Clinica Chimica Acta 437 (2014) 155–160

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Evaluation of glycosaminoglycans and heparanase in placentas of women with preeclampsia

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ARTICLE INFO

Article history:
Received 16 January 2014
Received in revised form 17 July 2014
Accepted 18 July 2014
Available online 30 July 2014

Keywords:
Preeclampsia
Heparan sulfate
Dermatan sulfate
Hyaluronic acid
Heparanase

ABSTRACT

Background: Preeclampsia is a multisystem disorder whose etiology remains unclear. It is already known that circulation of soluble fms-like tyrosine kinase-1 (sFlt-1) is directly involved in pre-eclampsia development. However, the molecular mechanisms involved with sFlt-1 shedding are still unidentified. We identified, quantified glycosaminoglycans and determined the enzymatic activity of heparanase in placentas of women with preeclampsia, in order to possibly explain if these compounds could be related to cellular processes involved with preeclampsia.
Methods: A total of 45 samples collected from placentas, 15 samples from placentas of preeclampsia women and 30 samples from non-affected women. Heparan sulfate and dermatan sulfate were identified and quantified by agarose gel electrophoresis, whilst hyaluronic acid was quantified by an ELISA like assay. Heparanase activity was determined using biotynilated heparan sulfate as substrate.
Results: The results showed that dermatan sulfate ($P = 0.019$), heparan sulfate levels ($P = 0.015$) and heparanase activity ($P = 0.006$) in preeclampsia were significantly higher than in the control group. There was no significant difference between the groups for hyaluronic acid expression in placentas ($P = 0.110$). The present study is the first to demonstrate directly the increase of heparan sulfate in human placentas from patients with preeclampsia, suggesting that endogenous heparan sulfate could be involved in the release of sFlt-1 from placenta, increasing the level of circulating sFlt-1.
Conclusion: Alterations of extracellular matrix components in placentas with preeclampsia raise the possibility that heparan sulfate released by heparanase is involved in mechanisms of preeclampsia development.
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1. Introduction

Preeclampsia is characterized by the presence of arterial hypertension and significant proteinuria after 20 weeks of pregnancy [1]. It is a multifactorial, multisystem disorder whose etiology has not been fully elucidated [2–5]. Decreased vascular endothelial growth factor (VEGF) levels and increased levels of the soluble fms-like tyrosine kinase-1 (sFlt-1) have been implicated in the pathophysiology of preeclampsia [6–9].

Glycosaminoglycans (GAG) participate in several biological signaling processes connecting intracellular and extracellular environments [10]. Marked differences in GAG sulfation patterns were observed in placental preeclampsia [11]. It is already known that exogenous heparan sulfate (HS) binds to sFlt-1, and that heparin can compete with HS for sFlt-1 binding. Interestingly, Low Molecular Weight Heparin (LMWH) administered for coagulation prophylaxis to women at risk of coagulation disorders increased circulating sFlt-1 levels compared to normal untreated pregnant controls [12].

It has already been shown that decorin, a chondroitin sulfate and dermatan sulfate proteoglycan inhibits the activity of transforming growth factor beta (TGF-β). TGF-β produced in the fetal–maternal interface plays a crucial role in the control of trophoblast invasion in the uterus [13]. Consequently, both chondroitin sulfate and dermatan sulfate may modulate trophoblast invasion.

Hyaluronan or hyaluronic acid (HA) is an extracellular matrix polysaccharide present at low concentrations in plasma. Normally, HA is rapidly eliminated from the blood by the liver. Increased concentration of circulating HA has been found in women with preeclampsia [14]. Histochemical analysis used to detect HA in placentas from uncomplicated pregnancies and patients with preeclampsia, showed enhanced staining in the stroma and blood vessel walls. HA was found within and on the surface of intervillous and perivillous fibrinoid deposits [15]. Since fibrinoid deposits of HA are increased in preeclampsia,

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http://dx.doi.org/10.1016/j.cca.2014.07.023
0009-8981/Published by Elsevier B.V.

resulting from infarcted villi, this HA from fibrinoid tissue is able to reach maternal blood and may explain increased levels of circulating HA in the plasma of women with preeclampsia.

Heparanase, an endo-β-glucuronidase, participates in the degradation of the heparan sulfate proteoglycan chains [16]. This enzyme presents two isoforms, a precursor with no apparent enzymatic activity (65 kDa) that undergoes proteolytic activity to form the mature active enzyme, a heterodimer containing a 50 kDa subunit associated with an 8 kDa. This posttranslational processing of heparanase is performed by a papain-like cysteine proteinase, called Cathepsin L [17].

