A putative role for telocytes in placental barrier impairment during preeclampsia

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Preeclampsia (PE) is a major health problem occurring in pregnant women and the principal cause of maternal morbidity and perinatal mortality. It is characterized by alteration of the extravilli trophoblast cell migration toward the endometrial spiral arteries with a concomitant reduction in maternal blood flow in the placenta. This result in a state of ischemia–hypoxia which triggers an oxidative stress stage with production of reactive oxygen species. A cascade of cellular and molecular events leads then to endothelial dysfunction, transduction pathway signal disruption and induction of apoptosis and necrosis mechanisms and therefore a significant reduction in the amount of nutrients required for normal fetal development. Placental anchoring chorionic and stem villi present a skeleton of myofibroblasts arranged in parallel disposition to its longitudinal axis. The intraplacental blood volume is controlled by the contraction/relaxation of these myofibroblasts, promoting the delivery of nutrients and metabolites to the fetus. Recently, a new mesodermal originated cell type has been described in the villous stroma, the so named “telocytes”. These cells are strategically located between the smooth muscle cells of the blood vessel wall and the myofibroblasts, and it is reasonable to hypothesize that they may play a pacemaker role, as in the intestine. This study provide new information supporting the notion that the occurrence of oxidative stress in PE is not only related to endothelial dysfunction and apoptosis of the trophoblast cells, but also involves telocytes and its putative role in the regulation of fetal blood flow and the intraplacental blood volume. Some ideas aimed at dilucidating the relationship between placental failure and the behavior of telocytes in pathological organs in adulthood, are also discussed.

Introduction

It is widely known that PE is a clinical syndrome that represents one of the major causes of maternal morbidity and fetal mortality in pregnancy. The incidence of this condition varies between 4% and 6% of the world population of pregnant women [1]. Affected women also present hypertension, proteinuria and edema [2], and those who have shown no previous signs of hypertension, develop this symptoms by the 20th week of pregnancy. These signs disappear after childbirth and the delivery of the placenta and, so far, the only treatment for PE consists in the termination of pregnancy [3,4]. The pathogenesis of PE has not been determined with certainty, although it is currently accepted that the syndrome would be triggered by some factors produced by the placenta [2]. PE is associated with a reduction of the utero-placental perfusion pressure, which leads to ischemia/hypoxia in the organ, which develops from the third trimester of pregnancy. The ischemia/hypoxia would be consequence of a failure in the migration of the extravilli trophoblast (EVT), during placentation, an event that takes place in the first trimester of pregnancy. EVT migration allows the replacement of both the endothelium and the muscle layer of the spiral arteries in the endometrium. By this mechanism, these vessels acquire high capacitance and low resistance, thus favoring flow of maternal blood to the placenta intervillous space [5–7]. Failure in EVT migration can increase the resistance of the blood vessels [8–10] producing placental ischemia/hypoxia that induces the release, towards the intervillous space, of a number of placental factors that will activate a cascade of cellular and...
molecular events that ultimately will produce dysfunctions in the endothelial wall and the smooth muscle cells of the blood vessel walls [11]. The same endothelial dysfunction has been described in cases of fetal growth restriction (IUGR), a fact that supports the hypotheses that both PE and IUGR share a common vascular etiology [9,12].

Background

Placental oxidative stress

Oxidative stress is consequence of an imbalance between excessive generation of reactive oxygen species (ROS) and the reduction of the antioxidant capacity; in other words, biological systems develop an inability to neutralize these molecules [13,14]. The production of ROS in the placenta [13,15,16] is particularly relevant since this is a highly vascular organ with large numbers of mitochondria [17], adding the presence of organ-specific macrophages or Hofbauer cells [18]. The vascular endothelium is highly sensitive to the effect of free radicals, which can cause damage at mitochondrial DNA level and endothelial cells. These radicals, in turn, react with nitric oxide (NO) to form the peroxynitrite anion [19], a well-known powerful oxidant. Evidence suggests that PE is a state of oxidative stress, and it has been reported that biochemical markers of lipid peroxidation, such as malondialdehyde and F2-isoprostane [11,20] show increased levels in PE placentas, while the plasma concentration of vitamins C and E [15,16,21] and the antioxidant capacity of the plasma (FRAP) are diminished [11]. The induction of antioxidant enzymes as a consequence of oxidative stress has been widely documented [22], however, there is no uniformity in the reported results of the activity of the antioxidant enzymes superoxide dismutase (SOD), catalase and glutathione peroxidase (GHS-ps) in PE [11,23-25]. In summary, an adaptation mechanism exists during gestation that activates maternal antioxidant defense mechanisms in order to counteract the attack of free radicals. This is achieved through enzyme or non-enzyme inductions that prevent the occurrence of oxidative stress. However, pregnancy is a state in which this balance and adaptation can easily be affected, as it has been clearly demonstrated in early abortions, membranes detachments [26] and IUGR [27].

