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Role of endoplasmic reticulum stress signalling in diabetic endothelial dysfunction and atherosclerosis

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Abstract

It is well established that diabetes mellitus accelerates atherosclerotic vascular disease. Endothelial injury has been proposed to be the initial event in the pathogenesis of atherosclerosis. Endothelium not only acts as a semi-selective barrier but also serves physiological and metabolic functions. Diabetes or high glucose in circulation triggers a series of intracellular responses and organ damage such as endothelial dysfunction and apoptosis. One such response is high glucose-induced chronic endoplasmic reticulum stress in the endothelium. The unfolded protein response is an acute reaction that enables cells to overcome endoplasmic reticulum stress. However, when chronically persistent, endoplasmic reticulum stress response could ultimately lead to endothelial dysfunction and atherosclerosis. Herein, we discuss the scientific advances in understanding endoplasmic reticulum stress-induced endothelial dysfunction, the pathogenesis of diabetes-accelerated atherosclerosis and endoplasmic reticulum stress as a potential target in therapies for diabetic atherosclerosis.

Keywords

Diabetes mellitus; endoplasmic reticulum stress; endothelial dysfunction; atherosclerosis

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Introduction

Diabetes mellitus is a disease where high blood sugar levels occur from decreased insulin signalling, either by a lack of insulin secretion in Type I diabetes or insulin resistance in Type II diabetes.[1] Diabetes is a complex group of diseases with a variety of causes including cardiovascular risk factors, such as obesity,[1] hypertension,[2] hypertriglyceridimia,[3] genetic alterations,[4] and lifestyle factors, such as smoking.[5] Further complications from the disease can often result in chronic kidney failure, brain damage, retinal blood vessel damage and other cardiovascular diseases.[6]

Atherosclerosis is marked by the appearance of plaques consisting of leukocytes, lipids, smooth muscle cells and low-density lipoprotein (LDL) cholesterol in the arterial walls.[4] When the condition worsens over time, it leads to ischemia-causing cerebrovascular accidents and myocardial infarctions in diabetic patients.[4] Along with coronary artery disease and congestive heart failures,[7] diabetic-induced atherosclerosis has been linked to endothelial dysfunction (ED) – a pathological imbalance between vasoconstriction and vasodilation in the endothelium.[8] Understanding the molecular mechanisms behind diabetic atherosclerosis is therefore of great clinical significance.

Healthy endothelium serves as a moderator producing autocrine and paracrine signals[9] to decide between vasodilation and vasoconstriction contractions in blood arteries. Nitric oxide (NO), formerly endothelium-derived relaxing factor,[10] bradykinin and prostacyclin are all potent vasodilators,[10] while thromboxanes,[8] serotonin,[8] endothelin[10] and angiotensin II[10] are vasoconstrictors involved in maintaining this equilibrium. In ED, a disorder of the endothelium lining the blood vessels, a decrease in bioavailability of NO[8,11] and an increase in vasoconstrictors[11] disrupt the equilibrium by increasing thrombus formation,[8] platelet aggregation,[8] chemokine production,[9] leukocyte adherence[9] and smooth muscle cell proliferation;[8,9] all of which contribute to atherosclerosis. The accumulation of cardiovascular risk factor superoxide, asymmetric dimethylarginine (ADMA, an endogenous NO inhibitor),[7,10] angiotensin II,[7,10] hyperhomocysteine (HHcy)[7,12] and impaired insulin signalling reduces endothelial nitric oxide synthase (eNOS) activity and levels of NO,[7] which are both major determinants of ED.[13]

ED has been suggested to predict the onset of atherosclerosis and is therefore considered to be an early marker for the disease.[10] Experiments using constriction of blood vessels in response to acetylcholine to test endothelial response have shown that ED occurs before and throughout the progression of atherosclerosis.[14,15] Similarly, plaque growth from ED is also part of the mechanism for the progression of the disease.[6,16] A dysfunctional endothelium promotes factors (mentioned above) such as migration of smooth muscle cells, chemokine and cytokine secretions (that promote plaque formation) and the development of cardiovascular diseases.[9] Even in the absence of inhibitors of smooth muscle cell proliferation like heparin, a blood thinner anticoagulant, one study found that administration of NO decreases the migration of vascular smooth muscle cells.[17]