Inhibition of heparanase with a neutralizing antibody resulted in a marked reduction in sFlt-1 secretion of normal and preeclampsia explants [18]. Moreover, the level of sFlt-1 in the serum of heparanase-overexpressing transgenic mice was nearly double that of wild-type mice [12].

Although many studies have already shown a larger reservoir of sFtl-1 in the placenta and the role of heparanase and heparan sulfate in modulating the release of sFlt-1 into the circulation, until now, no study has examined GAG and heparanase activity in placental tissues from preeclampsia patients.

2. Methods

2.1. Patients and tissue samples

A case–control study was conducted and placentas of pregnant women with preeclampsia (n = 15) and without preeclampsia (n = 30) were collected. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. The samples were collected after informed consent had been granted. The study procedures were approved by the Ethics Committee of the Women's Hospital and Faculdade de Medicina ABC (number 259/2009). Preeclampsia was defined as high blood pressure, above 140/90 mm Hg, associated with proteinuria ≥ 300 mg/24-h urine or dipstick ≥1+ after 20 weeks of gestation [1]. Severe preeclampsia was defined following clinical and laboratory features, such as hypertension > 160/110 mm Hg, signs of imminent eclampsia, eclampsia, HELLP syndrome and proteinuria ≥ 5 g/24-h urine. It is important to point out that the severe preeclampsia group was also evaluated for early-onset disease (gestational age < 34 weeks) and late-onset disease (≥34 weeks). Included in the case group were pregnant women diagnosed with preeclampsia, that present singleton pregnancy, regardless of the type of delivery (vaginal or abdominal) or gestational age. The control group was composed of single pregnancy without preeclampsia from any type of delivery (vaginal or abdominal) or gestational age. We excluded patients from both groups who presented multiple gestations, chronic hypertension, diabetes mellitus, chronic kidney disease, thrombophilia, collagenosis and illicit drug use. The placental sample was obtained from a square measuring 5 × 5 cm, around the center of the umbilical cord insertion. The material was rinsed with 0.9% saline solution to remove blood and amniotic fluid.

2.2. Identification and quantification of sulfated glycosaminoglycans

Tissue samples were homogenized and kept in acetone for 24 h, changing the solution 4 times. The obtained dry powder tissue

Table 1
Features of preeclampsia and non-affected women.

		Control group		Preeclampsia		*P*
Age (y)	N	30		15		NS[a]
	Mean	24.2		27.3		
	Median	25.0		2.0		
	Minimum	16.0		16.0		
	Maximum	35.0		38.0		
	Standard deviation	5.5		7.3		
Race	Caucasian	20	71.4%	12	80.0%	NS[b]
	Black	2	3.6%	1	6.7%	
	Others	8	25.0%	2	13.3%	
	Total	30	100.0%	15	100.0%	
Family history of arterial hypertension	Positive	10	34.5%	3	20.0%	NS[b]
	Negative	20	65.5%	12	80.0%	
	Total	30	100.0%	15	100.0%	
Family history of *Diabetes mellitus*	Positive	6	20.7%	1	6.7%	NS[b]
	Negative	24	79.3%	14	93.3%	
	Total	30	100.0%	15	100.0%	
Previous abortion	Positive	1	3.4%	–	–	NS[b]
	Negative	29	96.6%	15	100.0%	
	Total	30	100.0%	15	100.0%	
Smoking habit	Positive	3	10.3%	–	–	NS[b]
	Negative	27	89.7%	15	100.0%	
	Total	30	100.0%	15	100.0%	
Number of gestations	1	11	35.7%	6	40.0%	NS[a]
	2	10	32.1%	4	26.7%	
	3	7	25.0%	5	33.3%	
	4	1	3.6%	–	–	
	6	1	3.6%	–	–	
	Total	30	100.0%	15	100.0%	
Personal history of arterial hypertension	Positive	–	–	3	20.0%	0.034[b]
	Negative	30	100.0%	12	80.0%	
	Total	30	100.0%	15	100.0%	
Proteinuria (mg/24 h)	N	–		7		–
	Mean	–		554.3		
	Median	–		340.0		
	Minimum	–		300.0		
	Maximum	–		1400.0		
	Standard deviation	–		421.0		

[a] *t*-Student for independent samples.
[b] Fisher Exact Test or its extension.