Implications of Fundamental Signalling Alterations in Diabetes mellitus-associated Cardiovascular Disease

Pitchai Balakumar*
Pharmacology Unit, Faculty of Pharmacy, AIMST University, Semeling, 08100 Bedong, Kedah DarulAman, Malaysia

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The chronic diabetes mellitus (DM) is a major risk factor for cardiovascular disease. The incidence of cardiovascular disease might be a foremost cause of morbidity and mortality in patients afflicted with DM. In fact, DM is associated with multi-factorial cardiovascular signalling alterations via significant modulation of expression pattern, activation or release of PI3K, PKB, eNOS, EDRF, NADPH oxidase, EDHF, CGRP, adenosine, iNOS, ROCK, PKC-β2, CaMKII, microRNA (miR)-126 and miR-130a, which could result in inadequate maintenance of cardiovascular physiology and subsequent development of cardiovascular pathology. This review highlights the possible adverse implications of fundamental cardiovascular signalling alteration in DM-associated cardiovascular disease pathology.

Keywords: Diabetes mellitus, Signalling alterations, Cardiovascular disease pathology

Introduction
The prevalence rate of diabetes mellitus (DM) is high worldwide. The sixth edition of International Diabetes Federation (IDF) has recently reported that total number of patients with DM might increase from 382 million as of 2013 to 592 million by 20351,2. DM caused 5.1 million deaths in 2013 and every six seconds a person dies from DM1. It is a major risk factor for numerous cardiovascular disorders, including hypertension, atherosclerosis, coronary heart disease and cardiomyopathy3-7. Induction of vascular endothelial dysfunction (VED) might be a root cause of DM-associated cardiovascular disease3,8,9. The initial lesion of atherosclerosis as a result of VED during DM might be exaggerated by endothelial cellular blunting of nitric oxide (NO)-mediated vasodilatory response8. Vascular endothelium is an innermost lining of blood vessels that regulates vascular tone.

Furchgott and Zawadzki10 demonstrated the indispensable role of endothelium-derived relaxing factor (EDRF), also known as nitric oxide (NO) in the regulation of vascular relaxation10,11. The NO signals mediate anti-inflammatory, anti-atherogenic, anti-proliferative and anti-thrombotic actions12,13. DM-mediated reduction in the generation and bioavailability of NO due to inactivation of endothelial nitric oxide synthase (eNOS) and high vascular oxidative stress might account for the induction of VED and VED-associated cardiovascular disorders13. High glucose-induced oxidative stress induces VED, playing a central role in micro- and macro-vascular disease pathology. High oxidative stress brings out several phenotypic alterations in vascular smooth-muscle cell (VSMC) and promotes VSMC proliferation and migration in atherosclerotic lesions and VSMC apoptosis, contributing to atherosclerotic plaque instability and rupture14. The VED and increased generation of reactive oxygen species (ROS) are the underlying causes of hypertension and coronary artery disease in DM1,5.

It is worth mentioning that a marked alteration in cardiovascular defensive signalling system has been observed in numerous studies in the condition of DM. The phosphatidylinositol 3-kinase (PI3K) activates
serine/threonine protein kinase (Akt/protein kinase B), resulting in the activation of eNOS and subsequent production of endothelial NO. Studies have indicated that impairment of PI3K/Akt pathway might be one of important factors contributing to VED in DM. Both PI3K and Akt are essential components of ischemic preconditioning (IPC)-mediated cardioprotection against ischemia-reperfusion injury. The diabetic heart is known to be resistant to the myocardial infarct size-limiting effects of IPC because of DM-associated downregulation of PI3K-Akt signals. In addition, DM has been shown to be associated with altered endothelium-derived hyperpolarizing factor (EDHF), calcitonin gene-related peptide (CGRP) and adenosine signals in the cardiovascular system.

