Placenta 35 (2014) 718–723

Contents lists available at ScienceDirect

Placenta

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

Placental mRNA expression of angiopoietins (Ang)-1, Ang-2 and their receptor Tie-2 is altered in pregnancies complicated by preeclampsia

D. Kappou [a, b, *], S. Sifakis [b], V. Androutsopoulos [c], A. Konstantinidou [d], D.A. Spandidos [c], N. Papantoniou [a]

[a] 1st Department of Obstetrics and Gynaecology, University of Athens Medical School, Alexandra Hospital, Athens, Greece
[b] Department of Obstetrics & Gynecology, University Hospital of Heraklion, Crete, Greece
[c] Department of Clinical Virology, University of Crete, Medical School, Heraklion, Greece
[d] 1st Department of Pathology, University of Athens, Athens, Greece

ARTICLE INFO

Article history:
Accepted 2 July 2014

Keywords:
Angiopoietin-1
Angiopoietin-2
Angiogenesis
Placenta
Preeclampsia
Tie-2

ABSTRACT

Objective: To investigate the placental expression of angiopoietin (Ang)-1, Ang-2 and their receptor, Tie-2, in preeclampsia (PE) with or without intrauterine growth restriction (IUGR).
Methods: Case-control study including placentas from 28 PE pregnancies, 30 PE-IUGR pregnancies and 40 controls. The expression status of the genes was evaluated by quantitative real-time PCR.
Results: In both PE and PE-IUGR groups, compared to the control group, there was significantly higher expression of Ang-2 ($p < 0.001$) and Tie-2 ($p = 0.008$) and lower expression of Ang-1 ($p = 0.001$). The magnitude of the difference was similar for Ang-1 for both groups, whereas the magnitude of the differences was higher for Ang-2 and Tie-2 in PE-IUGR group compared to controls. Ang-2 and Tie-2 were correlated in both PE ($r = 0.8602$, $p < 0.001$) and PE-IUGR ($r = 0.6342$, $p < 0.001$) groups. In PE-IUGR group, Ang-1 was associated to Ang-2 ($r = 0.3458$, $p = 0.0452$) and Tie-2 ($r = 0.4448$, $p = 0.0084$). Log_{10}Ang-1 but not Ang-2 was gestational age dependent ($R2 = 0.40$, $p < 0.001$). After conversion in Multiples of the Median (MoM) log_{10} MoM Ang-1 was reduced in the PE group (mean $= -0.8181$, $p < 0.001$) and the PE-IUGR group (mean $= -1.2583$, $p < 0.001$) compared to control group (mean $= -0.0924$).
Discussion: We have demonstrated increased placental expression of Ang-2 and Tie-2 along with lower expression levels of Ang-1 in pregnancies with PE and PE-IUGR.
Conclusion: The angiopoietin axis seems to be disrupted in PE pregnancies. Whether the results of this study represent the angiogenic imbalance observed in PE pregnancies or they are part of the pathophysiology of this condition has to be further investigated.

1. Introduction

Preeclampsia (PE) affects approximately 2–7% of all pregnancies and is a major cause of maternal and perinatal morbidity and mortality [1]. There is robust evidence that PE is associated with impaired placentation and placental hypoxia followed by an angiogenic imbalance [2–4]. Successful placentation is dependent on the establishment of a competent vascular network formed by two processes: vasculogenesis, which involves the de novo formation of vessels and branching and non-branching angiogenesis, which is the remodeling of the existing vascular network [5,6].

Angiopoietin (Ang)-1 and Ang-2 are angiogenic growth factors that play a crucial role in the later stages of placental angiogenesis [7,8]. In addition, numerous reports implicate Ang-1 and Ang-2 in regulating the maturation and stabilization of vessels in different systems [6,8]. Both factors bind to the tyrosine phosphorylation of Tunica interna endothelial cell-kinase-2 (Tie-2) receptor on endothelial cells, which is activated by Ang-1 and blocked by Ang-2 [9,10]. The other known Tie receptor (Tie-1) is considered an orphan receptor [11]. Ang-1 promotes the structural integrity of blood vessels by recruiting and interacting with peri-endothelial

Abbreviations: Ang-1, angiopoietin-1; Ang-2, angiopoietin-2; CRL, crown-rump length; EFW, estimated fetal weight; GA, gestational age; UA–PI, umbilical artery pulsatility index; SUOG, International Society of Ultrasound in Obstetrics and Gynecology; IUGR, intrauterine growth restriction; MCA, middle cerebral artery; MoM, multiples of the median; PE, preeclampsia; PI, pulsatility index; Tie-2, Tunica interna endothelial cell-kinase-2 receptor; UA, umbilical artery.

* Corresponding author. 1st Department of Obstetrics and Gynaecology, Alexandra Hospital, Athens University Medical School, 80 Vasilissis Sofias Avenue, 11528 Athens, Greece. Tel.: +30 6985800945.
E-mail address: d.kappou@yahoo.com (D. Kappou).

http://dx.doi.org/10.1016/j.placenta.2014.07.001
0143-4004/

cells, exhibits anti-inflammatory properties and stimulates endothelial cell migration [12,13]. Ang-2 acts as the natural antagonist of Ang-1 on the endothelium, and overexpression of Ang-2 leads to disruption of vessel structure [9]. Independently from their well-described role in the vascular endothelium, angiopoietins are also involved in trophoblast migration, proliferation and regulation of nitric oxide release during placentation [14].

