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Is there a role for exosomes in foetoplacental endothelial dysfunction in gestational diabetes mellitus?

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A B S T R A C T  

Gestational diabetes mellitus (GDM) is a disease of pregnancy associated with endothelial dysfunction in the foetoplacental vasculature. Foetoplacental endothelial dysfunction is characterized by changes in the L-arginine—adenosine signalling pathway and inflammation. The mechanisms involved in these alterations are suggested to be hyperglycaemia, hyperinsulinaemia, and oxidative stress. These conditions increase the release of exosomes, nanovesicles that are generated from diverse cell types, including endothelial cells. Since exosomes can modulate vascular function, they may play an important role in foetoplacental endothelial dysfunction seen in GDM pregnancies. In this review, we summarized current knowledge on the potential role of exosomes in foetoplacental endothelial dysfunction seen in this disease of pregnancy.

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1. Introduction

Gestational diabetes mellitus (GDM) is a disease characterized by maternal glucose intolerance first appearing or diagnosed during pregnancy. It is associated with maternal hyperglycaemia, and
foetal hyperglycaemia and hyperinsulinemia [1]. Mothers diagnosed with GDM and children born to GDM pregnancies have a higher risk for developing metabolic syndrome, type 2 diabetes mellitus, or cardiovascular disease in later life [2,3]. GDM is associated with endothelial cell activation and dysfunction in both the microvasculature and macrovasculature of the placenta (i.e., foeto-placental vasculature) [4,5]. Endothelial cell activation is characterized by a proinflammatory, procoagulant state of the endothelial cells lining the lumen of blood vessels and is associated with increased expression of adhesion molecules [6–10], such as intracellular adhesion molecule type 1 (ICAM-1) as reported in GDM [11,12]. Also, a functional disassociation between the synthesis and bioavailability of nitric oxide (NO), a key vasodilator in the foeto-placental vasculature, is reported in this disease [5]. Endothelial activation is seen in early stages of vascular diseases such as atherosclerosis [13]. Thus, involvement of endothelial activation in the pathophysiology of foeto-placental endothelial dysfunction in GDM pregnancy is likely. Along with hyperglycaemia, inflammation, and oxidative stress in GDM-associated endothelial dysfunction, we here focus on the involvement of endothelial-derived exosomes in endothelial dysfunction and activation in the foeto-placental vasculature in GDM pregnancies.

2. Foeto-placental endothelial (dys)function in healthy pregnancy and GDM

The placenta is the organ responsible for maternal-foetal exchange of nutrients in the placental villi. Since the foeto-placental vasculature lacks innervation [14] the regulation of vascular tone in these vessels depends on the balance between the generation and bioactivity of endothelial cell-derived vasodilators (including NO and adenosine) and vasoconstrictors (including endothelin-1) [5,15]. To be able to generate NO, human umbilical vein endothelial cells (HUVECs) take up L-arginine via the human cationic amino acid transporters type 1 (hCAT-1) [16], and metabolize this amino acid through the endothelial NO synthase (eNOS) [5]. Adenosine is important in this pathway, since it upregulates the L-arginine/NO signalling pathway [5,17] (see Fig. 1A).

Endothelial dysfunction in GDM is characterized by an upregulated L-arginine/NO signalling pathway, i.e., by increased hCAT-1 expression and increased eNOS activity and expression [16–18]. This is associated with increased extracellular adenosine levels, likely due to decreased expression of the human equilibrative nucleoside transporter 1 (hENT1) [18] (see Fig. 1B). GDM is also characterized by a pro-inflammatory state in the foetus and in the mother, including higher levels of pro-inflammatory cytokines in the maternal circulation [19], the placenta [20], and umbilical cord vessels [11], and is associated with endothelial cell activation, characterized by increased expression of ICAM-1, compared with normal pregnancies. The endothelial expression of adhesion molecules on the cell membrane is involved in the adhesion of leukocytes to the endothelium [21] and this phenomenon may contribute to inflammation and endothelial dysfunction in GDM.