MicroRNAs (MiRs) have emerged as fundamental regulators of diverse cellular processes. Recent studies have reported that miR-126, miR-130a, miR-21, miR-27a and miR-27b are downregulated in endothelial progenitor cells (EPCs) from patients afflicted with type 2 DM. Downregulation of miR-126 and miR-130a is found to impair EPCs function, whereas dysfunction of EPCs contributes to diabetic vascular disease. Moreover, hyperglycemia is shown to cause a covalent modification of calcium/calmodulin-dependent protein kinase II (CaMKII) by O-linked N-acetylglucosamine (O-GlcNAc). In addition, O-GlcNAc-modified CaMKII is increased in the diabetic heart and is suggested to play a role in diabetic cardiac abnormalities.

In this review, the possible associations between key cardiovascular signalling alterations in DM and cardiovascular disease pathology have been discussed.

**Downregulation of cardiovascular PI3K-Akt-eNOS-NO signals during DM: A major concern**

The PI3K activates PKB/Akt, which enhances eNOS phosphorylation/activation and NO production to regulate cardiovascular function, while the cardiovascular PI3K-Akt-eNOS-NO signals are suppressed in the condition of DM. Such signalling alterations could make diabetic patients more vulnerable to cardiovascular disease induction and progression (Fig. 1). Below I discuss this fact in depth.

The PI3K-Akt-eNOS-NO signals play a fundamental role in the regulation of cardiovascular function. These signals regulate endothelial function, cardiomyocyte size and survival and angiogenesis.

Pharmacological agents that target PI3K-Akt-eNOS-NO have been shown to afford protection against cardiovascular disorders. Our group has recently reviewed that targeting eNOS might be a key mechanism involved in statins-mediated cardiovascular protection. The vascular endothelial protective potentials of statins are mediated by eNOS activation and subsequent generation of endothelial NO.

The PI3-K-Akt-eNOS-NO signals play a key role in preventing cardiovascular disease pathology. However, a marked suppression of these defensive signals has been noted in presence of hyperglycemia and DM in considerable number of studies. The hyperglycemia is shown to impair the activation of the PI3K-Akt pathway, resulting in deregulation of eNOS activity in human coronary artery endothelial cells. Likewise, another study has shown that hyperglycemia-induced impairment of PI3K-Akt signals might promote endothelial cellular proliferative dysfunction in DM.

Moreover, it is suggested that relaxation responses and NO production mediated via the PI3-K-Akt pathway are decreased in type 2 diabetic mice. The impairment of PI3K-Akt pathway underlies attenuated endothelial function in the aorta of mice with type 2 DM, suggesting that this might be a major cause of VED and resultant hypertension in type 2 DM. The expression level of total Akt protein is reported to decrease significantly in the diabetic aorta, while chronic simvastatin administration improves VED in type 2 diabetic mice via increasing Akt expression and phosphorylation.

Gender differences have been reported in the modulation of endothelial function in the aorta of type 2 diabetic mice. It has been reported that endothelial functions dependent on the Akt pathway are abrogated only in male mice with type 2 DM. Likewise, a recent study has suggested that endothelial functions mediated via the Akt-eNOS pathway are abrogated only in male mice with type 2 DM. In diabetic males as compared to diabetic females, systemic blood pressure is found to be elevated, insulin-induced Akt-dependent aortic relaxation is observed to be impaired and Akt and eNOS expression levels are observed to be lowered. The impairments are found in both the aortic relaxation and NO production induced by acetylcholine in male type 1 diabetic mice, which exhibit low adiponectin levels, while in female type 1
diabetic mice, impairments are observed of the aortic relaxations induced by both insulin and clonidine, suggesting that types of DM differentially affect male and female vascular beds.