Animal and human studies have shown that Ang-1, Ang-2 and Tie-2 are expressed in placenta even from the first trimester [7,15]. In normal pregnancies, placental expression of Ang-1 increases, whereas that of Ang-2 and Tie-2 decreases as the pregnancy advances [16,17]. In knockout models, mice deficient for the Ang-1 or Tie-2 genes die in utero, and those deficient for the Ang-2 gene die soon after birth due to vascular defects [18–20]. However, little is known about the role of angiopoietins in pregnancies with impaired placentation, such as PE and fetal growth restriction (IUGR). The majority of published studies examined the maternal serum levels of these factors in different trimesters (Table 1). To the best of our knowledge, only five studies have examined the placental expression of angiopoietins in PE pregnancies with rather small sample sizes and contradictory results [14,16,17,21,22].

Our aim was to investigate the placental expression of Ang-1, Ang-2 and Tie-2 genes in pregnancies complicated by PE with or without IUGR and in normotensive controls. Additionally, we examined the association between these factors in normal and preeclamptic pregnancies.

2. Methods

This is a case control study of 98 singleton pregnancies. The study population comprised of placentas obtained from 28 women with PE, 30 women with PE and IUGR (PE-IUGR) and 40 women with normotensive singleton pregnancies.

The gestational age (GA) in days was defined by the last menstrual period. In cases where the dates were uncertain or the difference between the menstrual dates and the CRL-derived dates was more than seven days, GA was corrected by CRL at 11–13 weeks of pregnancy. Demographic data, maternal and pregnancy characteristics and medical history were recorded from our maternity computerized records. In detail, age, parity, previous medical history, pregnancy-associated complications, medication, weight, height, gestation age at delivery, mode of delivery and smoking status were retrieved for our analysis.

According to local protocols, an ultrasound examination for fetal biometry and Doppler studies was carried out in the third trimester. All measurements were obtained according to International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) guidelines [32]. Estimated fetal weight (EFW) was calculated by the Hadlock formula [33]. Color Doppler was used to map the umbilical artery (UA) and the middle cerebral artery (MCA). Preeclamptic women in whom the fetal EFW was below the 5th centile with or without Doppler abnormalities were followed-up until delivery to obtain the PE-IUGR cohort.

Placental samples were collected at delivery from both groups. The biopsy specimens were taken immediately after the extraction of the placenta from the uterus, six basal plate biopsy specimens of the maternal–fetal interface, approximately 5 mm^3 in size, were randomly excised from each placenta in a way that each sample contained the decidua basalis and villous placenta. Areas involving calcification or infarcts were avoided. The tissue specimens were immediately frozen at −80 °C and stored until processing.

The Research and Ethics Committee of the University Hospital of Heraklion, Crete, Greece, approved the study, and written informed consent was obtained from all participants.

Table 1
Previous studies that examined the levels of Ang-1, Ang-2 and Tie-2 in placenta and maternal serum in pregnancies complicated with preeclampsia and/or intrauterine growth restriction.

Author & year	Specimen	Sample size	Gestational frame in weeks	Pregnancy complication	Ang-1	Ang-2	Tie-2
Dunk et al., 2000 [14]	Placenta	6	28–36	Severe IUGR		NS	
Zhang et al., 2001 [17]	Placenta	9	31–40	PE		↓	
Geva et al., 2002 [16]	Placenta	5	25–41	Severe PE		NS	
Sung et al., 2011 [22]	Placenta	19		PE	NS	NS	NS
Han et al., 2012 [21]	Placenta	16	28–41	Severe PE	NS	↑	
Vuorela et al., 1998 [23]	Serum	15	14–41	PE	↑	↓	↓
Hirokoshi et al., 2005 [24]	Serum	26	25–40	PE		↓	
Hirokoshi et al., 2007 [25]	Serum	30	25–40	PE		↓	
Wang et al., 2007 [26]	Serum	13	10–13	Severe IUGR		↓	
Gotsch et al., 2008 [27]	Serum	112 PE + 53 SGA	23.4–42.4	PE or SGA			↓
Akolekar et al., 2009 [28]	Serum	126	11–13	PE		NS	
Bolin et al., 2009 [29]	Serum	19	10–37	PE	↓	↑	
Leinonen et al., 2010 [30]	Serum	49 PE + 16 IUGR	12–15 and 16–20	PE or IUGR	NS	↑at 16–20	NS
Kamal et al., 2011 [31]	Serum	68	38 ± 2.1	PE	↓	NS	↓
Sung et al., 2011 [22]	Serum	19		PE			↓
Han et al., 2012 [21]	Serum	16	28–41	Severe PE	NS	↑	
Present study	Placenta	28 PE + 30 PE/IUGR	34–39	PE PE-IUGR	↓	↑	↑

PE: preeclampsia; IUGR: intrauterine growth restriction; SGA: small for gestational age; NS: non statistical.