Communication

Electrophoretic and chromatographic patterns of glycosaminoglycans of the umbilical cord vessels and their alteration in EPH-gestosis

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It was found that hyaluronic acid is the most abundant glycosaminoglycan (GAG) both in the umbilical cord arteries and in the umbilical cord veins. Chromatographic and as well as electrophoretic studies demonstrated that EPH-gestosis (Edema-Proteinuria-Hypertension), the most common pathological syndrome occurring in pregnancy, is accompanied by premature replacement of hyaluronic acid by sulphated GAGs in the investigated arteries but not in the veins. Such a replacement is a characteristic feature of the ageing process. One may conclude that EPH-gestosis is associated with a "premature ageing" of the umbilical cord arterial walls. The mechanism and possible role of this phenomenon in pathology are discussed.

The umbilical cord forms the connection between the placenta and the fetus. The cross section of the umbilical cord shows a specific gross morphology of one vein and two arteries surrounded by a distinct connective tissue region called Wharton's jelly.

Glycosaminoglycans (GAGs) belong to the main components of the extracellular matrix of the umbilical cord. In most cases the GAG chains (except hyaluronic acid) are covalently bound to core proteins, forming proteoglycans. They perform a wide range of biological functions. They are involved in: cell adhesion, migration and proliferation, protein secretion and gene expression. Most importantly, GAGs contribute to the general architecture and to

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permeability of connective tissue. In addition, they serve as an anchor for cell-specific growth factors and enzymes in the extracellular matrix and on the cell surface [1].

Some prenatal pathological processes may be caused by biochemical and morphological alterations occurring in the umbilical cord [2]. EPH-gestosis (Edema-Proteinuria-Hypertension) is the most common pregnancy-associated pathological process. For these reasons studies on the role of GAGs of the umbilical cord in the pathobiochemistry of EPH-gestosis seem to be important.

Preliminary chromatographic studies described in our previous paper [3] demonstrated that EPH-gestosis is accompanied by a reduction of hyaluronic acid content in the walls of umbilical cord arteries and its replacement by sulphated glycosaminoglycans. The aim of the present work was to compare both chromatographic and electrophoretic patterns of GAGs isolated from the umbilical cord arteries and veins, and to evaluate the effect of EPH-gestosis.

MATERIALS AND METHODS

Tissue material. Studies were performed on the umbilical cord arteries (UCAs) and umbilical cord veins (UCVs) taken from 10 control and 10 investigated newborns. Both control and investigated babies were born between 38 and 41 weeks of gestation by physiological delivery or by cesarean section.

The control material was taken from newborns delivered by healthy mothers, aged 18–35 with normal blood pressure (systolic 100–139 mm Hg, diastolic 70–89 mm Hg), with no symptoms of edema or renal failure. The mean body weight of the newborns was 3720 ± 428 g.

The investigated material was taken from newborns delivered by mothers with EPH-gestosis, diagnosed according to the criteria accepted by the Organisation Gestosis [4]. Severe and medium cases of polysymptomatic

gestosis (4 to 11 points of gestosis index) were investigated. Most of the mothers had all three symptoms of gestosis: edema, proteinuria and hypertension, the others demonstrated various combinations of two symptoms. The mean body weight of these newborns amounted to 3235 ± 610 g.

Immediately after delivery, about 20 cm long sections of the umbilical cords were excised beginning from their placental end. The UCAs and UCVs were carefully separated from the adjacent tissues.

Isolation of GAGs. The UCA and UCV wall tissues were cut into small pieces and washed with saline, suspended in anhydrous acetone, homogenized with a knife homogenizer, and dehydrated in the same solvent (4°C, 48 h). The homogenate was defatted by extraction with mixtures of acetone/ether (1:1, v/v) and methanol/chloroform (1:1, v/v) at room temperature for 48 h. The defatted material was dried at room temperature to a constant weight and submitted to isolation of GAGs as described in our previous paper [3].

Chromatographic fractionation of GAGs. The purified GAGs were submitted to fractionation on a CF11 cellulose microcolumn (0.3 cm × 6 cm), equilibrated with 1% cetylpyridinium chloride (CPC) [5] with a slight modification as described previously [3].

Briefly, samples of 0.4 ml containing 20–40 μg of uronic acids were applied to the column, equilibrated with 1% CPC. The elution was performed with the sequential use of the following solvents: A: 1% CPC, B: 0.3 M NaCl, C: 0.3 M $MgCl_2$, D: 0.5% CPC in a mixture of: n-propanol, metanol, acetic acid and water (40:20:1.5:38.5, by vol.), E: 0.75 M $MgCl_2$ in 0.6% acetic acid, F: 0.75 M $MgCl_2$, G: 1.25 M $MgCl_2$. The solvents B, C, E and F contained 0.05 % CPC.