It is important to note that hyperglycemia-induced impaired PI3K-Akt signalling could lead to migration, proliferation and angiogenesis dysfunction of endothelial cells in diabetes patients that are suggested to possibly contribute to the pathogenesis of diabetic vascular complications. A persistent reduction in vascular NO-sensitive guanylyl cyclase has been noted in genetically diabetic rats, suggesting that this defect might contribute to the elevation of blood pressure in the diabetic condition. A decrease in the bioavailability of NO within the vasculature in DM might occur due to hyperglycemia-mediated increase in the activation of NADPH oxidase and subsequent generation of superoxides. DM induces a cascade of events involved in the production of ROS from NADPH oxidase, leading to oxidation of tetrahydrobiopterin (BH4) and uncoupling of eNOS, promoting the oxidative inactivation of NO with subsequent formation of peroxynitrite. This mechanism could play a potentially important role in the pathogenesis of VED in DM. DM is often associated with diabetic dyslipidemia. The Akt pathway is noted to be hypoactivated by synergistic actions of DM and hypercholesterolemia, resulting in advanced coronary artery disease. DM and hypercholesterolemia are shown to synergistically induce the complex atherosclerosis associated with attenuated p-Akt (Ser473) levels, whereas aberrant Akt signalling is correlated with increased inflammation, cellular proliferation, apoptosis and vasa vasorum neovascularization.

The chronic and uncontrolled DM is associated with ischemic heart disease. Reperfusion to a previously ischemic myocardium could augment ischemic damage of the heart and this is known as ischemia-reperfusion injury. Ischemic preconditioning denotes short episodes of ischemia and reperfusion given before a sustained ischemia and reperfusion that can protect the
heart from detrimental ischemia-reperfusion injury\textsuperscript{17}. The cardio-protection against ischemia-reperfusion injury afforded by ischemic preconditioning is mediated through activation of PI3-K-Akt-eNOS signals in the heart\textsuperscript{43,45}. Moreover, apoptotic events in cardiac myocytes might be reduced by the activation of PI3K-Akt signals\textsuperscript{66}. However, the preconditioning-mediated cardio-protection might not be achieved in chronic diabetic myocardium\textsuperscript{17} because of DM-associated impairment in PI3K-Akt signaling\textsuperscript{47}. Both ischemic preconditioning and ischemic post-conditioning-associated myocardial protection are predominantly mediated by stimulation of PI3K-Akt signals and -associated GSK-3β pathway, while these signals are suppressed in DM, leaving both ischemic pre- and post-conditioning ineffective to afford myocardial protection against ischemia-reperfusion injury\textsuperscript{45}. In order to protect the diabetic myocardium, it appears necessary to increase the ischemic pre-conditioning stimulus for achieving the necessary threshold and the critical level of Akt phosphorylation to mediate myocardial protection\textsuperscript{48}.

It is demonstrated that glimepiride treatment facilitates ischemic preconditioning effect in the diabetic heart, as it lowers the threshold required to protect the diabetic heart by ischemic preconditioning\textsuperscript{48}. Another study has suggested that an increased susceptibility to ischemia-reperfusion injury in the aged diabetic heart could be a consequence of impaired Akt signalling (due to chronic Akt phosphorylation), while additional Akt phosphorylation required for ischemic preconditioning-mediated cardio-protection might not be possible in the aged diabetic rat heart, explaining the failure of this cardioprotective manoeuvre in the diabetic heart\textsuperscript{49}. Taken together, DM-associated suppression of PI3-K-Akt-eNOS-NO signalling system could significantly contribute to cardiovascular disease pathology associated with chronic DM (Fig. 1).

**Disparity in EDHF signals during DM**

Although NO regulates the vascular tone, it has become clear that EDHF is an important regulator of vascular tone majorly in small vessels, such as mesenteric arteries\textsuperscript{50}. DM is noted to be associated with reduced EDHF function in mesenteric arteries of diabetic mice\textsuperscript{19}. It is suggested that DM-induced reduction in EDHF function might not be compensated by increases in NO production\textsuperscript{19}. However, another study has suggested that when DM and hypercholesterolemia impair endothelium-dependent relaxation, due to a diminished contribution from NO, a compensatory contribution of EDHF to endothelium-dependent relaxation of the aorta could occur\textsuperscript{51}. The attenuation of NO-mediated relaxation in presence of both DM and hypercholesterolemia is suggested to be associated with enhanced superoxide generation\textsuperscript{51}.

