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HSP70-mediated control of endothelial cell apoptosis during pre-eclampsia

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ABSTRACT

Objective: Pre-eclampsia is a hypertensive disorder characterized by maternal vascular endothelial dysfunction. It is likely that this enhanced rate of endothelial cell stress is associated with the pre- and post-partum complications of both mother and fetus. Deciphering the expression pattern of factors involved in altering placental endothelial cell viability in pre-eclampsia aids in identifying components that may protect the fetus from the consequences of placental dysfunction and oxidative stress.
Study design: Expression of thioredoxin (Trx), an antioxidant protein; heat shock protein (HSP) 70, a cytoprotective protein; heat shock factor (HSF)1, a transcriptional factor of HSPs; and apoptosis signal-regulating kinase 1 (ASK1), a pro-apoptotic protein, was elucidated in endothelial cells from human term placentas of normotensive and pre-eclamptic subjects ($n = 35$).
Results: A significant increase in HSP70 ($p < 0.05$), HSF1 ($p < 0.05$), Trx ($p < 0.05$) and an insignificant increase in ASK1 were noted in pre-eclamptic endothelial cells.
Conclusion: This analysis supports the role of HSP70 expression in promoting cell survival by regulating ASK expression in pre-eclampsia.

1. Introduction

Pre-eclampsia, a pregnancy-specific disorder, is characterized by placental abnormalities and maternal vascular endothelial dysfunction [1]. The significance of pre-eclampsia is substantial when considering the requirement for regular antenatal monitoring of symptoms, preservation of maternal health and care of the premature or low birth weight fetus whose incidence of acute morbidity is increasing. This condition may be life-threatening to mother and fetus if it is not properly managed, but it usually ends when the baby and placenta are delivered [2]. A longer-term burden also exists, as pre-eclamptic women are two and half times more likely to develop ischaemic heart disease later in life [3]. The infants born to pre-eclamptic woman are at a higher risk of developing respiratory diseases and long-term neurological morbidity [4].

The increasing association between oxidative and nitrative stress with pre-eclampsia indicates that hypoxia is one of its common complications. The reactive oxygen species (ROS) formed contribute to the imbalance in antioxidant status and oxidative stress as seen in pre-eclampsia. This imbalance is known to induce cellular damage by altering cell integrity [5].

The present study focuses on the changes in the expression of the various signalling molecules involved in control of oxidative stress mediated via heat shock proteins (HSPs). HSPs are highly conserved, found in all cell types and expressed as a result of stressful environmental, pathological or physiological stimuli [6]. Apart from protein transport and degradation during unstressed conditions, they are involved in preventing stress-mediated accumulation of misfolded or damaged proteins [7]. HSP acts as an antioxidant in maintaining cellular redox homeostasis. HSPs are found to inhibit intracellular ROS sand increase the glutathione level [8]. There is evidence that HSPs are produced by the placental tissues and that they have a physiological role in stress management during pre-eclampsia [9,10]. HSP70 induction may involve different mechanisms under different circumstances. The regulation of heat shock gene expression in eukaryotes is mediated at the transcriptional level by transcriptional activators, heat shock factor (HSF) [11]. HSF1 is the ubiquitous stress-responsive transcriptional activator essential for the inducible transcription of genes encoding HSPs [12], by binding to regulatory heat shock elements present in the promoter region of all heat shock genes [13].

Thioredoxin (Trx) is a family of small proteins that contains a conserved redox active center [14]. It scavenges ROS such as H_2O_2 and free radicals through its direct reducing activity [15]. It has been reported that thioredoxin works as an antioxidant, stabilizing redox balance, and is expressed in the placenta [16].

Abbreviations: HSP, heat shock protein; Trx, thioredoxin; HSF, heat shock factor; ASK1, apoptosis signal-regulating kinase 1; ROS, reactive oxygen species; MAPKKK, mitogen activated protein kinase kinase kinase; BMI, body mass index; PROM, premature rupture of membrane; IUGR, intrauterine growth retardation.

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Table 1
Clinical characteristics of normotensive pregnant women and pre-eclamptic patients.

Criteria	Normotensive subjects ($n=35$)	Preeclamptic subjects ($n=35$)
Maternal age (years)	27 ± 5.2	30 ± 4.8^{NS}
Gestational age (weeks)	38.2 ± 0.4	$30.8 \pm 2.5^{*}$
Pregnancy weight at the time of admission (kg)	57.5 ± 7.8	$72.1 \pm 6.3^{*}$
Pre-pregnancy blood pressure (mmHg)		
Systolic	114.2 ± 5.1	117.2 ± 5.4^{NS}
Diastolic	76.2 ± 5.7	78.5 ± 4.9^{NS}
Pregnancy blood pressure at the time of delivery (mmHg)		
Systolic	122.6 ± 7.6	$167.5 \pm 7.9^{*}$
Diastolic	81.4 ± 7.2	$112.3 \pm 7.4^{*}$
Proteinuria (mg/dL)	Nil	$>300^{**}$
Xanthine oxidase (U/mg protein)	1.72 ± 0.84	$2.83 \pm 0.96^{*}$
Infant birth weight	3.42 ± 0.54	$2.19 \pm 0.54^{*}$

NS, non-significant.
* $p < 0.05$ when compared to normotensive subjects.
** $p < 0.01$ when compared to normotensive subjects.

