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Reactive Oxygen and Nitrogen Species and Functional Adaptation of the Placenta

Leslie Myatt

University of Texas Health Science Center San Antonio

Abstract

The placenta regulates fetal growth and development via transport of nutrients and gases, and synthesis and secretion of steroid and peptide hormones. These functions are determined by vascular development and blood flow and by growth and differentiation of the trophoblast, which contains receptors, transporters and enzymes. The placenta generates reactive oxygen species which may contribute to the oxidative stress seen even in normal pregnancy but this is increased in pregnancies complicated by preeclampsia, IUGR and pregestational diabetes where oxidative and nitritative stress have been clearly documented. Nitritative stress is the covalent modification of proteins and DNA by peroxynitrite formed by the interaction of superoxide and nitric oxide. We have demonstrated nitritative stress by localizing nitrotyrosine residues in these placentas and found increased expression of NADPH oxidase (NOX) enzyme isoforms 1 and 5 as a potential source of superoxide generation. The presence of nitritative stress was associated with diminished vascular reactivity of the fetal placental circulation, a situation that could be reproduced by treatment with peroxynitrite in vitro. We find many nitrated proteins in the placenta, including p38 MAP kinase which has a role in development of the villous vasculature. Nitration of p38 MAPK was increased in the preeclamptic placenta and associated with loss of catalytic activity. We hypothesize that nitration of proteins in the placenta including receptors, transporters, enzymes and structural proteins can alter protein and placental function and this influences fetal growth and development. Increasing nitritative stress but a decrease in oxidative stress, measured as protein carbonylation, is found in the placenta with increasing BMI. Formation of peroxynitrite may then consume superoxide, decreasing nitritative stress. As protein carbonylation is a covalent modification at Lys, Arg, Pro and Thr residues the switch from carbonylation to nitration at tyrosine residues may alter protein function and hence placental function.

1. Introduction

The placenta has crucial role in pregnancy as the interface between mother and fetus. It anchors the conceptus, provides an interface for the exchange and modification of nutrients and gases, synthesizes and secretes a range of steroid and peptide hormones as well as providing an immune barrier between the mother and the semi-allogeneic fetus. There is a large volume of literature that link abnormalities in each of these aspects of placental function with poor perinatal outcome. These include defective trophoblast invasion, which may have immune causes, and abnormalities in hormone production, placental blood flows and nutrient transfer

Corresponding author: Leslie Myatt PhD, Department of Obstetrics and Gynecology, Center for Pregnancy and Newborn Research, University of Texas Health Science Center San Antonio, Mail Code 7836, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900, Myattl@uthscsa.edu, Tel 210 567 7044 (office), Fax 210 567 5033.

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that are associated with poor fetal growth. Historically there has been a focus on the role of abnormal trophoblast invasion, maldevelopment of uterine blood flow and thus supply of nutrients, and generation of an ischemia/reperfusion type injury in regulation of fetal growth. More recently, however, there has been a renewed focus on the roles that expression and activity of nutrient transporters on the trophoblast surface play in fetal growth and development. The determinants of fetal growth are now recognized to be (a) those that are vascular (flow) dependent and regulated by remodeling, vasculogenesis and angiogenesis in uterine and fetal placental vasculatures [1–2] and (b) those that are trophoblast or membrane dependent and regulated by exchange surface area and thickness, transporter expression [3] and activity and hormone production [4] and metabolism. These in turn depend on trophoblast proliferation and differentiation.

2. The effect of timing of a placental “insult”

The placenta is in a constant state of growth and differentiation throughout gestation with development of the vasculature and the trophoblast occurring at different times in a highly regulated manner. An “insult” applied to this developmental process at any time may have an effect on subsequent placental function and hence fetal growth and development [5]. When the normal pattern of fetal growth and development is disrupted by an abnormal stimulus or “insult” applied at a critical point in in utero development “fetal programming” occurs. Fetal programming may determine the risk of disease in later life [6]. Equally the same insult applied to the placenta at different times may give divergent effects. For example in pregnancies complicated by insulin dependent diabetes mellitus with a LGA baby, increased Glut1 expression is seen in the basement membrane of trophoblast together with increased system A amino acid transporter [7]. However gestational diabetes plus an LGA baby shows no change in basement membrane Glut 1 but an increase in system A transporter [8]. This observation is consistent with hyperglycemia causing increased Glut 1 in the first trimester.