Endoplasmic reticulum stress and its role in atherosclerosis

The rough endoplasmic reticulum (RER) is the organelle in the cell responsible for the production and correct folding of proteins destined for the endomembrane system and secretion. Pathological stimuli disrupts the endoplasmic reticulum (ER) homeostasis and causes misfolded or unfolded proteins to accumulate in the ER until properly folded or destroyed.[18] In general, when the ER is overwhelmed by the accumulation of misfolded or unfolded proteins, a condition known as ER stress, the unfolded protein response (UPR) is then activated, and the expression of chaperones is upregulated to help fold ER proteins.[19] The UPR happens through the activation of evolutionally conserved signalling pathways involving inositol-requiring protein-1 (IRE-1), activating transcription factor 6 (ATF6) and protein kinase RNA-like ER kinase (PERK), where acute-responsible chaperone proteins are synthesized.[20,21] If not chronic, this response is adaptive and helps the cell recover to normal health.[22] However, if the UPR is sustained, apoptosis is induced through a pathway involving the transcription factor C/EBPα-homologous protein (CHOP) that induces DR5 expression.[23] This sustained ER stress is implicated in many disease processes and has been linked to Type II diabetes,[24] cardiovascular disease,[25] Alzheimer's disease,[26] as well as atherosclerosis.[25,27] In one study, administration of ER stress inhibitors in hypertensive mouse models improved endothelial function, indicating that ER stress may have been the cause of ED.[28] Later, Lenna et al.[29] linked ER stress to dysfunctional eNOS in endothelial cells (ECs), and Galan et al.[30] showed that ER stress causes ED through oxidative stress. A link between ER stress and atherosclerosis was also established in atheroma ECs and macrophages.[31] Because ER stress is linked to ED and poor endothelium health is linked to atherosclerosis, the mechanisms that cause ER stress in diabetic conditions could be of great therapeutic potential. In this review, we will focus on the progress made in understanding molecular mechanisms involving reactive oxygen species (ROS)/reactive nitrogen species (RNS), mitochondrial dysfunction, intracellular calcium homeostasis, free fatty acids (FFAs), advanced glycation end products (AGEs), inflammation and insulin resistance. Therapeutic potentials of targeting ER stress signalling pathways will also be examined. For mechanisms not discussed in this review, such as abnormal disposal of unwanted proteins in ER, defects in autophagy, inhibition of the proteasome and flaws in ER–Golgi trafficking, readers should refer to other reviews.[32–38]

ROS and RNS

High glucose in diabetes stresses the electron transport chain in which electrons are directly passed from coenzyme Q to oxygen in generating superoxides or ROS.[39] As reviewed earlier, ED is linked to a reduction in NO bioavailability, either caused by reduced production or increased degradation of NO. One of the mechanisms for degradation of NO comes from its reaction with ROS[40] to generate peroxynitrite – a RNS.[41] This reaction occurs faster than reactions that normally remove ROS [which are catalysed by superoxide dismutase (SOD)][42] and produces elevated levels of ROS, a key downregulator of NO bioavailability. During chronic ER stress, the UPR generates ROS,[43] which suggests that ER stress is connected to ED and the decrease in NO concentration. The generation of ROS partly comes from ER oxidoreductin 1 protein (Ero1p), an oxidative thiol enzyme that produces H_2O_2 when it oxides protein disulphide isomerase (PDI) to fold proteins in the

UPR.[44] When non-chronic, the UPR actually reduces ROS by upregulating antioxidant pathways,[45] however, but only when the response is overwhelmed by ER stress. In fact, ROS can even cause ER stress, as one study showed that the UPR triggered by TNFα actually required ROS to be present.[45] This indicates that in some cases, the UPR is activated by ROS, where ROS also activates the NF-κB pathway[46] (which will be discussed later as a mechanism for ER stress response).

