biotin was carried out (Ripellino et al., 1985). NHS–biotin 1 mg dissolved in dimethylsulphoxide (DMSO) was mixed with 1 mg of the hyaluronic acid–hyaluronan binding region complex dissolved in 0.1 mol/l sodium phosphate buffer (pH 7.4) and incubated at room temperature for 4 h. After dialysis against water and lyophilization, the material was applied to a Sephacryl S400-HR (Pharmacia) column. Separation of hyaluronan binding region from hyaluronic acid was done under dissociative conditions (4 mol/l guanidinium chloride in 50 mmol/l sodium acetate buffer, pH 5.8). Fractions were pooled, dialysed against water and stored at −80°C, either lyophilized or dissolved in phosphate-buffered saline (PBS) containing 6% bovine serum albumin (BSA).

The efficiency of the chromatographic steps was confirmed by dot blotting of different bound and non-bound fractions using a monoclonal antibody against the HABR (1C6) as described (Sorrell et al., 1999). Protein content was determined by the Bradford method (BioRad; Bradford, 1976) using BSA as a standard. The amount of purified HABR obtained out of 1 g cartilage ranged between 0.27 mg and 0.35 mg.

Histochemistry

Skin was taken from the nuchal region of fetuses immediately after surgical termination of pregnancy and was then embedded in Tissue Tek (Leica, Bensheim, Germany) without prior fixation and frozen in liquid nitrogen, as described earlier (von Kaisenberg et al., 1998a,b). Controls were carried out on paraformaldehyde-fixed sections that did not differ in result. Frozen sections (20 μm) were collected on gelatin-coated slides. After air drying, cryosections of nuchal skin from trisomic and normal fetuses were treated with 1% BSA in 0.1 mol/l potassium phosphate buffer (pH 7.6) for 10 min to block non-specific binding, and afterwards incubated with biotinylated HABR for 1 h. The HABR is the region of the core protein of aggrecan, which binds the hyaluronan side chains in association with a link protein. After washing with potassium phosphate buffer, the cryosections were stained in the dark for 1 h with streptavidin–Texas Red (1:100) at room temperature. Cryosections incubated with hyaluronidase as well as cryosections incubated with biotinylated HABR and an excess of soluble hyaluronan did not show any staining. The sections were examined by epifluorescence microscopy and photographed using TMY-400 black-and-white film (Ektachrome).

Results

In the chromosomally normal fetuses, hyaluronan was detected using a biotinylated fragment of aggregan containing the HABR. Hyaluronan was localized mainly in the upper dermis, and, to a lesser extent, in the epidermis; the subcutis and the basement membrane were barely stained (Figure 1).

In trisomy 21 fetuses there was strong staining of hyaluronan, especially in the dermis and to a lesser extent in the subcutis (Figure 1a,b). In fetuses with trisomy 18 the nuchal skin showed disseminated staining, mostly in the dermis, but this was less intense than in trisomy 21 (Figure 1c). In skin from trisomy 13 fetuses there was an irregular pattern of hyaluronan with a cluster-like distribution in the upper and lower dermis, subcutis and basement membrane; the epidermis showed no staining (Figure 1d). In the skin from fetuses with Turner’s syndrome, staining was restricted to the upper dermis and did not differ from fetuses with normal karyotype in its distribution to (Figure 1e,f).

The distribution of hyaluronan relates to our previous immunohistochemical findings on the distribution of collagen type VI (Table 1), which showed a similar distribution in the dermis, subcutis and basement membrane (von Kaisenberg et al., 1998b).

Discussion

We examined the distribution of hyaluronan in the nuchal skin of chromosomally abnormal fetuses and normal age-matched controls using a biotinylated probe for hyaluronan (HABR) prepared from cartilage.

In fetuses with trisomy 21, but not in trisomies 18 or 13 and Turner’s syndrome, hyaluronan is abundant in the nuchal skin, especially in the dermis. These findings suggest that hyaluronan may be implicated in the pathogenesis of increased NT in fetuses with trisomy 21, but the common phenotypic expression of increased translucency in different chromosomal abnormalities may be the consequence of other mechanisms (von Kaisenberg et al., 1999b). It is of interest that also in postnatal life, conditions with increased amounts of hyaluronan, such as inflammatory rheumatic diseases or cirrhotic liver disease (Engström-Laurent et al., 1989), are associated with interstitial oedema (Hay, 1991).