3. Generation of oxidative and nitrate stress in the placenta

The defective trophoblast invasion seen with preeclampsia or IUGR is thought to lead to a relative hypoxia [9] in the villous placenta or an ischemia/reperfusion type injury [10]. This then leads to a heightened inflammatory response in turn giving oxidative [11] and nitrate stress [12]. We hypothesize that oxidative and nitrate stress can lead to altered placental function via covalent modification of protein structure and function. Measurements of markers of oxidative stress in maternal blood and urine [13–15] show that pregnancy per se is a state of oxidative stress due to the high metabolic activity of the placenta and maternal metabolism in pregnancy. However, this is heightened in pregnancies complicated by preeclampsia, IUGR and diabetes as measured by increased markers of production of reactive oxygen species and decreases in antioxidant defences [11,16–18].

The placenta produces many reactive oxygen species but the focus of this discussion will be superoxide, nitric oxide and peroxynitrite. These reactive oxygen species have differing half lives which in turn determine their potential diffusion distances in the placenta. The diffusion distance of superoxide is 0.4 μm in one half life, this limiting it to an intracrine action within the cell of synthesis. In contrast the diffusion distance of NO is 100 μm allowing it to cross the distance of an intermediate stem villous and act as a paracrine mediator in adjacent cells. Indeed NO produced by endothelial cells can act on underlying smooth muscle. The diffusion distance of peroxynitrite is 5 μm (the thickness of the trophoblast membrane) [19], meaning again it acts locally. Indeed we find evidence of peroxynitrite action very locally in the vascular endothelium and surrounding stroma of pregnancies complicated by pregestational diabetes or preeclampsia [12,20]. Placental production of superoxide is increased in preeclampsia. There may be several cellular sources in the placenta including the mitochondrial electron transport

chain [21], xanthine oxidase [22] and NADPH oxidase (NOX) [23]. Previously Raijmakers et al [24] had provided evidence for an increase in NOX-dependent superoxide generation in the placenta in preeclampsia. There is now recognized to be a superfamily of NOX enzymes [25]. In addition to their well recognized role in host defence the NOX enzymes may function in oxygen sensing, control of cell proliferation and intercellular signaling but may also have roles in pathologies such as atherosclerosis and vascular smooth muscle hypertrophy [26]. We have cloned NOX 1 and 5 isoforms from human trophoblast [27] and found that expression of both isoforms was increased in syncytiotrophoblast, vascular endothelium and stromal cells of the placenta in preeclampsia and may account for the increased superoxide generation.

4. Peroxynitrite and nitrotyrosine

When endothelium derived relaxing factor was identified as nitric oxide it was pharmacologically defined by the ability of superoxide to scavenge EDRF or NO and the ability of superoxide dismutase (which scavenges superoxide) to prolong the half life of NO [28]. Under conditions of increased superoxide and NO production they react together to produce a more long-lived and potent pro-oxidant, peroxynitrite [19]. Peroxynitrite characteristically nitrates tyrosine residues in proteins to produce nitrotyrosine [29]. Nitrotyrosine is measured, either by ELISA or immunohistochemistry, as an index of peroxynitrite production and action. This nitration and covalent modification of proteins and DNA is described as nitrative stress. Nitrotyrosine residues were first described in atherosclerotic plaques [30] but have now been described in many inflammatory situations including by ourselves in the placenta of pregnancies complicated by preeclampsia [12] or pre-gestational diabetes [20]. What then are the consequences of protein nitration? The description of the occurrence of nitrotyrosine in many pathologic situations led to the belief that nitration was a terminal event, where proteins lost activity and there was cellular damage. Indeed nitration targets proteins to the proteasome [31]. However nitration is increasingly thought of as a selective, reversible physiologic process with beneficial as well as detrimental effects. Indeed covalent modification by nitration gives loss of function in many proteins eg SOD [32] and PGI₂ synthase [33], but a gain of function in others eg PARP [34] and COX-2 [35]. Within any cell many proteins are found to be nitrated and include cytosolic, membrane, mitochondrial and structural proteins [36]. The covalent modification by nitration is akin to a phosphorylation. Indeed the addition of a negatively charged nitrate group can in some cases mimic phosphorylation. However to date no equivalent of the phosphatase i.e. the denitrase activity has been isolated although there is evidence that cellular homogenates possess a denitrase-like activity [37]. There is also evidence from in vitro studies of a denitrase-like activity in other cell systems [38–39]. Recent work with mitochondrial protein nitration has revealed a nitration consensus sequence [40]. Protein nitration may also be residue, protein and tissue specific with not all tyrosine residues of a protein being nitrated and not all proteins nitrated [41] depending on cellular location of protein, and the peroxynitrite generating system [42], the concentration of peroxynitrite and interaction with other molecules.

