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Endothelial Nitric Oxide Synthase (eNOS) as a Therapeutic Target in Type 2 Diabetes Mellitus and Its Vascular Complications: A Narrative Review

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ABSTRACT

Diabetes mellitus type 2 (T2DM) has been a global health problem. Current studies have shown that the increased mortality and morbidity in T2DM are related to vascular complications. The vascular complications were caused by increased reactive oxygen species (ROS) associated with chronic hyperglycemia and insulin resistance. The increase of ROS in T2DM was influenced by the p38 MAPK pathway which is directly related to the modulation of nitric oxide (NO) produced by endothelial nitric oxide synthase (eNOS) of endothelium cells. The decrease of NO by eNOS also has a connection with an event known as eNOS uncoupling. The decrease of eNOS plays a role in the pathogenesis of T2DM and its vascular complications such as increased inflammatory pro-cytokine, activation of NADPH pathway, increased of AGEs, VCAM-1, ICAM-1, and also the activation of protein kinase c and Rho-kinase pathway. Some interventions indirectly or directly have modulated NO relayed to its work targets such as oral antidiabetic drugs (metformin, sulfonylurea, and acarbose) or some polyphenol compounds such as emodin, α-Lipoic acid, curcumin, and olive oil. Modulation of NO in these interventions can be strong evidence that NO can be a target for further therapy in the management of T2DM and its complications.

Keywords: eNOS, vascular complication, Type 2 Diabetes mellitus

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia. Hyperglycemia is defined as a condition of increasing blood sugar levels exceeding the normal limit with one of the parameters, namely fasting blood sugar (GDP) levels of more than 126 mg/dl.[1] DM is a health problem that is a threat worldwide. Based on data from the World Health Organization (WHO) in 2014, the number of DM sufferers worldwide is around 4.7%. This figure is estimated to double to 8.5% in 2016.[2] Data from the International Diabetes Federation (IDF) in 2019 showed 9.3% of the world's population was diagnosed with DM and this figure is also estimated to increase by around 51% by 2045.[3]

Diabetes mellitus consists of type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). T1DM is DM caused by an autoimmune reaction that attacks pancreatic beta cells so that insulin production is disrupted, while T2DM is DM which is identical to insulin resistance. Of the two types of DM, T2DM is the most common DM.[2,4] As a chronic disease, T2DM can cause various complications that affect the mortality and morbidity of the sufferer.[5] Complications in T2DM can be vascular complications and nonvascular complications. Nonvascular complications in T2DM include gastroparesis, infection,

skin changes, and loss of hearing loss, while vascular complications are further subdivided into microvascular and macrovascular.[6] Microvascular complications include diabetic retinopathy, diabetic nephropathy, and diabetic neuropathy. Macrovascular complications of diabetes mellitus include coronary artery disease (CAD), peripheral arterial disease (PAD), and cerebrovascular disease.[6] Research shows that 70% of DM deaths or mortality are related to vascular complications.[7] Data from the IDF also shows that 21% of CAD and 32% of other heart diseases are found in people with DM in countries with upper-middle income.[3]

The association of vascular complications in T2DM cannot be separated from the p38 mitogen-activated protein kinase (p38MAPK) pathway which triggers an increase in reactive oxygen species (ROS) levels. In addition, this pathway also increases the activity of inflammatory pro-cytokines such as tumor necrosis alpha (TNF-α), IL-1beta, and IL-6.[8-12] This pathway also modulates the NADPH pathway so that oxidative stress increases. The activation of these pathways is supported by the hyperglycemia and insulin resistance that occurs in T2DM. Consequently, these conditions affect the bioavailability of NO.[13] Reduced NO levels were accompanied by an increase in various molecules such as advanced glycosylated end products (AGEs), vascular adhesion molecule 1 (VCAM-1), intracellular adhesion molecule 1 (ICAM-1), cyclooxygenase-2 (COX-2), and reduced insulin sensitivity.[14-16] Decreased NO levels in T2DM are also associated with a mechanism known as eNOS uncoupling. eNOS uncoupling is associated with various cardiovascular diseases in DM in general. eNOS uncoupling can increase oxidative stress by producing superoxide (SO) instead of NO production. An increase in SO and a decrease in NO will activate an increase in ICAM-1 and the activation of several pathways such as protein kinase C and Rho-kinase that can induce vascular complications such as diabetic nephropathy and retinopathy.[17]

The relationship between eNOS in the pathogenesis of T2DM can be used as a therapeutic target in developing advanced treatments or interventions for T2DM and its vascular complications. As a therapeutic target, NO production by eNOS can be modulated by various interventions. Even some of the interventions currently used, such as oral antidiabetic drugs, including sulfonylureas, acarbose, and metformin, work by influencing NO levels in T2DM patients. In addition, various studies prove that NO has an association in the treatment of T2DM and its complications. Further understanding of NO as a therapeutic target could lead to a comprehensive development of the management of T2DM and its complications in the future.[18-19]

METHOD

The literature search was conducted from November, 22nd to December, 15th 2021 using two databases, namely Pubmed and Google Scholar with the keywords used were "diabetes mellitus and nitric oxide", "Diabetes mellitus and cardiovascular", and "Diabetes mellitus and cardiovascular". vascular and complications." The inclusion criteria used were all studies that discussed T2DM which is associated with eNOS and vascular complications. After that, screening was carried out so that the appropriate X was obtained, seven from Google Scholar and 50 from Pubmed. Furthermore, the entire literature obtained was critically examined by considering the aspects of validity, importance, and applicability. The data obtained are then synthesized systematically and logically.