Placental apoptosis

The proper balance between proliferation, differentiation and apoptosis in the placenta depends on the normal functioning as well as on the adequate architectural morphology of the organ. Maintenance of the homeostasis of these basic processes is fundamental for the proper growth and development of the fetus [28]. When these processes are altered, spontaneous abortion, PE, premature birth and/or reduced fetal growth occur [29]. Huppertz et al. [30], have described the apoptotic cascade that undergoes the cytotrophoblast during its differentiation to syncytiotrophoblast. A large number of signaling molecules, such as the family genes bcl-2, caspase-3, and NFkappaB are involved in apoptosis [31,32], and have been found in increased levels in PE placentas or IUGR, concomitant with an increase in apoptosis [33,34]. Huppertz et al. [26] showed that in placental explants subjected to hypoxia, as occurs in PE, there is a concomitant increase in the activity of caspase 3, which eventually causes necrosis to exceed the apoptosis in the syncytiotrophoblast, releasing syncytiotrophoblast cellular debris which, according to these authors and also in agreement with the results reported by Tjoa et al. [35], would be responsible for the signs of PE manifested in the mother. Although the fact that placental hypoxia causes apoptosis of the trophoblast, endothelium and stroma has been very well documented [36], there is no publication whatsoever regarding the apoptosis of placental telocytes in cases of PE caused by placental hypoxia. In this regard, it is reasonable to assume that the placenta functions as gut in the absorption of metabolites. It has also been found that when the intestinal telocytes undergo apoptosis by different mechanisms, disturbances will occur in the normal functioning of the organ [37]. This intestinal disorder is due to failures in the pacemaker function of telocytes related to the organ peristalsis [38].

Telocytes

In the 19th century the great neuropathologist Santiago Ramón y Cajal found a special type of cells in the gut that he named “interstitial neurons”. It was only in 1970 and using transmission electron microscopy that scientists confirmed that these cells were indeed neurons and were renamed as interstitial cells of Cajal (ICC), [39]. These cells possess few and long cytoplasmic projections emerging from an elongated cell body, with little perinuclear cytoplasm and oval nucleus presenting one or more nucleoli [40]. It has also been reported that ICC has large numbers of mitochondria, smooth endoplasmic reticulum and caveolae [41]. On other line of evidence, the study of Wallace and Burns [42] concluded that gut ICC participate in the mechanism of intestinal peristalsis, observation supported by the fact that these cells form a network located among neurons from the nerve plexus that runs between the smooth muscle layers. Today ICC are considered gastrointestinal pacemaker, connected with smooth muscle cells and nerves [38]. Recent studies in the pancreas, where there is no presence of muscle layers, suggest that telocytes may constitute the pancreatic pacemaker, considering that these cells are strategically located between neurons and pancreatic exocrine ducts and between neurons and blood vessels, thus providing rhythmicity to the exocrine ducts and flow regulation in the blood vessels [43,44].

ICC are currently named telocytes after Popescu and Faussone-Pellegrini [39] argued the need to unify criteria in its designation. Telocytes communicate between themselves through their long, slim cytoplasmic extensions called telopodes which can present wide endings, or podomos, or narrow endings denoted as podomes. Caveolae, mitochondria and endoplasmic reticulum vesicles are accumulated inside podomes [45]. Telocyte communication established through telopodes is denominated homocellular junction, but if the communication is established with another cell type it is denoted as heterocellular junction. These junctions could be established either by direct communication (synapses stromal) or mediated via microvesicles or exosomes [46]. It is also important to note that in the heart telocytes have some functions related to intercellular signaling, cardiac repair and remodeling, forming also part of the reserve pool of undifferentiated cells [47,48]. Furthermore, studies of Suciu et al. [45] performed in human normal term placenta have shown evidence that telocytes are located between the smooth muscle cells of the blood vessel wall and myofibroblasts, the latter arranged in parallel to the longitudinal axis of the anchoring or stem villous [49,50].