The mechanisms by which GDM induces foetal endothelial dysfunction and endothelial cell activation are not completely known, but include hyperglycaemia [6,22] and oxidative stress [23–28]. HUVECs from normal pregnancies incubated with high D-glucose (12–26 mmol/L) showed endothelial dysfunction and activation as in HUVECs from GDM patients. This was characterized by an increased L-arginine/NO signalling pathway [16], and decreased hENT1-mediated adenosine transport [22], increased expression of ICAM-1 [6] and toll-like receptors 2 and 4 [23]. The involvement of oxidative stress has been shown by the fact that high D-glucose also increased the generation of superoxide anion (O₂⁻) in HUVECs [24,25]. Moreover, foetal tissues from pregnant women with GDM show oxidative stress as well as changes in radical scavenger function [26], such as increased glutathione and glutathione peroxidase activity, but decreased superoxide dismutase and vitamin E levels in placentas and umbilical cord plasma from GDM pregnancies [27,28]. However, a low level of nitrite, an indirect index of NO level, in maternal plasma and in cord blood from these pregnancies was reported [29]. This finding may suggest that in vivo in GDM there is a decreased bioavailability of NO. The latter may result from increased conversion of NO by O₂⁻ into peroxynitrite (ONOO⁻) which results in a higher abundance of nitrotyrosine in proteins in GDM [28,30,31]. The reported consequence of this phenomenon is endothelial dysfunction as seen in GDM [31–33].

3. Exosomes

Exosomes are nanovesicles (~40–100 nm diameter) [34] released into the extracellular space by different cell types, including endothelial cells [35] (Fig. 2). Microvesicles are larger vesicles (up to ~1000 nm diameter) and bud, in contrast to exosomes, directly from the plasma membrane [34]. Exosomes contain proteins and lipids, but also miRNA, microRNA and noncoding RNA [36], and are involved in cell-cell communication [37]. In endothelial cells, exosomes modulate a broad spectrum of cellular functions, including migration [38,39], proliferation [40], and angiogenesis [38,41–44]. Although the largest population of extracellular vesicles in the circulation is derived from platelets, an important subpopulation of extracellular vesicles in the circulation is derived from endothelial cells [45]. Therefore, an important role for exosomes in physiological processes, including intercellular communication between endothelial cells and inflammatory cells, has been suggested. Since high D-glucose increased the number of exosomes and alters the exosome protein and RNA composition in endothelial cells [34], a role for exosomes in GDM in the maternal and foetal circulation is likely. In this review, we focus on the potential role of exosomes in endothelial cell dysfunction and activation in the foeto-placental circulation. Although, little data are available on the role of exosomes in foeto-placental endothelial dysfunction specifically, based on data in existing literature on effects of exosomes on endothelial cells in general, we hypothesize that exosomes play a role in the foetal-placental endothelial dysfunction.

3.1. Exosomes and the L-arginine/NO signalling pathway

A few studies suggested a role for endothelial exosomes as modulators of the L-arginine/NO signalling pathway. Endothelial exosomes modulate the PI3K/eNOS signalling pathway [46], which is altered in the foeto-placental endothelium in GDM pregnancies [47]. Moreover, considering that exosomes may contain miRNA-203 [48], which induces NO activity [49], suggests that exosomes in the foetal circulation in GDM may also contain this miRNA and therefore may affect foetal endothelial cell function. Interestingly, a recent study showed that exosomes derived from syncytiotrophoblast cells carry eNOS [50], which could also contribute to NO production. We recently showed that exosomes derived from HUVECs from GDM pregnancies increased L-arginine transport, most likely via hCAT-1, in this cell type from normal pregnancies reaching comparable values to those in cells from GDM [51]. This study shows a potential role for exosomes in the foetal circulation in GDM in inducing foeto-placental endothelial dysfunction.

3.2. Exosomes and adenosine signalling

Studies suggesting a role for exosomes in adenosine signalling...
Fig. 1. Adenosine/L-Arginine/Nitric Oxide (ALANO) signalling pathway in HUVECs from normal and gestational diabetes mellitus pregnancies. A, In HUVECs from normal pregnancies (healthy pregnancy), transport of L-arginine is mainly mediated by the human cationic amino acid transporter 1 (hCAT-1). L-Arginine is used by the endothelial nitric oxide synthase (eNOS) for the generation of L-citrulline and nitric oxide (NO), which reaches the vascular smooth cells to cause vasodilation. hCAT-1 and eNOS activity is modulated by extracellular adenosine through the activation of A₁, A₂A, A₂B, and A₃ adenosine receptors. Adenosine is generated from ATP/ADP catabolism via the CD39/CD73 axis, and its extracellular concentration is kept in physiological ranges by its transport into the cell mediated by the human equilibrative nucleoside transporter 1 (hENT1). The physiological
showed that circulating microparticles and exosomes express CD39, an ecto-enzyme for the generation of adenosine [52]. Similarly, tumour cells secrete exosomes that express both CD73 and CD39 [53], while exosomes from human T cells contain CD73 [54]. These findings suggest that exosomes carry the enzymatic machinery required to generate adenosine and that exosomes could be a mechanism promoting the production or the increase in the extracellular concentration of adenosine.