In addition to ER stress causing ED from producing peroxynitrite (through the reaction of ROS with NO), peroxynitrite itself can lead to ER stress. When ECs were treated with Sin-1, a peroxynitrite generator, or with uric acid (which reacts with peroxynitrite), the expression of chaperones characteristic of ER stress were induced with the former but not with the latter,[47] indicating that peroxynitrite (a RNS) is capable of causing ER stress.[47] Furthermore, peroxynitrite has a more direct effect on endothelial function through decreasing production of NO from eNOS.[42] Oxidized LDL can then decrease eNOS activity by reducing the dimer/monomer ratio and the total amount via calpain-mediated, ROS-dependent and Ca^{2+}-dependent manners.[48] Although NO is important for endothelial health as a RNS, excessive amounts of the compound can cause a disruption of ER chaperones that promote ER stress,[49] nitrosylate Akt, and inhibit insulin signalling in contributing to diabetes, where the effect can be further amplified in the presence of ROS.[50] In summary, both ROS and RNS can cause and be caused by ER stress, linking them to ED and atherosclerosis in diabetic patients.

The mitochondria's electron transport chain normally functions to produce adenosine triphosphate (ATP) for the cell. However, the mitochondria generate ROS as a by-product and can induce apoptosis through the activation of the caspase system by cytochrome C,[51] which is normally a component of the electron transport chain. Constant leakage of Ca^{2+} through inositol 1,4,5-trisphosphate receptor (IP3R) is required to generate ATP in the mitochondria, and when this pathway is blocked, ATP production decreases substantially.[52] During the early stages of ER stress, Ca^{2+} leakage from the ER and subsequent uptake by the mitochondria increase the rate of energy production[53] and help the cell in responding to ER stress. In chronic ER stress, small amount of cytochrome C is solubilized (partially by ROS oxidation of cardiolipin[54]) and leak from mitochondrial pores through apoptotic stimuli.[54] The small amount of cytochrome C binds to IP3R and causes a release of Ca^{2+}, which then activates a positive feedback loop to release more cytochrome C[54,55] to induce apoptosis (see further discussion in 'Intracellular calcium homeostasis').

Mitochondrial dysfunction is common in diabetic patients, and it has been suggested to be a cause of insulin resistance and impaired insulin secretion[56] having multiple effects on insulin signalling. It has been well established that mitochondrial dysfunction and ROS production accelerate diabetes, and because these two conditions could also be caused by the high glucose and fatty acid states of obesity-induced diabetes,[57] it demonstrates that there is a reciprocal causality between the two. This mitochondrial dysfunction has been linked to ED in humans[58,59] and also to ER stress through its activation of c-Jun N-terminal kinase (JNK)[60] with dependency on Ca^{2+}. Win et al.[60] reported that activation of JNK by ER stress correlated with increased ROS production and inhibited mitochondrial respiration. Additionally, mitochondrial dysfunction has been shown to induce inflammation by

activating redox-sensitive inflammatory pathways (since dysfunctional mitochondria generate high levels of ROS) or by activating the inflammosome.[61] Mitochondrial dysfunction directly promotes insulin resistance, diabetes and ED by generating ROS and activating inflammatory pathways that stress the ER and induce apoptosis.