Further, it is demonstrated that acetylcholine-induced relaxations are significantly impaired in mesenteric arteries from both male and female diabetic rats at weeks 1 and 8, whereas at week 8 the extent of impairment is significantly high in diabetic females as compared to diabetic males\textsuperscript{50}. The study has revealed that predisposition of female rat mesenteric arteries to vascular injury after the induction of DM might be due to a shift away from a putative EDHF, initially the major vasodilatory factor towards a greater reliance on NO\textsuperscript{50}. Recent study has investigated whether DM affects either or both NO-mediated and EDHF-type endothelium-dependent relaxation of mesenteric arteries from streptozotocin-induced diabetic rats\textsuperscript{52}. In this study, superoxide levels are found to be significantly increased in diabetic mesenteric arteries when compared with normal arteries, while both NO and EDHF-type relaxations are impaired in DM that are caused by increased oxidative stress\textsuperscript{52}. Taken together, such disparity in EDHF signals during DM might contribute to vascular abnormalities (Fig. 1).

**Alteration in CGRP signals during DM**

CGRP has a regulatory action on cardiovascular function\textsuperscript{53}. It is reported to have potent vasodilatory action on different vessels of humans (internal mammary artery) and animals (rabbit coronary arteries)\textsuperscript{54}. CGRP has been shown to be involved in exercise-induced cardioprotection\textsuperscript{55}. A recent study has indicated that long-term exercise preconditioning increases CGRP synthesis in the dorsal root ganglion and promotes CGRP release in the blood and heart\textsuperscript{56}. CGRP could play an important role in the cardioprotective effect of long-term exercise preconditioning\textsuperscript{56}. It is in fact an endogenous mediator of preconditioning\textsuperscript{57,58}. It is suggested that protective effect of ischemic post-conditioning is also related to stimulation of endogenous CGRP release in the rat heart\textsuperscript{58}. CGRP is a major transmitter of capsaicin-sensitive sensory nerves and plays a key role in mediating preconditioning effect by inhibiting the production of cardiac tumor necrosis factor alpha (TNFα)\textsuperscript{59}. 
Interestingly, cardioprotective effect of CGRP-mediated ischemic pre-conditioning is shown to be related to inhibition of cardiac TNF-α production, but not to activation of the K(ATP) channel. The cardioprotection via pre-conditioning-induced CGRP release can be mimicked by exogenous CGRP and both can be blocked by a CGRP antagonist, indicating strongly an important regulatory role for CGRP in ischemic preconditioning. Pretreatment with exogenous CGRP exerts a cardioprotective action against myocardial ischemia-reperfusion injury. Heat stress possesses cardioprotection, which is suggested to be related to the synthesis and release of CGRP via activation of capsaicin receptor (vanilloid receptor subtype 1) on the capsaicin-sensitive sensory neurons.

In addition to the cardioprotective role of CGRP, the endothelial cell-derived CGRP is shown to contribute to heat stress-induced vascular protection of endothelial function. These studies suggest cardiovascular defensive regulatory roles of CGRP. However, it should be noted that expression of transient receptor potential vanilloid 1 and CGRP is decreased in diabetic heart. Interestingly, CGRP gene transfer is shown to protect against ischemia-reperfusion-induced injury in diabetic hearts. Adenovirus-mediated up-regulation of CGRP gene expression could protect the diabetic heart against ischemia-reperfusion injury. Interestingly, serum CGRP level is observed to be significantly lower in diabetic patients with coronary artery disease as compared to control group patients, suggesting strongly that CGRP could have a role in the pathogenesis of coronary artery disease in patients with DM. Taken together, DM-associated suppression of CGRP level could play a key role in DM-induced cardiovascular disease.

