Smith-Jackson, Kate and Hentschke, Marta and Poli-de-Figueiredo, Carlos and Pinheiro da Costa, Bartira and Broughton Pipkin, Fiona and Czajka, Anna and Mistry, Hiten D. (2015) Placental expression of eNOS, iNOS and the major protein components of caveolae in women with pre-eclampsia. Placenta, 36 (5). pp. 607-610. ISSN 1532-3102

Placental expression of eNOS,iNOS and the major protein components of caveolae in women with pre-eclampsia

Kate-Smith-Jackson[1,2*],Marta R. Hentschke[1,2,3*],Carlos E. Poli-de-Figueiredo[3], Bartira E. Pinheiro da Costa[3], Lesia O. Kurlak[2], Fiona Broughton Pipkin[2], Anna Czajka[4*], Hiten D. Mistry[1,2*]

[1]Division of Women's Health, King's College London, Women's Health Academic Centre, UK;[2]Department of Obstetrics & Gynaecology, School of Medicine, University of Nottingham, UK;[3]Laboratory of Nephrology - IPB, School of Medicine, PUCRS, Brazil;[4]Division of Diabetes and Nutritional Sciences, Diabetes Research Group, King's College London, UK.

*Authors made equal contribution

Correspondence author: Dr. Hiten D. Mistry

Division of Women's Health

King's College London

Women's Health Academic Centre, KHP

St Thomas' Hospital

Westminster Bridge Road

London, SE1 7EH, UK

Tel: 020 71888151; Fax: 020 7620 1227

Email: hiten.mistry@kcl.ac.uk

Abstract:

Caveolae regulate many cardiovascular functions and thus could be of interest in relation to pre-eclampsia, a pregnancy specific disorder characterised by hypertension and proteinuria. We examined placental mRNA and protein expression/localisation of the caveolae components Caveolin 1-3, Cavin 1-4 as well as eNOS/ iNOS in normotensive control (n=24) and pre-eclamptic pregnancies (n=19). Placental mRNA expression of *caveolin-1*, *cavin 1-3*, was lower and *eNOS* expression was increased in pre-eclampsia ($P<0.05$ for all). Additionally Caveolin-1 protein expression was also reduced in pre-eclampsia ($P=0.007$); this could be an adaptive response in pre-eclampsia, possibly to attenuate the oxidative stress/inflammation.

Keywords: Hypertension; cavin; caveolin; pre-eclampsia; placenta.

Introduction

Pre-eclampsia is a hypertensive disorder of pregnancy. As the placenta receives no autonomic input, it relies upon vasoactive mediators to regulate its vascular reactivity. Nitric oxide (NO) plays an integral role in controlling vascular resistance within the placenta; a disruption of this pathway has been identified in pre-eclampsia [1]. NO production is catalysed by the conversion of L-arginine to NO by NO synthases (NOS), two isoforms being present in the placenta: endothelial and inducible NOS (eNOS /iNOS)[2].

Caveolae are invaginations of the plasma membrane present in most mammalian cell types [3]. Caveolins (Cav-1, Cav-2, Cav-3) and cavins (1 to 4), participate in the formation of the caveolae and the coordination of the signal transduction [4]. Cavins (adapter proteins) are responsible for caveolae assembly and Cav protein expression and stabilisation [5]. Four isoforms of cavins have been identified (cavin-1 to 4).

Caveolae and Cavs, in particular *Cav-1* expressed in endothelial cells (EC), have been shown to have regulatory roles in pathological angiogenesis and in vascular disease such as atherosclerosis and pulmonary hypertension [6]. Studies have confirmed protein expression of Cav-1 and 2 in the endothelium placental capillaries and syncytiotrophoblast in term human placental tissue [7-9]. Cav-1 is an organiser of redox-sensitive signalling pathways, specifically involved in reactive oxygen species (ROS)-dependent signalling events [10]. Co-localisation of NADPH oxidase with eNOS in Cav-1 rich-caveolae in ECs both sustains ROS-mediated activation of eNOS by Angiotensin II and simultaneously promotes eNOS uncoupling.

58 We examined placental mRNA and protein expression/localisation of the caveolae
59 components and eNOS/iNOS in normotensive control and pre-eclamptic women.

60

Materials and Methods

Two groups of white European women (24 normotensive, 19 pre-eclamptic) were analysed; detailed demographics and outcome data have previously been published [11]. The study was approved by the Nottingham University Hospitals Ethics Committee (LREC-Q2090312) and written, informed consent was obtained. Pre-eclampsia was stringently defined as per the International Society for the Study of Hypertension in Pregnancy guidelines [12]; full depth placental tissue samples were collected [11].

Evaluation of the mRNA expression was conducted as per previously published methods [11]. Immunostaining of paraffin-embedded placental sections were performed as previously described [13]. All slides were assessed by the same observers (KS-J & MRH) and quantified using the Positive Pixel Algorithm of Aperio Image Scope software [13].