Intracellular calcium homeostasis

Intracellular calcium homeostasis is critical to the maintenance of normal physiological functions of the cell. The process is mainly modulated through plasma membrane calcium pumps and ER-resident calcium pumps and receptors. Mitochondrial Ca^{2+} uniporters, Na-Ca^{2+} and H-Ca^{2+} exchangers also play an important role in intracellular calcium homeostasis. The ER normally serves as the major Ca^{2+} reservoir to maintain intracellular Ca^{2+} homeostasis.[62] It accomplishes this mainly through the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA) and ER-resident receptors such as IP3R. SERCA utilizes ATP hydrolysis to actively transport Ca^{2+} into the ER,[63] and oxidation of SERCA at a specific thiol group of cysteine increases atherosclerosis.[64] AMP-activated kinase alpha 2 (AMPKα2)-knockout mice exhibit augmented ER stress by changing the redox status via SERCA oxidation that disrupts the intracellular calcium homeostasis.[65] On the one hand, ox-LDL (the mediator for atherosclerosis) decreases SERCA activity by oxidizing with it and disturbing intracellular calcium balance, leading to EC ER stress.[66] On the other hand, Ca^{2+} is released from the ER during certain processes by Ca^{2+} release channels such as the ryanodine receptors (RyRs),[67] the IP3R[67] and translocon (TLC).[68] However, Ca^{2+} also plays a role in maintaining the environment of the ER,[63] where the balance of Ca^{2+} in the ER is disrupted and released into the cell, causing major problems in both the mitochondria[69] and ER.[70] In the mitochondria, excessive Ca^{2+} can lead to increased production of ROS and induce ED, linking Ca^{2+} leakage from ER to vascular complications.[69] Disruption of Ca^{2+} homeostasis in the ER can further lead to ER stress and activation of the UPR,[70] providing another link between intracellular Ca^{2+} and ED. This over-release of Ca^{2+} can then induce apoptosis in the cell.[71]

As mentioned earlier, when there are exceeding unfolded proteins in the ER, its stress is reduced when the cell activates the UPR. One of the mechanisms the cell uses to signal ER stress involves Ca^{2+}, which binds to chaperones such as calreticulin.[72] These and other chaperones are unable to keep up with the protein load during ER stress, where they appear to signal Ca^{2+} leak channels in causing release of Ca^{2+} into the cytosol. Hammadi et al.[68] found such a relationship between TLC and the ER chaperone glucose-regulated protein 78 or BiP (GRP78). The release of Ca^{2+} then leads to further misfolding of proteins similar to when Ca^{2+} homeostasis is disrupted, resulting in the dysfunction of these chaperones and protein misfolding.[72] When protein misfolding activates the UPR, a vicious cycle eventually ends in apoptosis where more Ca^{2+} are released.[35] However, with an abundance of chaperones around, they can signal the release channels to close. This is evidenced when increased concentrations of BiP closes Sec61, preventing ER Ca^{2+} leakage through the Sec61 complex by binding to Sec61α.[73] Loss of IP3R causes ER stress-induced apoptosis and brain damage,[74] and inhibiting SERCA activity also augments ER stress and apoptosis.[75]

Calcium also interacts with other pathways of ER stress as mentioned before. Both Ca^{2+} release and ROS production were necessary to stimulate NF-κB activation in cells under a condition of ER stress.[76] Ca^{2+} is also released in response to treatment with FFAs, which is another way in which FFAs cause ER stress.[77,78] In summary, intracellular calcium disruption causes ER stress and ED.

FFAs and AGEs

Obese individuals tend to have higher levels of FFAs, which are thought to play a role in the development of insulin resistance and diabetes.[79] While not all individuals with diabetes have elevated FFAs, many individuals with Type II diabetes often do. High concentrations of saturated FFAs are a contributing factor to ED.[80] These saturated fatty acids appear to cause ER stress,[81] while unsaturated fatty acids do not have the same effect.[82] When β-cells were treated for an extended time with palmitate, a saturated fatty acid, ER stress was induced, and the UPR was activated via a Ca^{2+}-dependent pathway.[77] Another study found that palmitate-induced ER stress decreased when the lipids were packed into triglycerides via overexpression of PPAR-γ and ACSL-1, while lipases increased ER stress.[83]

Including the ER, FFAs are packaged into phospholipids that are incorporated into the cell's membranes.[84] IRE-1 and PERK can sense the lipid composition of the ER membrane, and when the degree of saturation of the membrane's fatty acids become excessive, the UPR will be activated.[85] This occurs even when the protein-sensing domains of IRE-1 and PERK are non-functional.[85] Taken together, these results indicate a mechanism for the induction of ER stress through FFAs. High levels of saturated fatty acids increase the saturation of the ER membrane (which causes the UPR to activate). In obese and diabetic patients, this stress is chronic, causing apoptosis, ED and therefore atherosclerosis.