3.3. Exosomes and endothelial activation

It has been shown that exosomes from various cell types in patients with uncontrolled diabetes mellitus contain TNF-α [55,56]. Since TNFα is a major mediator in inflammation [57], it is likely that exosomes play a role in inflammation and endothelial cell dysfunction. Interestingly, HUVECs exposed to exosomes from monocytes show increased endothelial thrombogenicity, apoptosis and angiogenesis [58], indicating that monocytes-derived exosomes could also be involved in endothelial dysfunction. Since cytokines increase the release of exosomes [59], inflammatory molecules may promote the generation of exosomes from the endothelium leading to endothelial activation.

3.4. Exosomes and hyperglycaemia

Different studies have correlated the effect of high d-glucose with the release of exosomes and changes in exosomal cargo. High d-glucose increases the release of exosomes from first trimester trophoblast cells, and these nanovesicles induce the secretion of cytokines, such as interleukin-8 (IL-8) and TNF-α in HUVECs [60]. Preliminary findings suggest the possibility that high d-glucose-induced exosomes generate a high d-glucose phenotype in HUVECs resembling the GDM phenotype [61]. Other studies showed that exposure of glomerular endothelial cells to high d-glucose results in increased release of exosomes, while these exosomes in their turn promote renal fibrosis by activating transforming growth factor-β1/mothers against decapentaplegic homolog 3 (TGF-β1/Smad3) signalling pathway in glomerular mesangial cells [62]. It seems thus likely that high d-glucose-increased release of exosomes may contribute to foetoplacental endothelial dysfunction in GDM pregnancies.

3.5. Exosomes and oxidative stress

Various studies suggest that exosomes affect oxidative stress. The biological actions of exosomes on oxidative stress in target cells is most likely tissue dependent, since for instance exosomes from melanoma increase [55], while exosomes secreted by mesenchymal stem cells decrease [63] reactive oxygen species (ROS) generation. Moreover, ROS lead to the secretion of exosomes by tumour cells [64] indicating that generation of exosomes or ROS may result in a vicious circle involving ROS and exosome generation.

Exosomes from various tissues have been shown to induce the generation of ROS in target cells. For instance, exosomes induced ROS in human microvascular endothelial cells, a phenomenon associated with increased NADPH oxidase in this cell type [65]. Also, platelet-derived exosomes [66] or microvesicles [67] from patients with septic vascular dysfunction induced increased NO and O2- levels and expression of NADPH oxidase. We have recently shown that in HUVECs from normal pregnancies, which were intracellular level of NO is not enough or just enough to maintain (segmented arrow) the activation of the transcription factors C/EBP homologous protein 10 (hCHOP) and C/EBPs transcription factor (C/EBPα) to a minimum. B. In HUVECs from gestational diabetes mellitus (GDM) pregnancies, transport of l-arginine is increased (⇧) due to higher expression and activity of hCAT-1, which also increases eNOS activity and NO synthesis. NO activates hCHOP–C/EBPα complex reducing (⇩) the transcription of SLC29A1 for hENT-1, decreasing hENT-1 expression and adenosine transport. This phenomenon leads to increased extracellular adenosine level activating the l-arginine/NO signalling via preferential activation of A2A adenosine receptors. From data in Refs. [17,24,25,72].
exposed to exosomes from HUVECs from GDM pregnancies a GDM phenotype was induced, with increased eNOS total protein and NO synthesis [12,51]. However, exosomes may also have beneficial effects, as we have shown, for example, that exosomes from HUVECs from normal pregnancies may revert the high d-glucose-induced dysfunction of these cells back to normal function [61]. This corroborates findings from others who showed in vivo that exosomes from mouse mesenchymal stem cells may induce liver regeneration [68].