Each of the solvents (A through F) eluted one type of GAGs. The each fraction containing a particular GAG was collected into one test tube and submitted to a quantitative assay. The concentration of keratan sulphate was estimated by the assay of hexosamines [6] and

the concentration of other GAGs by the assay of uronic acids (UA) [7]. Commercial preparations of highly purified GAGs (Sigma) were used as standards.

Mean values and S.D. for each group were calculated and presented in the form of bars.

Electrophoretic fractionation of GAGs was performed in the following conditions. The samples of GAG solutions, containing 1.5 μg of uronic acids, were applied to cellulose acetate strips and submitted to electrophoresis in 0.1 M barium acetate buffer, pH 5.0, at a constant current of 1 mA/cm, at room temperature, for 2 h. The electrophoregrams were stained with Alcian blue and submitted to densitometric analysis with the use of a Marcel d180 densitometer. Commercial preparations of highly purified GAGs (Sigma) were used as standards.

Statistical analysis. Mean values from 10 assays ± S.D. were calculated. Statistical analysis was performed with the use of Student's *t*-test, accepting $P < 0.05$ as significant.

RESULTS

Almost the same amounts of GAGs were found in the walls of all the investigated vessels. As can be seen from Fig. 1, they contain about 12.2–12.9 mg of GAG-bound uronic acids per gram of dry, defatted tissue. No significant differences between the UCAs and UCVs in control and EPH-gestosis groups were observed.

It is of interest that both control and EPH-gestosis UCAs and UCVs contain several GAGs (Fig. 2) with hyaluronic acid (HA) being the main component. In control UCAs the mean value of HA exceeds 40% of the total GAG content. They also contain keratan sulphate, heparan sulphate, chondroitin-4-sulphate, chondroitin-6-sulphate, dermatan sulphate and heparin. However, significant differences between the content of particular GAG in control and investigated UCAs were observed. First of all, EPH-gestosis is accom-

panied by a significant decrease in hyaluronic acid content (to about 29% of all GAGs) and increase in most sulphated GAGs, namely KS, C6S, DS and Hep (Fig. 2).

In contrast to UCAs, there are no significant differences between the content of particular GAG in control and EPH-gestosis UCVs (Fig. 2).

The densitometric scans of electrophoregrams of total GAGs isolated from the UCAs and UCVs in newborns delivered by healthy mothers (control) and by mothers with EPH-gestosis applied in our study made possible the separation of isolated GAGs into three fractions. Fraction 1 contains HS, DS and Hep, fraction 2 HA and fraction 3 KS C4S and C6S. The relative amounts of these fractions are presented in Table 1. As can be seen from Table 1, the EPH-gestosis was associated with a significant reduction of hyaluronic acid content in the UCAs, accompanied by a simultaneous increase in the relative amount of sulphated GAGs contained in fractions 1 and 3. In contrast, no significant differences between control and EPH-gestosis in veins were observed. Both absolute and relative amounts of particular GAGs were similar in the veins of both groups (Fig. 2, Table 1, respectively).
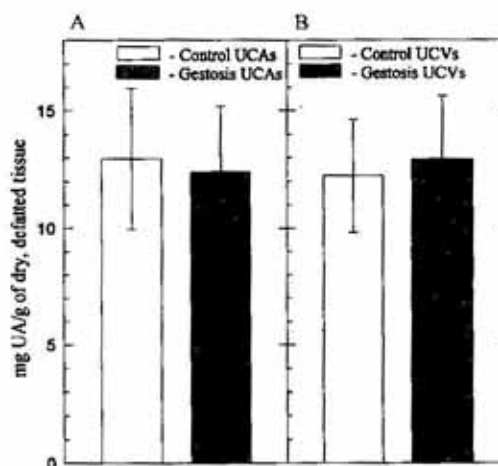

Figure 1. Total amounts of GAGs in the UCAs (A) and UCVs (B) in newborns delivered by healthy mothers (control) and by mothers with EPH-gestosis.