High glucose state of diabetes also causes an increase in the formation of AGEs.[86] These molecules are proteins, nucleic acids and lipids with abnormal glycosylations.[86] AGEs are linked to ED through binding with receptors for AGE (RAGEs) and activating responses that inhibit eNOS downstream.[87] Linden et al. found that patients with diabetic nephropathy have higher AGE levels correlated with higher levels of ADMA and impaired endothelial function, further linking ED to AGE.[88] Like FFAs, AGEs have been linked to and are speculated to cause ER stress in ECs[89], suggesting yet another link between ER stress and ED.

In the same study with ADMA, AGEs were found to increase ROS production,[88] contributing to ER stress and the activation of the UPR. When AGEs increase ROS production, NF-κB is activated and TNFα is produced, causing a further increase in superoxide production.[46,90–92] Furthermore, AGEs have also been suggested to phosphorylate eNOS and reduce the production of NO.[93] Through these mechanisms, AGEs cause ED, and ER stress is implicated in this process.

Inflammation

It is clear that obesity promotes states of both chronic low-grade inflammation and insulin resistance.[94] However, even in the absence of obesity, infusion of animals with

proinflammatory cytokines or lipids can cause insulin resistance.[94,95] Elevated levels of TNFα,[96] interleukin-6 (IL-6)[97] and IL-1β[97] are often seen in patients with diabetes. The adipose tissue of obese patients produced higher levels of TNFα as assessed by mRNA levels, and these patients also exhibited hyperinsulinemia, indicative of diabetes.[96] TNFα promotes ROS production[91] and inflammation.[98] Similarly, an enzyme-linked immunosorbent assay (ELISA) assay of samples from the EPIC-Potsdam study revealed that patients with elevated levels of IL-6 and IL-1β together had an increased risk of Type II diabetes.[97] Interestingly, having IL-1β or IL-6 levels elevated alone did not lead to a significant increase in risk, indicating that the interplay of cytokines is important for the development of diabetes.[97]

In turn, the high glucose state of diabetes promotes inflammation. Cytokines (such as TNFα and IL-1β) and receptors IL-1R or tumour necrosis factor receptor (TNFR) are produced by monocytes,[90] often working together in a positive feedback loop in obesity-induced diabetes. Proinflammatory cytokines produced by adipose tissue recruit monocytes and other leukocytes, and these cells in turn secrete more cytokines, suggesting an interaction between adipocytes and immune cells. Additionally, the NF-κB pathway (which activates and produces these cytokines[90]) is also activated in monocytes,[99] and the high glucose state causes the monocytes in diabetic patients to exhibit increased expression of toll-like receptor 2 (TLR2) and toll-like receptor 4 (TLR4)[100] (which then activates these inflammatory pathways through their recognition of heat-shock protein 60 (HSP60)[101]). These results are significant because TNFα, IL-1 and NF-κB signalling pathways are critical to promote cardiovascular complications.[100,102]

The role of proinflammatory cytokines in atherosclerosis has been well established.[103–106] The mechanism for proinflammatory cytokines affecting endothelial function again involves ER stress. ER stress has been shown to induce the NF-κB pathway through its activation of IRE-1 in myocytes,[107] leading to inflammatory responses. CHOP, a transcription factor during chronic stress, induces cell death and inflammatory responses after saturated FFA exposure by activating NF-κB through a pathway involving IRAK2 expression, resulting in secretion of cytokines IL-8 and TNFα.[108] The UPR has also been shown to selectively increase levels of IL-1α in hepatocytes[109] and IL-1β in macrophages[110] even though translation is normally inhibited during the response. Interestingly, upregulation of IL-1β has been shown to be independent of the UPR, since ER stress activates the NLRP3 (nucleotide-binding domain and leucine-rich repeat containing proteins) inflammasome[111] in providing a more direct link between it and inflammation. Akerfeldt et al.[112] demonstrated that cytokine-induced β-cell death is independent of ER stress signalling in INS-1 cells. This data further suggest a complexity between ER stress-induced and proinflammatory cytokine-induced apoptosis in Type I diabetes. To summarize, chronic ER stress may upregulate the expression of proinflammatory cytokines in diabetes or obesity. High level of proinflammatory cytokines in circulation is one of the key mediators for ER stress-induced ED[113] in atherosclerosis.