**Alteration in PKCβ2 and ROCK signals during DM**

Protein kinase C (PKC)β2 is overexpressed in the diabetic myocardium, which induces cardiomyocyte hypertrophy and involves in diabetic cardiomyopathy. Hyperglycemia-associated PKCβ2 activation induces diastolic cardiac dysfunction in diabetic rats by impairing caveolin-3 expression and Akt-eNOS signalling. Pharmacological inhibition of PKCβ2 using LY333531 prevents PKCβ2 activation and subsequently attenuates cardiac diastolic dysfunction in diabetic rats by restoring caveolin-3 expression and rescuing Akt-eNOS-NO signaling. An increased expression of the small GTP-binding protein RhoA and activation of RhoA/rho kinase (ROCK) pathway is shown to be associated with diabetic cardiomyopathy in diabetic rats. Importantly, ROCK pathway contributes to diabetic cardiomyopathy by promoting the sustained activation of PKCβ2. Expression of ROCK2 and iNOS inhibition improved cardiac function by preventing the upregulation of RhoA and its availability for activation. Activation of ROCK is also implicated in acute vasospasm and chronic vasoconstriction in major organ systems. In fact, ROCK-mediated vasoconstriction contributes to coronary vasomotor tone in early diabetic rats, while acute ROCK inhibition using fasudil improves coronary vasomotor tone in early diabetic microcirculation. These studies collectively suggest that DM-associated overexpression of iNOS, ROCK and PKCβ2 might contribute to contractile dysfunction and cardiomyopathy.

**Alteration in CaMKII signals during DM**

CaMKII has multiple downstream targets that might promote vascular disease, heart failure and arrhythmias. The CaMKII has emerged as an important transducer of vasoactive peptide-associated responses in VSMC. For instance, endothelin-1 is a potent vasoactive peptide that at high level has a pathogenic role in vascular diseases, whereas endothelin-1-induced growth promoting responses are reported to be mediated by CaMKII in VSMC. Further, Ca²⁺ signalling through CaMKII promotes vascular remodelling. In addition, CaMKII has emerged as a key signalling protein having a pivotal role in cardiac physiology and pathology. CaMKII overactivation can directly induce
pathological changes in ion channels, Ca$^{2+}$ handling and gene transcription$^{26}$.

Erickson et al.$^{26}$ have identified in human and rodents a novel mechanism linking hyperglycemic signalling in DM with CaMKII. Acute hyperglycemia causes a covalent modification of CaMKII by O-GlcNAc and O-GlcNAc-modified CaMKII is noted to be increased in the heart of diabetic humans and rats. In cardiomyocytes, high glucose concentration is shown to significantly enhance CaMKII-dependent activation of spontaneous sarcoplasmic reticulum Ca$^{2+}$ release events that could contribute to cardiac mechanical dysfunction and arrhythmias$^{26}$. Intriguingly, these effects are prevented either by pharmacological inhibition of O-GlcNAc signalling or genetic ablation of CaMKII$\delta$. It is also noted in diabetic animals that acute blockade of O-GlcNAc inhibits arrhythmogenesis$^{26}$. Therefore, O-GlcNAc modification of CaMKII might be a novel signalling event that could contribute to DM-associated cardiac disorders (Fig. 1).

Other signalling alterations in DM

DM is known to adversely affect the number and function of circulating endothelial progenitor cells (EPCs), which is an initiating factor in the development of diabetic vascular disorders$^{24}$. Dysfunction of EPCs might contribute to diabetic vascular complications. The miRs have been recognized as key regulators of diverse cellular processes, including angiogenesis. Importantly, miR-126, miR-130a, miR-21, miR-27a and miR-27b are downregulated in EPCs of type 2 DM patients, whereas downregulation of miR-126 in EPCs impairs their functional properties via target gene Spred-1$^{24,25}$.