Although exosomes from specific cells may carry phosphorylated proteins involved in modulation of apoptosis, cell survival and cell metabolism, such as protein kinase B/Akt and lactate dehydrogenase [69], it is unknown whether exosomes contain the enzymatic machinery to generate free radicals. A recent study suggest that they do, since exosomes released from the STB in preeclampsia show reduced synthesis of NO from eNOS compared with exosomes from normal pregnancies [50]. The reasons for this observation was not addressed in this study but since eNOS uncoupling is known to generate O2•−, which scavenges NO, it is likely that exosomes generated by cells from the foetoplacental vasculature in diseases of pregnancy could carry a deficient eNOS resulting in increased oxidative stress. At present, therefore, there is not enough evidence to suggest that exosomes carry the enzymatic machinery to generate free radicals. A recent study suggest that they do, since exosomes released from the STB in preeclampsia show reduced synthesis of NO from eNOS compared with exosomes from normal pregnancies [50]. The reasons for this observation was not addressed in this study but since eNOS uncoupling is known to generate O2•−, which scavenges NO, it is likely that exosomes generated by cells from the foetoplacental vasculature in diseases of pregnancy could carry a deficient eNOS resulting in increased oxidative stress. At present, therefore, there is not enough evidence to suggest that exosomes carry the enzymatic machinery to generate free radicals. A recent study suggest that they do, since exosomes released from the STB in preeclampsia show reduced synthesis of NO from eNOS compared with exosomes from normal pregnancies [50]. The reasons for this observation was not addressed in this study but since eNOS uncoupling is known to generate O2•−, which scavenges NO, it is likely that exosomes generated by cells from the foetoplacental vasculature in diseases of pregnancy could carry a deficient eNOS resulting in increased oxidative stress. At present, therefore, there is not enough evidence to suggest that exosomes carry the enzymatic machinery to generate free radicals. A recent study suggest that they do, since exosomes released from the STB in preeclampsia show reduced synthesis of NO from eNOS compared with exosomes from normal pregnancies [50]. The reasons for this observation was not addressed in this study but since eNOS uncoupling is known to generate O2•−, which scavenges NO, it is likely that exosomes generated by cells from the foetoplacental vasculature in diseases of pregnancy could carry a deficient eNOS resulting in increased oxidative stress. At present, therefore, there is not enough evidence to suggest that exosomes carry the enzymatic machinery to generate free radicals. A recent study suggest that they do, since exosomes released from the STB in preeclampsia show reduced synthesis of NO from eNOS compared with exosomes from normal pregnancies [50].

4. Concluding remarks

Exosomes are synthesized and released by endothelial cells, and hyperglycaemia and oxidative stress change their cargo [55,60,69]. Exosomes released in response to hyperglycaemia or oxidative stress induce dysfunction of various types of endothelia [55,60], including foetoplacental endothelium [12,51]. We propose that hyperglycaemia and oxidative stress may induce exosomes generation affecting their cargo in the foetoplacental vasculature in GDM leading to endothelial dysfunction and activation (Fig. 3). Since endothelial activation could result in an adverse outcome including immunological activation leading to clinical conditions such as hypertension, oedema, thrombosis, and infarction [70], or neonatal morbidity [71], foetoplacental endothelial dysfunction may be indicative of a metabolic disturbed foetus. Knowledge of the mechanisms behind this phenomenon may allow planning of future therapeutic strategies to promote long-term vascular health in the children from mothers with GDM.

Fig. 3. Possible role of foetoplacental exosomes in foetoplacental endothelial dysfunction in gestational diabetes mellitus. Foetoplacental exosomes from different cell types such as endothelium, immune, and other cells (A) are secreted into the blood circulation reaching foetoplacental endothelial cells, such as human umbilical vein endothelium (HUVECs). Both the release of exosomes and their cargo are influenced by abnormal environments, such as high blood d-glucose concentration (Hyperglycaemia) (B), and increased oxidative stress (C) seen in the foetoplacental tissue in gestational diabetes mellitus (GDM). The foetoplacental exosomes could be involved in modulation of the activity of CD39 and CD73 for ATP and ADP catabolism (D,E) increasing the extracellular concentration of adenosine (F) and the activity of adenosine receptors. Foetoplacental exosomes also could be involved in the modulation of the activity or expression of endothelial nitric oxide (eNOS) (G) and L-arginine transport via the human cationic amino acid transporter 1 (hCAT-1) (H) HUVECs from GDM. A1, A2A, A2B, A3, adenosine receptors; hENT-1, human equilibrative nucleoside transporter 1.
Conflicts of interest

There is no conflict of interest.

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