Origin of placental telocytes

Events that ensure the normal development of the placenta also ensure normal fetal development and growth. All these events are highly related between them and are susceptible to the effects of intra and extra-placental environmental factors, including the nutritional status of the mother and nutrient intake during pregnancy [51-53]. The placenta begins to originate when blastocyst implantation occurs, at the end of the first week of gestation. The blastocyst is constituted by the outer trophoblast and the internal embryonic pole. Upon implantation, the external region undergoes differentiation into two cell types, the externally
disposed syncytiotrophoblast and the cytotrophoblast located underneath the former. Both cell types are present in the placental primary villous [54]. Gastrulation occurs during the second week of gestation and it is characterized by the movement of cells that eventually will form both the extraembryonic and intraembryonic mesoderm. The former is introduced into the primary villous to form secondary villi, and the latter becomes the tissue from which the cardiovascular and urinary systems will subsequently develop.

Blood vessels are formed in situ on the third week, in all areas where the extra- and intra-embryonic mesoderm is present. In this way the tertiary villi are formed, which latter will grow into stem villous, anchor villous and free villi or exchange villus. It is precisely in the stroma of these tertiary villi where mesenchymal cells differentiate into telocytes. This is evidenced by positive immunohistochemical reaction to vimentin and CD34 as well as immunofluorescence to c-kit/CD117 [55]. Further studies of Suciu et al. [45] performed in human normal term placentas have shown evidence that telocytes are located between the smooth muscle cells of the blood vessel wall and myofibroblasts, the latter arranged in parallel to the longitudinal axis of the anchoring or stem villous [49,50].

Hypotheses

In this context, we propose that the apoptosis experienced by placental telocytes in PE would be related to the failure of its pacemaker function that modulates the contraction/relaxation of chorionic villi in the intervillous space as well as the blood flow to the fetus.

Evaluation of the hypotheses and results

In order to validate this hypotheses, we are analyzing a number of data already provided in the literature and complemented it with observations and findings from preliminary studies at immunohistochemical and transmission electron microscopy (TEM) level, performed at our lab.

Preliminary study

Samples from 2 human term normal and 2 PE placentas were routinely processed for immunohistochmical assay as previously reported [11]. Briefly, for caspase 3(CPP32) determination, mouse monoclonal antibody (Novocastra JHM62, 1:50 v/v), was applied to the tissue sections for 30 min at 37 °C. In negative controls the antibody was omitted. In addition, small tissue samples from the same placentas were routinely processed for TEM as previously described [44]. Women included in the study were required to sign an informed consent form and all procedures were performed according to protocols approved by the local ethic committees of the Faculty of Medicine of the University of Chile and the Clinical Hospital.

The results show that in PE placentas the expression of antiactive caspase-3 monoclonal antibody was observed as an intense mark in the trophoblast layer, endothelium and stromal cells, both in the villous core connective tissue, as well as in the perivascular cells (Fig. 1A). In contrast, in the normal placentas the expression of the same antibody yielded a moderate reaction restricted only to the trophoblast layer (Fig. 1B). At TEM level, relatively abundant deposits of collagen fibers were observed surrounding the telopodes of nearby telocytes in PE placental villi, as shown in Fig. 1C. Little or none collagen fibers were found in the normal placental villi (Fig. 1D).

Bibliographic evidence

Endothelial dysfunction

As placental blood vessels are not innervated, the fetal/placental flow is regulated by autocrine/paracrine agents such as NO [18,56–58], whose production by placental telocytes and endothelium has been already demonstrated [45,56]. This potent vasodilator is normally produced by the endothelium, and it has been found in low concentrations in PE [59,60]. Furthermore, it is known that the placental endothelium expresses some factors that prevent the activation of the coagulation cascade, such as the membrane glycoprotein thrombomodulin (TM), a plasma protein that activates anticoagulant protein C. In this regard, Bosco et al. [11] found no significant differences in the expression of TM in the placental syncytiotrophoblast from normal pregnancies versus PE placentas. However, a TM decrease in fetal endothelium in PE cases and TM increase in cases of severe PE was observed both in the stromal myofibroblasts as well as in the perivascular cells in the stem villous [11]. The authors concluded that the expression of TM in myofibroblasts and perivascular cells of these placentas are somehow related to the maintenance of normal fetal blood flow, taking into consideration that in severe PE no cases of IUGR were observed. Notably, this was the first link that led us to consider the significant role exerted by myofibroblasts and perivascular cells in the modulation of fetal blood flow. In 2005 there was no available evidence that placental perivascular cells may corresponded to telocytes, or that their possible function was related to pacemaker activity, as it has been recently postulated in pancreas [43,44]. Suciu et al. [45,55] demonstrated the presence of telocytes in the human placenta and they postulated that placental telocytes were the pacemaker cells related to the smooth muscle fibers of fetal blood vessels and to the stromal myofibroblasts in order to control the contraction/relaxation cycle of the fetal vessels and the chorionic villous of the placenta (Fig. 2).