5. Functional consequences of protein nitration

What then is the functional consequence of protein nitration in the placenta? We first described the presence of nitrotyrosine residues in the vascular endothelium and surrounding stroma and in syncytiotrophoblast of placentas from pregnancies complicated by preeclampsia and pregestational diabetes [12,20]. This was associated with an attenuated vascular reactivity in these placentas [43]. We could recapitulate these findings in the placental vasculature treated in vitro with peroxynitrite which led to attenuated vascular reactivity and the presence of nitrotyrosine residues [43] suggesting that peroxynitrite did indeed cause alterations in vascular reactivity by nitration of proteins in the fetal placental vasculature. The search for nitrated proteins in the placenta can be performed in two ways, either by screening for all nitrated

proteins or by examination of selected proteins using immunoblotting, immunoprecipitation and mass spectrometry techniques. P38MAP kinase is critical for placental development as p38 MAP kinase null mice show embryonic lethality with decreased labyrinthine and spongiotrophoblast layers and decreased vascularization of the labyrinth [44]. We subsequently identified p38 MAP kinase as a nitrated protein in the placenta [45], that in vitro p38 MAP kinase could be nitrated with peroxynitrite at tyrosine residues, and further that this was associated with a loss of catalytic activity [46]. This data strongly suggests that nitrative stress and covalent modification of protein function may have an effect on placental function.

Subsequently we have identified many nitrated proteins in the placenta. Nitrated proteins are found in the normal placenta and in increased amounts in placentas from pregnancies complicated by preeclampsia and pregestational diabetes. These include acetyl CoA acyltransferase, p53, P2X4 purinergic receptors [47] and the taurine transporter among others. Potentially any protein in the placenta is a target for nitration including enzymes, receptors, transporters, signal transduction molecules, structural proteins and steroid hormones. The covalent modification of any of these molecules potentially can alter protein and then placental function and thus fetal growth and development (Figure 1).

6. Obesity and oxidative and nitrative stress

Obesity is a growing problem in the US and many other developed countries. Fifty seven percent of women of reproductive age in the US are overweight (BMI>25) and 30% are obese (BMI>30). Obesity during pregnancy is associated with maternal complications and poor perinatal outcome, including development of pregnancy induced hypertension, diabetes, an increased rate of cesarean section and its complications, prematurity, stillbirth and macrosomia [48–49]. As adults the offspring of such pregnancies show an increased incidence of obesity, insulin resistance, hypertension and cardiovascular disease [50]. Obesity also leads to increased production of inflammatory cytokines [51–52] which in turn can lead to increased oxidative and nitrative stress. We hypothesized that with increasing BMI there would be increased oxidative and nitrative stress in the placenta. We collected placental tissue from lean (BMI 18.5–24.9), overweight (BMI 25–25.9) and obese (BMI 30–40) and measured oxidative stress via measurement of protein carbonyls and nitrative stress by measurement of nitrotyrosine residues. Interestingly we found that with increasing BMI there was increasing concentrations of nitrotyrosine residues but conversely decreasing concentrations of protein carbonyls [53]. We hypothesize that increasing nitrative stress draws superoxide radicals away from oxidative effects towards formation of peroxynitrite. Protein carbonylation is another covalent modification but with carbonyls being placed on lysine arginine, proline and threonine residues. Modification of proteins at these residues may give distinct functional effects from that of nitration at tyrosine residues. Thus the balance between oxidative and nitrative stress or carbonylation vs nitration may alter placental function. Our data therefore suggest that in addition to under- or overnutrition, changes in diet or exposure to inappropriate developmental signals or stress that ischemia/reperfusion injury and oxidative/nitrative stress may affect placental function and hence fetal growth and development.

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Figure 1.
Targets and Effects of Protein Nitration In the Placenta