ASK1 (apoptosis signal regulating kinase 1) is the member of the mitogen-activated protein kinase kinase kinase (MAPKKK) that was shown to be an important signalling kinase in apoptotic cell death [17,18]. Over-expression of ASK1 induces apoptosis through multiple cell death pathway (intrinsic and extrinsic, caspase-dependent and independent) induced by various pro-apoptotic stimuli such as lipopolysaccharide, reactive oxygen species (ROS), ischaemic insult and genotoxic stress [19,20].

This paper aims to identify factors that may protect the fetus from the consequences of placental dysfunction and oxidative stress. It is investigating the extent to which the expression of certain cytoprotective and apoptotic proteins could be involved in altering placental endothelial cell viability, thus promoting fetal survival.

2. Materials and methods

2.1. Selection of subjects

Patients registered in the Department of Obstetrics & Gynecology of a public sector hospital at Chennai in India were enrolled in the study. The study was carried out for a period of 1 year. The sample consisted of 35 mild pre-eclamptic patients and 35 normotensive subjects of the age 18–40 years. Patients with mild pre-eclampsia were defined on the basis of the following clinical and laboratory criteria: systolic blood pressure in the range of 140–160 mmHg and diastolic blood pressure in the range of 90–110 mmHg noted on at least two occasions; proteinuria concentrations >300 mg/dL measured on at least two random specimens and xanthine oxidase activity of approximately 2.6 U/mg protein [21]. Patients with severe pre-eclampsia were excluded from the study as most of them were advised immediate medication to avoid further complications. Healthy volunteers who were normotensive, of similar race, body mass index (BMI) and without maternal and fetal complications during pregnancy were selected as control subjects. Clearance was obtained from the Hospital Ethical Committee prior to the commencement of the study and informed consent was obtained from all subjects. Pregnant women with other complications such as PROM, IUGR, gestational diabetes, chorioamnionitis, other clinical infections and those undergoing medication were excluded. The clinical characteristics of the pre-eclamptic patients were tabulated and compared with the normotensive pregnant subjects and the data are presented in Table 1.

2.2. Isolation of endothelial cells

Placental endothelial cells were prepared by the method previously mentioned [22]. The cells were cultured overnight at 1 million cells per culture flask (125 mm^2) in M199 medium containing 20% fetal calf serum in a 5% CO_2 atmosphere at 37 °C. Non-adherent cells and debris were removed by washing three times with PBS the following day. Viability of the endothelial cells was assessed using trypan blue exclusion test [23].

2.3. Scanning electron microscopy (SEM) analysis of endothelial cells

Endothelial cells were fixed for scanning electron microscopy with 4% glutaraldehyde overnight at 4 °C followed by centrifugation at 100 × *g* for 5 min. The supernatant was discarded and the pellet was resuspended in 2% osmium tetroxide for 2 h at room temperature. The centrifugation process was repeated and the samples were dehydrated with an ascending ethanol series (10–100%). The absolute ethanol was finally displaced by liquid carbon dioxide which served as the transitional fluid for critical point drying. Dried samples were mounted on aluminium stubs and sputter coated with gold (JEC-1100). Electron accelerators for SEM were operated at 15 kV and the samples were viewed with an AMR100 scanning electron microscope (Jeol JSM-6360, Jeol Ltd., Tokyo, Japan).

2.4. Co-immunofluorescence of HSP70 and HSF1

Indirect immunofluorescence technique was performed with the placental sections taken from the subjects of the study according to the method of Pringle et al. [24] with some modifications. The

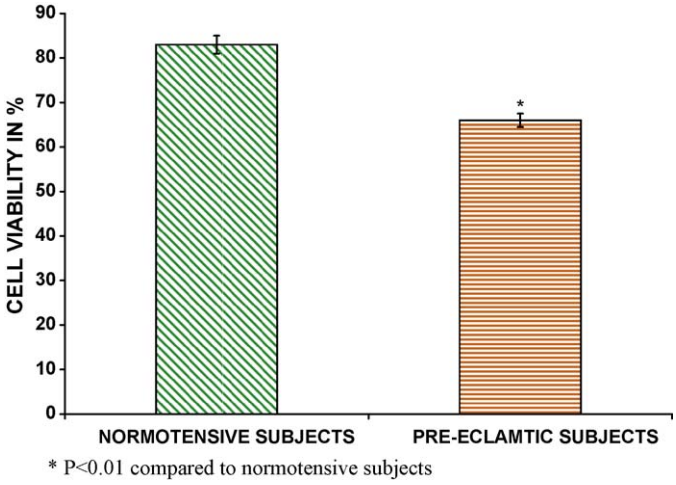

Fig. 1. Cell viability of endothelial cells isolated from placentas of normotensive and pre-eclamptic subjects.