Placental vascularization

Placental angiogenesis is mediated by VEGF and its receptors, which play a fundamental role in this process [61,62]. Therefore, VEGF expression increases during pregnancy, nonetheless, immunohistochemical studies in placentas from women presenting PE have been inconclusive: some of these works reported reduction of VEGF [63], some reported increased levels [64] and some other reported no changes [65]. Other functions of VEGF are related to the activation of the synthesis of the vasodilator NO [66–68], the regulation of the invasion, proliferation and differentiation of TEV [64]. VEGF also acts as a protective vascular factor, maintaining the survival of endothelial cells, suppressing thrombosis and producing anti-inflammatory effects [69]. VEGF expression has also been demonstrated in the muscle layer of large blood vessels in PE placentas [70], which the authors attributed to a compensatory effect of hypoxia by oxidative stress. The same effect has been observed in the muscle layer of the pulmonary arteries of smokers and/or chronic obstructive pulmonary disease [71], evidence that allows us to conclude that under hypoxic conditions smooth muscle cells of the blood vessels wall express VEGF. Finally, it is noteworthy to mention that Suciu et al. [45], found that placental telocytes located in the vicinity of perivascular smooth muscle cells also express VEGF. Therefore it is plausible to conclude that the degree of immunohistochemical expression in telocytes could be an indicator of the degree of oxidative stress to which these cells are exposed.

Function of telocytes in the placental villi

Farley et al. [72], demonstrated that myofibroblasts arranged in parallel disposition to the longitudinal axes of anchoring villi of the
Fig. 1. PE and normal human placental barriers. Upper panel: (A) Expression of the anti-active caspase-3 monoclonal antibody in PE placenta, displaying an intense immunohistochemical mark in the cytotrophoblast (C), endothelium (E) and telocytes (T) in the stroma. (B) Expression of the same antibody in normal placenta. Note that in this case only the syncytiotrophoblast evidenced a moderate reaction. Lower panel: (C) Electron micrograph of a PE placental villi showing the syncytiotrophoblast (S) toward the maternal space (MS), the fetal capillary (FC) and telocytes (T). Note the abundant collagen fibrils (Co) surrounded some telocytes located in the vicinity of a fetal capillary. (D) Electron micrograph of a normal placental villi showing the syncytiotrophoblast (S), the maternal space (MS), the cytotrophoblast and telocytes (T) of a telocyte. Note that in this case almost no collagen fibers are observed. Calibration bars: A and B = 50 µm; C and D = 2.4 µm. Antibody working dilution was 1:50.

Fig. 2. Hypothetical model of the interstitial telocyte network acting in the regulation of the placental exchange function in normal and preeclamptic placentas.
placenta present the ability to contract and relax longitudinally. The authors postulated that myofibroblast regulated intraplacental blood volume, and hence fetal-maternal oxygen-nutrient exchange. In this regard, Suciu et al. [45] postulated that, in a similar way as in the intestine, telocytes would act as pacemaker for the contraction/relaxation of the placental villi (myofibroblasts) as well as the contraction/relaxation of smooth muscle cells in the blood vessels wall (perivascular cells), which would have implications not only in the regulation of blood flow within the placenta but also in the regulation of the length and width of the villi in the intervillous space. The latter would have a direct implication in the regulation of the maternal blood pressure in the hematic chamber (see Fig. 2). The relaxation mechanism is probably mediated by NO [49,72]. Obviously, placental telocytes represent a particular case of telocytes, since in the placenta they do not contact nerve fibers [45,55] as they do in other organs [38,44,45]. Accordingly, it seems reasonable to assume that alteration of placental telocytes in PE would be affecting the primary role of the placenta which is to supply nutrients to the fetus.

Discussion and conclusions

Regarding the preliminary results communicated here, it is important to mention that Caspase 3 is implicated in the cleavage of various cellular components and the morphological changes associated to apoptosis [36]. In PE placenta we have found an intense immunohistochemical expression of the anti-active caspase-3 monoclonal antibody, displaying intense mark in the cytotrophoblast layer, endothelium and telocytes (Fig. 1A and B). This data are in the same direction of the study of Cobellis et al. [38], who found an abnormal level of apoptosis in the stroma of PE placentas.