Results

The placental mRNA expression of *Cav-1-3, cavin-1-4, eNOS and iNOS* is presented in Figure 1a-c. *Cav-1 and cavin-1-3* had significantly lower expression in pre-eclamptic women ($P<0.05$ for all; Figs. 1a & 1b) whereas *Cav-2* and *3* and *cavin-4* were not statistically different between groups ($P> 0.05$ for all). *eNOS* was increased in pre-eclampsia ($P = 0.045$; Fig. 1c) but *iNOS* did not differ between groups ($P> 0.05$).

The protein expression and localisationin placental tissue are shown in Figure. 2. Immunohistochemical staining for Cav and cavin isoforms were localised around fetal vessels and fibroblasts, with staining also in syncytiotrophoblasts. Both eNOS and iNOS expression was localised to the syncytiotrophoblast, with some staining in the endothelium. Cav-1 placental protein expression was significantly reduced in pre-eclampsia (median [IQR]: 0.78 [0.73, 0.82] *vs.* 0.87 [0.82, 0.90] respectively; $P=0.001$). No significant differences were observed for any other proteins ($P>0.05$).

Discussion

This is the first report of a detailed expression profile of Cavs and cavins, together with both eNOS and iNOS in placentae from women who had pre-eclampsia. This study demonstrates that the mRNA expression of *cavin-1-3* and *Cav-1* are down regulated in this tissue. *eNOS* mRNA is upregulated in pre-eclamptic placentae in agreement with the literature [14]. As with previous studies [15, 16], no other differences in eNOS protein expression were observed between groups. The placental localisation of Cav-1 and eNOS in this study also coincides with previous observations [7-9].

The reduction in placental Cav-1 protein expression in pre-eclamptic pregnancies may have effects on eNOS uncoupling via Angiotensin type 1 receptor (AT1R). We have previously reported increased placental AT1R protein expression in pre-eclampsia [17]; a partial down regulation in Cav-1 could reduce eNOS uncoupling through attenuation of NADPH oxidase assembly [18] and activation of eNOS in response to Angiotensin II, whilst still maintaining functional eNOS at the membrane, as has been reported in ECs [19]. This could explain why eNOS protein, but not mRNA expression, was unchanged between groups. Caveolae have been implicated as mediators of vascular inflammation, as well as determinants of intracellular redox status; the latter accomplished by facilitating the formation of ROS and decreasing NO bioavailability in response to EC injury or inflammatory stimuli [20].

We have previously reported increased maternal Thiobarbituric acid reactive substances (TBARS) [11] and placental oxidative stress markers (xanthine oxidase and NADPH oxidase) [21] and reduced placental antioxidant glutathione peroxidase activities in the women with pre-eclampsia [11]. The reduction of Cav-1 in pre-eclampsia could be an adaptive response,

independent of eNOS, to attenuate the increased oxidative stress and inflammation, as seen in models of atherosclerosis [22].

Disruption and progressive loss of Cav-1 have been associated with pulmonary hypertension outside of pregnancy [18], but detailed analysis in relation to normotensive pregnancy is sparse [7-9] and lacking altogether in pre-eclampsia. In order to determine if similar differences antedate the clinical onset of the disease, future longitudinal studies are needed to determine whether the results are cause or effect. Examination of first and second trimester placentae would enable us to trace the ontogeny of mRNA and protein expression of caveolae through pregnancy.

Acknowledgments: We thank all the women who participated in the study and the midwives and doctors whose support made this study possible. We also thank Dr. Geneviève Escher and Mr Yosef Mansour for proof reading the manuscript and help with images. Some of this work was funded by Tommy's Charity (Charity number: 1060508), CAPES/CNPq, Brazil (MRH); CEPF is a CNq researcher and KS-J was funded by a Society for Endocrinology Summer studentship.

Conflict of Interest: No conflict of interest for all authors.

References

[1] Ghabour MS, Eis AL, Brockman DE, Pollock JS and Myatt L. Immunohistochemical characterization of placental nitric oxide synthase expression in preeclampsia. Am J Obstet Gynecol. 1995;173(3 Pt 1):687-94.

[2] Alderton WK, Cooper CE and Knowles RG. Nitric oxide synthases: structure, function and inhibition. Biochem J. 2001;357(Pt 3):593-615.

[3] Parton RG. Caveolae and caveolins. Curr Opin Cell Biol. 1996;8(4):542-8.

[4] Briand N, Dugail I and Le Lay S. Cavin proteins: New players in the caveolae field. Biochimie. 2011;93(1):71-7.

[5] Liu L and Pilch PF. A critical role of cavin (polymerase I and transcript release factor) in caveolae formation and organization. J Biol Chem. 2008;283(7):4314-22.

