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Pre-eclampsia-associated alterations in Wharton's jelly proteoglycans*

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Proteoglycans of Wharton's jelly contain mainly chondroitin/dermatan sulphate chains. The predominant proteoglycan is decorin (core proteins of 45 and 47 kDa), although the core proteins of biglycan (45 kDa), versican (260 kDa) and of other proteoglycans (90, 110, 220 kDa) were also detected (Gogiel *et al.*, 2003). The aim of the present study was to compare the proteoglycan composition of Wharton's jelly of newborns delivered by healthy mothers and those with pre-eclampsia. Proteoglycans from pre-eclamptic Wharton's jelly had a higher sulphated glycosaminoglycan/protein ratio than those of normal tissue. Pre-eclampsia is associated with a lower level of all proteoglycan core proteins, especially those of higher molecular mass (such as versican), although the same set of core proteins were found in normal and pre-eclamptic Wharton's jelly. The alterations in the proteoglycan composition of Wharton's jelly may affect the mechanical properties of the umbilical cord and, in the case of pre-eclampsia, disturb foetal blood circulation.

Keywords: glycosaminoglycans, pre-eclampsia, proteoglycans, umbilical cord, Wharton's jelly

The intrauterine foetal development is highly dependent on the vascular system of the mother, foetus and placenta (Howard, 1987). It is severely affected in pre-eclampsia (PE), also known as edema, proteinuria, and hypertension (EPH)-gestosis, the most common pregnancy-associated pathological syndrome (Barthel-Wottke & Goecke, 1978; Bańkowski *et al.*, 1993; Bańkowski, 1999).

The umbilical cord forms a connection between the placenta and foetus. It contains one vein and two arteries surrounded by Wharton's jelly and covered by simple amniotic epithelium. Some prenatal pathological processes may be caused by biochemical and morphological alterations in the umbilical cord, especially in Wharton's jelly (Bańkowski, 1999).

Proteoglycans (PGs) are macromolecules built of protein cores covalently attached to sulphated glycosaminoglycans (GAGs) (Iozzo & Murdoch, 1996; Hascall *et al.*, 1997). PGs perform numerous functions. They affect the mechanical properties of tissues, regulate collagen matrix organization, participate in cell–cell and cell–extracellular matrix interactions, bind growth factors, enzymes, viruses, yetc. (Iozzo & Murdoch, 1996; Hascall *et al.*, 1997). We have recently described that PE is associated with alterations in PG composition of the umbilical cord arteries (UCAs). The feature of pre-eclamptic UCA-PGs is a higher sulphated GAG/protein ratio. PE is also associated with a higher content of small PG core proteins (especially decorin) and a lower level of large PG core proteins. Furthermore, a decreased content of biglycan core protein was found (Gogiel *et al.*, 2001).

The role of the Wharton's jelly proteoglycans in the pathomechanism of PE has not hitherto been studied. Therefore, we have decided to compare the PG composition of normal Wharton's jelly with that of newborns delivered by mothers with pre-eclampsia.

MATERIALS AND METHODS

Tissue material. Studies were performed on 20 samples of Wharton's jelly taken from 20 newborns. The control material (normal Wharton's jelly) was taken from 10 newborns delivered by healthy

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Abbreviations: CS, chondroitin sulphate; CS/DS, chondroitin/dermatan sulphate; DS, dermatan sulphate; EPH, edema, proteinuria, hypertension; GAG, glycosaminoglycan; PE, pre-eclampsia; PG, proteoglycan; UCA, umbilical cord artery.

mothers, aged 23–36, with normal blood pressure (systolic 110–135 mm Hg, diastolic 60–80 mm Hg), without any symptoms of edema or renal failure. The babies were born between the 38th and 41st week of gestation. Their mean body mass was 3560 ± 396 g. The pre-eclamptic material was taken from 10 newborns delivered by mothers, aged 19-31, with pre-eclampsia (EPH-gestosis). The mothers demonstrated an elevated blood pressure (systolic >140 mm Hg, diastolic > 90 mm Hg), proteinuria (above 500 mg/l) and edema after bed rest (Barthel-Wottke & Goecke, 1978). Their babies were born between the 35th and 40th week of gestation. The mean body mass of these newborns was 3205 ± 597 g. In all cases, sections (20 cm long) of the umbilical cords were excised beginning at their placental end. Wharton's jelly was carefully dissected from the umbilical cord vessels immediately after delivery and stored at –20°C.