On the other hand, Ratts et al. [73], studying the expression of the pro-apoptotic BAX protein in placentas found that this protein was undetectable in the syncytiotrophoblast, but was expressed in cytotrophoblast and was prominent in connective tissue and perivascular cells within the villous core. They also described that BAX protein was uniformly localized in cells from the villous core connective tissue and in perivascular cells adjacent to vessels in both intermediate and terminal villi, but they only remarked about the functional importance of trophoblast apoptosis.

The results of our TEM study showed a considerable amount of collagen deposits located near the telopodes of the telocytes of PE placentas (see Fig. 1C and D). These findings are in accordance with those described by Kostin [47] who in a model of cardiac hypoxia found that increased interstitial fibrosis and fibrillar collagen lead to telocyte cell death via apoptosis with shrinkage and shortening of telopodes. Apoptosis of placental telocytes from the chorionic villi leads to alteration of the telopods and therefore to a possible loss of the synaptic-like connections that relate telocytes with themselves, with myofioblasts and with smooth muscle cells from fetal blood vessels [45]. The loss of these synapses would lead to loss of the hypothetical pacemaker function of these cells, affecting the contraction/relaxation cycle of the chorionic villi which, in turn, would cause decrease of the contact surface of the villi with the maternal blood in the intervillous space, with a consequent reduction of maternal metabolic contribution to the fetus. Our results, although preliminary, seem to suggest that deregulation of the apoptotic process of placental trophoblast, endothelium, myofioblast and telocytes may lead to placental dysfunction and disturbance of the normal fetal blood flow.

In conclusion, taking our experimental results and the bibliographic evidences together, we postulate here that telocytes in the hypoxic placenta experience the same apoptotic fate of endothelium and trophoblast as a consequence of oxidative stress (Fig. 2). As placental apoptosis has so far been studied with regard to syncytiotrophoblast and EVT apoptosis [6,26,31,74] further analysis of the apoptotic state of telocytes in the PE placenta would help to complete the picture regarding the apoptotic cycle. In our opinion, the finding of placental telocytes supports the notion of the existence of a network, either by direct cell to cell contact, or indirectly, by secreting paracrine signaling molecules in order to regulate placental function, blood flow and the length and width of the villi in the intervillous space. We have summarized a number of recent evidence aimed at shedding some light on the relationship between placental telocyte failure and the possible ulcer failure of telocytes in pathological organs in adulthood. Furthermore, we propose that it is possible that placentas in which telocytes are damaged as a consequence of oxidative stress, could represent a reflection of the condition of other organs later in adult life, as those telocytes had the same mesodermic origin during the embryogenesis. More experimental studies are needed to further substantiate this notion.

Conflict of interest statement

None.

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References

Bosco C, Díaz E, Gutiérrez R, González J, Pérez J. Ganglionar nervous cells and
Nicolescu MI, Popescu LM. Telocytes in the interstitium of human exocrine
Wallace AS, Burns AJ. Development of the enteric nervous system, smooth
Faussone-Pellegrini MS, Thuneberg L. Guide to the identification of interstitial
Wang XY, Diamant NE, Huizinga JD. Interstitial cells of Cajal: pacemaker cells
Tjoa ML, Cindrova-Davies T, Spasic-Boskovic O, Bianchi DW, Burton GJ. Telocytoid cells: a case of serendipity: the
Heazell AE, Crocker IP. Live and let die – regulation of villous trophoblast
Hyazell AE, Crocker IP. Live and let die – regulation of villous trophoblast
Smith SC, Baker PN, Symonds EM. Placental apoptosis in normal human
Sharp AN, Heazell AE, Crocker IP, Mor G. Placental apoptosis in normal human
Wang Y, Walsh SW. Antioxidant activities and mRNA expression of superoxide
Bae I, Fan S, Meng Q, et al. BRCA1 induces antioxidant gene expression and
Davidge ST. Oxidative stress and altered endothelial cell function in
Liochev SI, Fridovich I. How does superoxide dismutase protect against tumor
Davidge ST. Oxidative stress and altered endothelial cell function in
Wang XY, Diamant NE, Huizinga JD. Interstitial cells of Cajal: pacemaker cells
Zachary I. Signaling mechanisms mediating vascular protective actions of
Sgambati E, Marini M, Zappoli Thyrion GD, et al. VEGF expression in the
Sgambari E, Marini M, Zappoli Thyrion GD, et al. VEGF expression in the
Heazell AE, Crocker IP. Live and let die – regulation of villous trophoblast
Wang Y, Walsh SW. Antioxidant activities and mRNA expression of superoxide