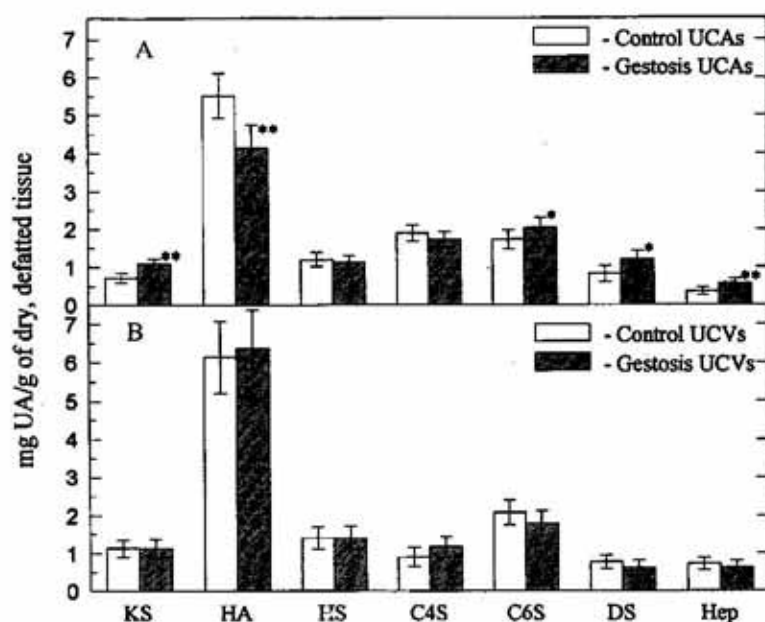Mean values ±S.D. are presented; UA, uronic acid.

Figure 2. Contents of particular GAG in the UCAs (A) and UCVs (B) in newborns delivered by healthy mothers (control) and by mothers with EPH-gestosis.

*$P < 0.01$; **$P < 0.001$. Mean values ±S.D. are presented; UA, uronic acid (see abbreviations).

DISCUSSION

Hyaluronic acid is known to be commonly present in embryonic tissues, particularly at early stages of their development, later it is replaced by sulphated GAGs. It has physical and chemical properties which are important both for the integrity of early embryonic tissues and for the morphogenetic processes taking place within them. HA ensures high hydration of the extracellular matrix, regulates cell to cell adhesion, stimulates cell movement, and participates in the regulation of cell differentiation. Some of the effects exerted by HA are mediated by specific cell-surface HA receptors [8].

Morphogenesis is accompanied by dramatic changes in the composition of extracellular matrix. A common pattern in developing tissues is accumulation of HA along the pathways of migrating mesenchymal cells, followed by its removal and replacement by sulphated GAGs during subsequent differentiation [9].

It is apparent from our results that HA is the most abundant GAG component both in the UCAs and in the UCVs. Chromatographic and electrophoretic studies allow to conclude that EPH-gestosis, the most common pathological syndrome occurring in pregnancy, is accompanied by premature replacement of hyaluronic acid by sulphated GAGs in the UCAs,

Table 1. Relative distribution (%) of particular electrophoretic fractions of GAGs isolated from control and EPH-gestosis UCAs and UCVs.

Mean values ±S.D. are presented (see abbreviations). Total content of GAGs was taken as 100.

GAGs	Percent			
	UCAs		UCVs	
	Control	EPH-gestosis	Control	EPH-gestosis
C4S, C6S, KS	36.5 ± 6.5	48.4 ± 7.2	32.3 ± 4.8	33.2 ± 5.1
HA	46.5 ± 6.9	29.4 ± 5.6	43.8 ± 6.5	46.6 ± 6.9
HS, DS, Hep	17.4 ± 2.8	21.6 ± 3.2	23.8 ± 3.5	19.9 ± 3.2

but not in the UCVs. Such a replacement is a characteristic feature of the ageing process. One may conclude that EPH-gestosis is associated with "premature ageing" of the umbilical cord arterial walls. In contrast, the GAG composition of the umbilical cord veins does not change.

It is apparent from our results and those reported by others [10, 11] that both the UCA and the UCV walls contain amounts of GAGs similar to those in the walls of internal mammary artery and saphenous vein of an adult human. In all cases GAGs constitute about 1% of dry defatted tissue [10]. This allows to conclude that there are no significant changes in total amounts of GAGs in extracellular matrix of the blood vessel wall during the life of an individual human. However, the proportions between some of them do distinctly change.

HA constitutes about 45% of total GAGs in the wall of umbilical cord vessels, but only 9% in an adult human artery and vein. On the other hand, in the umbilical cord vessels the CS and DS constitute about 30%, while in corresponding tissues of adult subjects, more than 80% of total GAGs [10].

Accumulation of sulphated GAGs in extracellular matrix of the umbilical cord artery may affect many biological functions of the arterial tissues. The resulting changes may exert an effect on collagen fibrillogenesis [12] and interact with some growth factors (FGF, PDGF, TNF-I) which may promote smooth muscle proliferation, gene expression, protein (collagen) biosynthesis and other processes [1].

The EPH-gestosis-associated decrease of HA content in the UCAs with a simultaneous increase of collagen [13] and reduction in elastin (unpublished) contents in these arteries may affect mechanical properties of the umbilical cord and disturb the fetal blood circulation.

It might be possible that the observed changes are the response of the increased blood pressure in mothers during pregnancy.

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