Extraction and isolation of Wharton's jelly proteoglycans. The general schema of PG extraction, described in our previous paper (Gogiel & Jaworski, 2000), was applied. Briefly, samples of Wharton's jelly were homogenized and subjected to two-step extraction with a buffer containing 4 M guanidine-HCl and 2% (v/v) Triton X-100 in 0.1 M sodium acetate (pH 5.8), and a mixture of protease inhibitors (Gogiel & Jaworski, 2000; Gogiel *et al.*, 2001). The non-extractable residues were digested with papain and tested for sulphated GAGs (see below) to estimate the amount of PGs unsusceptible to extraction (Gogiel & Jaworski, 2000; Gogiel *et al.*, 2001). The extracts were dialyzed against a 7 M urea-containing buffer, purified on a Q-Sepharose Fast Flow column, dialyzed against double distilled water at 4°C, frozen (–20°C) and lyophilized (Gogiel & Jaworski, 2000; Gogiel *et al.*, 2001).

SDS/PAGE of digested and non-digested PG samples was performed under reducing conditions on 0.75 mm-thick, 80 mm-wide isocratic gels (10% separating with 4% stacking gels) according to the procedure of Laemmli (1970). Protein molecular mass markers from Sigma (SDS-6H) were used. Electrophoresis was run at a constant current of 25 mA. The gels were stained with Alcian Blue and Coomassie Brilliant Blue R-250 followed by silver nitrate as described in a previous paper (Gogiel & Jaworski, 2000).

Western immunoblotting to detect decorin, biglycan, versican and perlecan was performed as we previously described (Gogiel *et al.*, 2001; 2003). Molecular mass of PG core proteins were estimated using pre-stained molecular mass markers (Sigma) which were pre-calibrated with the SDS-6H markers by SDS/PAGE.

The polyclonal antibodies used for immunodetection included anti-human decorin antiserum (LF-136) and anti-human biglycan antiserum (LF-51). The polyclonal antisera were raised in rabbits against a specific synthetic peptide corresponding to the N-terminal fragments of the two PGs (Fisher *et al.*, 1995). The antisera were a generous gift of Dr. Larry W. Fisher (National Institute of Dental and Craniofacial Research, NIH, Bethesda, MD, USA). Monoclonal antibodies included anti-large proteoglycan antibody, clone 2-B-1 (Calbiochem®, a brand of EMD Biosciences, Inc., an Affiliate of Merck KGaA, Darmstadt, Germany) that recognizes human versican (Isogai *et al.*, 1996), and anti-perlecan antibody, clone 7B5 (Zymed Laboratories, Inc., South San Francisco, CA, USA), that recognizes perlecan domain III.

Sulphated GAGs were assayed in duplicate samples by the dimethylmethylene blue binding method (Farndale *et al.*, 1982) using chondroitin 4-sulphate (Sigma) as a standard.

Chondroitinase ABC digestion and protein determination (Zor & Selinger, 1996) were performed as described in a previous paper (Gogiel *et al.*, 2001).

Statistical analysis. Mean values from ten experiments ±S.D. were calculated. The results were submitted to statistical analysis with the use of Student's *t*-test, accepting $P < 0.05$ as significant.

RESULTS AND DISCUSSION

As can be seen from Fig. 1a, similar amounts of sulphated GAGs (reflecting the presence of PGs) were isolated from normal and pre-eclamptic Whar-

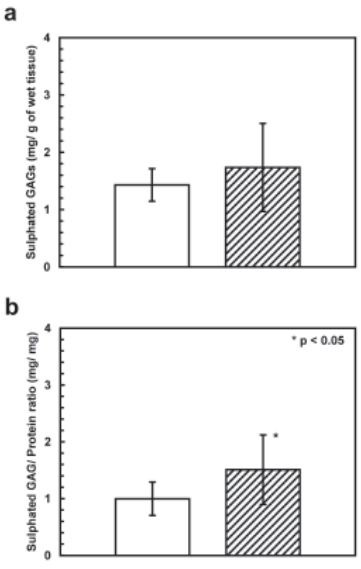

Figure 1. The amount of sulphated GAGs (a) and sulphated GAG/protein ratio (b) in purified proteoglycans from normal and pre-eclamptic Wharton's jelly.

Sulphated GAGs (reflecting the presence of PGs) were measured by the dimethylmethylene blue binding method. Open bars, PGs from normal Wharton's jelly; dashed bars, PGs from pre-eclamptic Wharton's jelly.

ton's jelly. However, a significantly higher sulphated GAG/protein ratio was found in PG preparations purified from pre-eclamptic material than from normal Wharton's jelly (Fig. 1b). This phenomenon may result from slower degradation of sulphated GAGs that was found in pre-eclamptic UCAs (Romanowicz *et al.*, 1999a) and Wharton's jelly (Romanowicz *et al.*, 1999b).