Insulin resistance

Type II diabetes is characterized by insensitivity to insulin that keeps blood glucose level elevated. ED has been linked to this insulin insensitivity, since normal insulin signalling is important in the production of NO and in the process of vasodilation.[114] In normal physiological functions, insulin signalling activates the kinase Akt (also known as protein kinase B), which phosphorylates many downstream targets with varying effects. One of these targets is eNOS,[115] where Akt causes an increase in the production of NO from the EC, which is important for vascular health. As a consequence of the disrupted insulin signalling, the activation of eNOS cannot occur, and the bioavailability of NO decreases, explaining a link between insulin and ED.

Extensive studies have demonstrated that insulin resistance is one of the key factors for ED in vitro and in vivo.[116–119] Furthermore, high level of insulin in circulation under the conditions of insulin resistance has been shown to upregulate ER stress and the UPR in humans.[120] Conversely, ER stress is implicated as a potential disrupter of insulin signalling itself, likely through JNK-interacting protein 1 (JIP-1).[94] As recently suggested by Zhang et al.,[121] ER stress potentiates insulin resistance through PERK-mediated FOXO phosphorylation and has also been shown to induce phosphorylation of rictor, a crucial phosphorylation site on mTORC-2, which inactivates and prevents the activation of Akt,[122] a key molecule in insulin signalling. Taken together, insulin resistance leads to ER stress and ED. In turn, ER stress feedback augments insulin resistance in providing another link between ER stress and ED.

Interplay among different mechanisms and pathways

As discussed above, diabetic-induced ER stress mechanisms for ED and atherosclerosis are complex. These pathways not only interact with ER stress signalling but also affect each other. For example, ROS/RNS promotes ER stress, while ER stress also induces oxidative species production.[123] Insulin resistance can induce ER stress, while chronic ER stress also adversely increases insulin resistance.[124] Similarly, ER stress enhances inflammation,[125] while chronic inflammation further promotes ER stress.[124,126] Because chronic ER stress in diabetic patients can lead to ED and apoptosis in atherosclerosis,[127] reducing or controlling one or several of the pathways to restore endothelial function would be beneficial to halt diabetic atherosclerosis.

Therapeutic potential of ER stress as target in diabetic atherosclerosis

ER stress has been proposed to be a potential therapeutic target in different diseases.[128] Indeed, targeting ER stress has shown promise.[27,129–132] GRP78 and CHOP have been suggested to be important targets to reduce ER stress.[133,134] Chemical ER chaperones have also been tested in animal models.[135] For example, recent studies have shown that chemical chaperones 4-PBA (phenylbutyrate acid) and TUDCA (tauroursodeoxycholic acid) reduce ER stress and restore glucose homeostasis in a mouse model with Type 2 diabetes[135,136] and cardiovascular disease.[65,137] Erbay et al.[138] have shown that ER modification from chemical chaperones in macrophages and adipocytes has therapeutic efficacy against atherosclerosis

in mouse models. We have previously reported that deletion of AMPKα2 significantly increases the level of ER stress and reduces ATPase (SERCA) activity in ECs due to thiol group oxidations and intracellular calcium imbalances.[65] Furthermore, administration of an ER stress alleviator can significantly reverse the progression of atherosclerosis in an AMPKα2-deficient mouse model.[65] AICAR and metformin attenuate atherosclerosis by AMPK activation and reduction in ER stress.[65,66] All these results suggest that ER stress is a promising target to treat diabetic atherosclerosis.

Closing remarks

Although the link between ER stress, ED and atherosclerosis has been established, diabetic-induced atherosclerotic mechanisms remain elusive. Future studies need to address several critical questions: (1) the molecular mechanisms underlying ED and the new pathways yet to be identified, (2) the interaction between endothelium and immune cells in pathological conditions, (3) identification of the main targeting molecules in ER stress signalling, (4) the possibility of combinational therapy by targeting multiple pathways in ER stress signalling and (5) the development of efficient technologies to specifically deliver candidate drugs to the atheroma.

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