[6] Mathew R. Cell-specific dual role of caveolin-1 in pulmonary hypertension. Pulm Med. 2011;2011:573432.

[7] Byrne S, Cheent A, Dimond J, Fisher G and Ockleford CD. Immunocytochemical localization of a caveolin-1 isoform in human term extra-embryonic membranes using confocal laser scanning microscopy: implications for the complexity of the materno-fetal junction. Placenta. 2001;22(6):499-510.

[8] Lyden TW, Anderson CL and Robinson JM. The endothelium but not the syncytiotrophoblast of human placenta expresses caveolae. Placenta. 2002;23(8-9):640-52.

[9] Linton EA, Rodriguez-Linares B, Rashid-Doubell F, Ferguson DJ and Redman CW. Caveolae and caveolin-1 in human term villous trophoblast. Placenta. 2003;24(7):745-57.

[10] Ushio-Fukai M and Alexander RW. Caveolin-dependent angiotensin II type 1 receptor signaling in vascular smooth muscle. Hypertension. 2006;48(5):797-803.

[11] Mistry HD, Wilson V, Ramsay MM, Symonds ME and Broughton Pipkin F. Reduced selenium concentrations and glutathione peroxidase activity in pre-eclamptic pregnancies. Hypertension. 2008;52:881-8.

[12] Brown MA, Lindheimer MD, de Swiet M, Van Assche A and Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). Hypertens Pregnancy. 2001;20(1):IX-XIV.

[13] Mistry HD, McCallum LA, Kurlak LO, Greenwood IA, Broughton Pipkin F and Tribe RM. Novel expression and regulation of voltage-dependent potassium channels in placentas from women with preeclampsia. Hypertension. 2011;58(3):497-504.

[14] Dotsch J, Hogen N, Nyul Z, Hanze J, Knerr I, Kirschbaum M and Rascher W. Increase of endothelial nitric oxide synthase and endothelin-1 mRNA expression in human placenta during gestation. Eur J Obstet Gynecol Reprod Biol. 2001;97(2):163-7.

[15] Corthorn J, Germain AA, Chacon C, Rey S, Soto GX, Figueroa CD, Muller-Esterl W, Duarte I and Valdes G. Expression of kallikrein, bradykinin b2 receptor, and endothelial nitric oxide synthase in placenta in normal gestation, preeclampsia, and placenta accreta. Endocrine. 2006;29(3):491-9.

[16] Matsubara S, Takizawa T, Takayama T, Izumi A, Watanabe T and Sato I. Immuno-electron microscopic localization of endothelial nitric oxide synthase in human placental terminal villous trophoblasts-normal and pre-eclamptic pregnancy. Placenta. 2001;22(8-9):782-6.

[17] Mistry HD, Kurlak LO and Broughton Pipkin F. The placental renin-angiotensin system and oxidative stress in pre-eclampsia. Placenta. 2013;34(2):182-6.

[18] Chen F, Barman S, Yu Y, Haigh S, Wang Y, Dou H, Bagi Z, Han W, Su Y and Fulton DJ. Caveolin-1 is a negative regulator of NADPH oxidase-derived reactive oxygen species. Free Radic Biol Med. 2014;73:201-13.

[19] Lobysheva I, Rath G, Sekkali B, Bouzin C, Feron O, Gallez B, Dessy C and Balligand JL. Moderate caveolin-1 downregulation prevents NADPH oxidase-dependent endothelial nitric oxide synthase uncoupling by angiotensin II in endothelial cells. Arterioscler Thromb Vasc Biol. 2011;31(9):2098-105.

[20] Layne J, Majkova Z, Smart EJ, Toborek M and Hennig B. Caveolae: a regulatory platform for nutritional modulation of inflammatory diseases. J Nutr Biochem. 2011;22(9):807-11.

[21] Williams PJ, Mistry HD, Innes BA, Bulmer JN and Broughton Pipkin F. Expression of AT1R, AT2R and AT4R and their roles in extravillous trophoblast invasion in the human. Placenta. 2010;31(5):448-55.

[22] Frank PG, Lee H, Park DS, Tandon NN, Scherer PE and Lisanti MP. Genetic ablation of caveolin-1 confers protection against atherosclerosis. Arterioscler Thromb Vasc Biol. 2004;24(1):98-105.

Figure Legends

Figure 1: Normalised mRNA expression (copy number) of a) Cav-1-3; b) cavin-1-4 and c) eNOS and iNOS in placentae from normotensive and pre-eclamptic pregnancies. Boxplots represent median [interquartile range].

Figure 2: Placental protein expression and localisation of Cav-1, 2 and 3; cavin-1, 2, 3 and 4; eNOS and iNOS , in normotensive control and pre-eclamptic women. Expression was significantly downregulated in pre-eclamptic placentae ($P<0.05$). Positive staining was localised mainly around fetal vessels (black arrows) with some weak staining in syncytiotrophoblasts (red arrow).