Combined PGs, containing equal amounts of sulphated GAGs, were subjected to SDS/PAGE, both as intact PGs and after digestion with chondroitinase ABC. A representative electrophoregram is shown in Fig. 2.

Electrophoresis of intact PGs of normal (lane 1) and pre-eclamptic (lane 3) Wharton's jelly revealed two broad bands, one of about 120–190 kDa and the other higher than 220 kDa. These bands appear to correspond to decorin and biglycan, respectively. A very wide range of molecular masses has been reported for intact forms of both biglycan (about 120–140, 200, 220, and 250 kDa) (Wight, 1989; Järveläinen *et al.*, 1991; Nelimarkka *et al.*, 1997) and decorin (about 130, 90–150 and 120–180 kDa) (Wight, 1989; Järveläinen *et al.*, 1991; Delorme *et al.*, 1998). An intensive broad band in the stacking gel, close to the top of the separating gel, is also visible (Fig. 2, lanes 1 and 3). It probably reflects the presence of large PGs, such as versican. Versican is a large PG containing multiple chondroitin sulphate/ dermatan sulphate (CS/DS) chains (Iozzo & Murdoch, 1996) and because of its molecular mass (over 1000 kDa) it hardly penetrated the separating gel.

SDS/PAGE of intact PGs from both the normal and pre-eclamptic Wharton's jelly revealed bands of almost the same intensity and mobility. This was not surprising because these samples contained equal amounts of sulphated GAGs that stain more strongly than protein with the staining method applied.

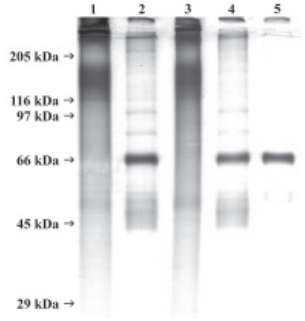

Figure 2. SDS/PAGE of intact proteoglycans and their core proteins from normal and pre-eclamptic Wharton's jelly.

Intact PG samples, containing equal amounts (1.5 μg) of sulphated GAGs were applied undigested and after treatment with chondroitinase ABC (for details see Methods). Lane 1, intact PGs from normal Wharton's jelly; lane 2, PGs from normal Wharton's jelly treated with chondroitinase ABC; lane 3, intact PGs from pre-eclamptic Wharton's jelly; lane 4, PGs from pre-eclamptic Wharton's jelly treated with chondroitinase ABC; lane 5, chondroitinase ABC.

The action of chondroitinase ABC resulted in an almost complete disappearance of all broad PG bands, both in the case of normal (lane 2) and pre-eclamptic Wharton's jelly PGs (lane 4). This points to a significant predominance of CS/DS-PGs in Wharton's jelly. Treatment with the enzyme resulted in the appearance of new bands of about 260, 220, 110, 90, 47 and 45 kDa (lanes 2 and 4). The intensive bands of 45 and 47 kDa are typical for two variants of the decorin core protein, having two or three N-linked oligosaccharides (Fisher, 1999a). The 45-kDa band is also characteristic of the core protein of biglycan (Iozzo & Murdoch, 1996; Fisher, 1999b) and the band of 260 kDa was shown to be immunologically related to versican (see below). The other, faint bands (of 220, 110 and 90 kDa) do not correspond to perlecan as no bands were detected by anti-perlecan

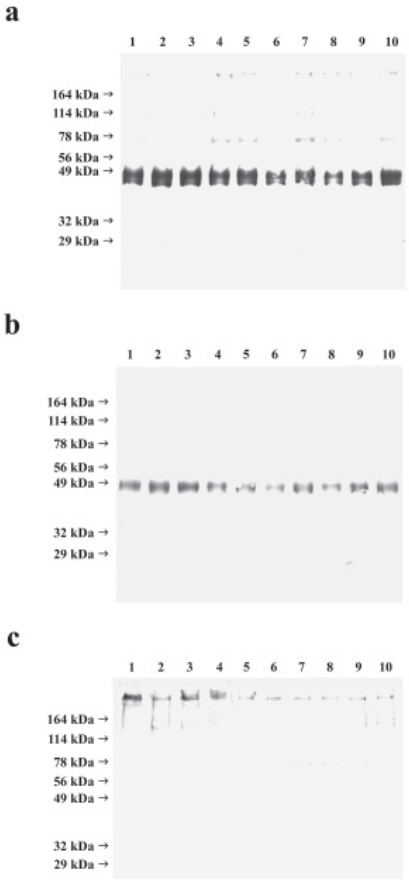

Figure 3. Western blot analysis to detect decorin (a), biglycan (b), and versican (c) in proteoglycan core proteins from normal and pre-eclamptic Wharton's jelly.