In addition, a recent study has demonstrated that miR-130a plays an important role in maintaining normal function of EPCs, while DM-associated decreased miR-130a in EPCs could contribute to impaired EPCs function likely via its target, runt-related transcription factor 3 (Runx3)$^{25}$. Moreover, miR-130a is important for maintaining normal autophagy levels and promoting the survival of EPCs via regulation of Bcl-2 and Beclin1 expression via Runx3$^{23}$. The downregulated miR-130a in patients with type 2 DM results in dysfunction of EPCs, including increased apoptosis$^{23}$. Taken together, DM-associated downregulation of miR-126 and miR-130a might cause EPCs dysfunction, which might lead to diabetic vascular complications (Fig. 1).

Adenosine acts as a key triggering molecule in ischemic preconditioning-mediated cardioprotection$^{17,23,74,75}$. In response to the ischemic pre-conditioning stimulus, numerous triggers, including adenosine are released in the myocardium, leading to the activation of various mediators, including PI3K$^{44,76,77}$. However, DM has been noted to be associated with enhanced unidirectional uptake of interstitial adenosine and reduced ability to release adenosine by cardiac cells during ATP deprivation$^{22}$, suggesting that the reduced myocardial extracellular availability due to increased adenosine uptake might play a role in the suppressed cardio-protective action of ischemic preconditioning in diabetic subjects.

Our study group has recently shown that adenosine preconditioning is unsuccessful to afford cardioprotection in the diabetic rat heart subjected to ischemia and reperfusion$^{23}$. The reduced myocardial availability of extracellular adenosine might possibly explain the inability of adenosine preconditioning to protect the diabetic rat heart against ischemia-reperfusion-induced myocardial injury. However, pharmacological elevation of extracellular adenosine using dipyridamole restores the adenosine preconditioning-mediated cardioprotection in the diabetic myocardium$^{23}$. Thus, DM-associated reduced extracellular availability of adenosine might contribute to cardiovascular disease pathology in presence of DM (Fig. 1).

Expression of transforming growth factor-β (TGF-β) is increased in blood vessels of many vascular beds in DM that is a causative factor for development of fibrosis in the myocardium and other tissues$^{78}$. Platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) receptor signaling promotes cell survival. However, in diabetes, activation of Src homology-2 domain-containing phosphatase-1 (SHP-1) can dephosphorylate these receptors and contribute to apoptosis$^{78}$. It is shown that aortic VSMCs from diabetic rats exposed to high glucose exhibit increased levels of Gqα and PLCβ proteins$^{79}$. It has been suggested that hyperglycemia-induced enhanced expression of Gq/11α and PLCβ proteins and signaling might be attributed to the enhanced oxidative stress$^{79}$. In addition, oxidative stress through the transactivation of growth factor receptor might contribute to high glucose-induced enhanced expression of Gq/11α/PLC and -associated cell signaling through MAPK/PI3K pathway$^{80}$.
Concluding remarks

A marked alteration in cardiovascular survival signalling pathways like PI3K-Akt-eNOS-NO could cause cardiovascular dysfunction in chronic DM. Likewise, disparities in NADPH oxidase, EDHF, CGRP and adenosine signals could play key roles in chronic DM-associated cardiovascular abnormalities. In addition, DM-associated upregulation of iNOS, ROCK and PKCβ2 could contribute to contractile dysfunction and cardiomyopathy in DM. Moreover, O-GlcNAc modification of cardiac CaMKII might contribute to DM-associated cardiac disorders. Furthermore, DM-associated decreased miR-126 and miR-130a in EPCs could contribute to impaired EPCs function and subsequently the diabetic vascular disease. Pharmacological agents modulating and improving these signalling alterations might prevent the pathogenesis of cardiovascular disease associated with chronic DM.

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References