The wide distribution and prolonged presence of hyaluronan in the skin of trisomy 21 fetuses may be the consequence of increased synthesis or decreased degradation. The metabolism of hyaluronan is a complex process that involves hyaluronan synthase, the hyaladherin CD44 receptor, and catabolic enzymes. Known hyaluronan synthases are HAS1 (Itano and Kimata, 1996; Shyjan et al., 1996), HAS2 (Watanabe and Yamaguchi, 1996), and HAS3 (Povey, 1996), but the gene loci for these enzymes have not yet been mapped. The CD44 receptor, which mediates adhesion of hyaluronan, has been mapped to 11p13 (Goodfellow et al., 1982; Ala-Kapee et al.,
Hyaluronan in nuchal skin of aneuploid fetuses

Figure 1. Visualization of hyaluronan, using biotinylated hyaluronan binding region and detection with Texas Red–streptavidin, in nuchal skin from fetuses with trisomy 21 (a, b), trisomy 18 (c), trisomy 13 (d), Turner’s syndrome (e) and normal karyotype (f). Bar = 50 mm.

Table 1. Immunohistochemical findings in the extracellular matrix of chromosomally abnormal fetuses

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 2)</th>
<th>Trisomy 21 (n = 4)</th>
<th>Trisomy 18 (n = 4)</th>
<th>Trisomy 13 (n = 3)</th>
<th>Turner’s syndrome (45,XO) (n = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hyaluronan</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epidermis</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>(+)</td>
<td>−</td>
</tr>
<tr>
<td>Dermis</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Subcutis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Basement</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td><strong>Collagen type VIa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epidermis</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>nd</td>
</tr>
<tr>
<td>Dermis</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>nd</td>
</tr>
<tr>
<td>Subcutis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>nd</td>
</tr>
<tr>
<td>Basement</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>nd</td>
</tr>
</tbody>
</table>

*Previously published results (von Kaisenberg et al., 1998b).
nd = not determined.

1989) and it is therefore unlikely to be altered in trisomy 21. Catabolic enzymes involved in hyaluronan degradation, located predominantly in the liver, include hyaluronidase, glucosaminidase, acetylhexosaminidase and a number of glucuronidases (Nakamura et al., 1996). The genes for these enzymes have not yet been mapped. However, superoxide dismutase, which protects against free radical mediated degradation of hyaluronan, is encoded by chromosome 21 (21q22.1) (De La Torre et al., 1996). Consequently, the increased amount of hyaluronan in the skin of trisomy 21 fetuses (i.e. having three copies of the gene relating to superoxide dismutase instead of two) may be due to decrease in degradation.
Another possible mechanism for increase of hyaluronan in the skin of trisomy 21 fetuses is the presence of a high amount of collagen type VI (von Kaisenberg et al., 1998a,b). This collagen, which has a high capacity for binding hyaluronan (McDevitt et al., 1991), is composed of three chains and two of these are encoded by genes located on chromosome 21 (Weil et al., 1988).

However, an increased amount of hyaluronan was also found to be present in the nuchal skin of murine trisomy 16 fetuses (the animal model for human trisomy 21), which do not overexpress collagen type VI (von Kaisenberg et al., 1998b).

In trisomy 21, the abundance of hyaluronan may not be restricted to the nuchal skin but it is probably found in other tissues and organs contributing to the pathogenesis of the various abnormalities in this chromosomal defect. A single chain of hyaluronan, through link proteins, is bound to a large number of aggregan monomers and these large aggregates can inhibit cell movement. For example, matrices containing hyaluronan-aggregating proteoglycans inhibit the migration of neural crest cells by interacting with the cell surface, to which the molecules bind via their hyaluronic acid binding regions to cell membrane-anchored hyaluronan (Perris and Johansson, 1990). This may be the underlying mechanism for Hirschsprung’s disease, which is associated with trisomy 21 and is thought to be the consequence of failure of migration of neural crest cells to the bowel segments which generally occurs between weeks 6 and 12 of gestation.

The availability of hyaluronan seems to be a prerequisite for normal migration of enteric ganglia precursors to occur, since its presence precedes that of neural crest cells on their pathway (Fujimoto et al., 1989). Moreover, altered distribution or composition of the extracellular matrix has been reported to occur in the aganglionic segments of the bowel in the murine ls/ls (lethal spotted) mutant (Payette et al., 1988).

Acknowledgements

We thank Drs H.-D. Hofmann and M. Kirsch for their kind advice during the preparation of HABR and for providing the cold laboratory. The IC6-antibody was obtained from the Developmental Studies Hybridoma Bank, Iowa, USA. We wish to thank Ellen Gimbel for her technical assistance and Christa Micucci for photographic assistance. This publication was supported by a grant from the Deutsche Forschungsgemeinschaft (Ka 1136/1–1) and from the Fetal Medicine Foundation.

References


Povey, S. (1996) Nomenclature Committee reserved symbol.


Received on August 31, 1999; accepted on December 6, 1999.