Proteoglycan core proteins were obtained by treatment of intact PGs, containing equal amounts of sulphated GAGs, i.e. 1.5 μg (a, b) or 4 μg (c), with chondroitinase ABC (for details see Methods). Lanes 1–5, PG core proteins from five individual normal samples; lanes 6–10, PG core proteins from five individual pre-eclamptic samples.

antiserum. The band of 220 kDa may represent an alternative splicing variant of versican, not reacting with the anti-versican antibody, and the bands of 90 and 110 kDa — the core proteins of transmembrane PGs, beta-glycan and CD44 proteoglycan, as we previously discussed (Gogiel *et al.*, 2001). However, the core proteins of 90, 110 and 220 kDa were not detected by any antibody used, therefore their identity remains to be established. As can be seen, all the bands are less intensive for the chondroitinase ABC digest of pre-eclamptic Wharton's jelly PGs (lane 4) than of normal material (lane 2; for discussion see below).

The most prominent 66-kDa band (lanes 2 and 4) is also present in the sample containing chondroitinase ABC alone (lane 5), and it probably corresponds to bovine serum albumin present in the enzyme preparation as a stabilizer.

Western blots to detect decorin, biglycan and versican in chondroitinase ABC digestion products of individual PG preparations, containing equal amounts of sulphated GAGs, are shown in Fig. 3. A double band of 45 and 47 kDa, typical for decorin core protein (Fig. 3a), and the 45-kDa band characteristic of biglycan core protein (Fig. 3b) are visible in chondroitinase ABC digestion products of both normal (lanes 1–5) and pre-eclamptic (lanes 6–10) Wharton's jelly PGs. As can be seen, a slightly lower signal was observed for both PGs in most pre-eclamptic samples (Figs. 3a and 3b, lanes 6–10). A Western blot to detect versican is shown in Fig. 3c. A band of 260 kDa was detected in chondroitinase ABC digestion products of both normal (lanes 1–5) and pre-eclamptic (lanes 6–10) Wharton's jelly PGs. A much lower signal was observed in all pre-eclamptic samples (Fig. 3c, lanes 6–10). The level of decorin core proteins (Fig. 3a) was much higher than that of the core proteins of biglycan and versican (Figs. 3b and 3c, respectively), both in the case of normal (lanes 1–5) and pre-eclamptic (lanes 6–10) material.

Neither normal nor pre-eclamptic intact PG preparations reacted with anti-decorin and anti-biglycan antisera, or with anti-versican antibody. Also, normal and pre-eclamptic (intact or digested) PGs did not react with anti-perlecan antibody (not shown).

It is of interest that versican and biglycan are localized close to the cell surface (Iozzo & Murdoch, 1996; Fisher, 1999a). Versican is also known to destabilize cell adhesion and facilitate cell growth and proliferation (Yang *et al.*, 1999). It can not be excluded that lower expression of the core protein of versican and (to a lesser degree) of biglycan may impair Wharton's jelly cell function. This is in line with previous findings that PE-associated soluble factors, contained in the umbilical cord blood, reduce cell division (Pawlicka *et al.*, 2002) and stimulate sulphated GAG biosynthesis (Romanowicz *et al.*, 2000) in cultured fibroblasts. Decorin specifically binds to collagen fibrils regulating their diameter and organisation (Iozzo & Murdoch, 1996; Fisher, 1999b). The decrease in the level of decorin core proteins was relatively moderate, but because of their strong predominance among other PG core proteins, it may be of greater significance. One may conclude that the lower content of decorin core protein may disturb proper collagen fibril formation and function in Wharton's jelly. Indeed, microscopic observations have demonstrated that bundles of collagen fibers and numerous chaotically composed microfibrils are more compactly arranged in Wharton's jelly of newborns delivered by mothers with pre-eclampsia in comparison to a control group (Bańkowski, 1999).

It may be concluded that the pre-eclampsia-associated alterations in Wharton's jelly proteoglycans may contribute to the rearrangement of the extracellular matrix of the umbilical cord. They may affect the mechanical properties of Wharton's jelly by decreasing the elasticity and adaptability of this tissue. This may, in turn, reduce the ability of the umbilical cord vessels to properly regulate their diameter in response to the alterations of transmural pressure during the prenatal period or delivery and disturb foetal blood circulation.

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