DISCUSSION

Diabetes mellitus and cardiovascular disease

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia due to impaired insulin secretion, insulin function, or both.[1] DM is divided into two types, namely diabetes

mellitus type 1 (T1DM) and diabetes mellitus type 2 (T2DM). Based on the types of DM, T2DM is the most common type of diabetes Mellitus.[2]

Type 2 diabetes mellitus is a type of DM associated with insulin resistance. Insulin resistance is a condition when insulin cannot work properly, even though insulin has been secreted in normal amounts. This results in impaired glucose uptake in peripheral tissues.[20] If this condition is maintained, then T2DM can have an impact on vascular and nonvascular complications. Non-vascular complications can be in the form of gastroparesis, infection, changes in the skin, and loss of hearing loss, while vascular complications are subdivided into macrovascular and microvascular.

Macrovascular complications in T2DM can be in the form of coronary heart disease, blood vessel disease in the brain and peripherals, while microvascular complications are related to diabetic retinopathy, neuropathy, myopathy, and nephropathy.[6] Studies have shown that patients with T2DM have an accelerated and increased incidence of atherosclerotic vascular lesions.[21] In addition, cardiovascular diseases such as myocardial infarction and stroke are two to four times higher in people with T2DM than in people with or without T2DM.[21-22]

Epidemiology of Diabetes Mellitus

Diabetes mellitus is one of the health problems in the world today. Data from the world health organization (WHO) shows the prevalence of DM sufferers in the world population over 18 years was 8.5% in 2014. This data tends to increase when compared to 1980 with 4.7%.[2] In addition to WHO, data from the international diabetes federation (IDF) in 2019 showed as many as 463 million or 9.3% of the world's population were diagnosed with DM and it is estimated that this number will continue to increase by 51% in 2045 to 700 million sufferers.[3] In terms of aging itself, it is known that DM increases as a person ages.[3] This situation is supported by data that in adults aged 20 to 24 years the prevalence of DM was found to be around 1.4% while in adults aged 75-79 years the prevalence of DM was 19.9%.[3]

In addition to the high incidence, DM also affects morbidity and mortality in the world's population. Data from the IDF shows that 11.3% of deaths that occur in the world are related to DM.[3] These data also show that 4.2 million adults aged 20 to 79 years are estimated to die from DM and its complications. These high numbers are associated with comorbid diseases that are often experienced by DM patients such as CAD. Research has shown that there is a 21% association between CAD and adults with DM in upper-middle-income countries.[23] In addition, it was found that 32% of other cardiovascular diseases also had a similar relationship with DM sufferers in upper-middle-income countries.[23] Elevated blood sugar levels or hyperglycemia has also been associated with a 15% responsibility for deaths related to cardiovascular disease and kidney disease.[24] Another study also showed that 32.2% of patients with cardiovascular disease, 29.1% of people with atherosclerosis, 21.2% of coronary heart disease, 14.9% of heart failure, 14.6% of angina, 10% of myocardial infarction, and 7, 6% of strokes in a cross-sectional study of 57 research articles were associated with T2DM. The total number of samples in this cross-sectional study was 4,548,481 T2DM patients.[25]

Nitric oxide synthase (NOS)

Nitric oxide (NO) is an endothelial-derived relaxing factor (EDRF) compound produced by endothelial cells. NO is a potent vasodilator synthesized due to the stimulation of bradykinin. NO has various roles with different molecular targets. NO can act as a regulator of neurotransmission, vascular tone, regulate gene transcription and mRNA translation, and post-translational protein production.[26] Nitric oxide synthase consists of three types of

isoenzymes, namely endothelial nitric oxide synthase (eNOS), inducible nitric oxide synthase (iNOS), and neuronal nitric oxide synthase (nNOS). eNOS enzyme is a type of enzyme that acts as a producer of most of the NO in normal vessels by endothelial cells of arteries, veins, and also platelets that are dependent on calcium (Ca). nNOS is a special form of eNOS that plays a role in the nervous system while iNOS is a type of enzyme that is present and induced by macrophage cells, myocytes, and small blood vessel cells which are regulated by pro-cytokines and endotoxins. NO itself is synthesized by endothelial cells from L-arginine and molecular oxygen with the help of cofactors bihydrobiopterin (BH4) and flavin adenine dinucleotide (FAD).

All NOS isoenzymes use L-arginine as a substrate and molecular oxygen and reduce NADPH as a co-substrate. In addition, the NOS synthesis process requires various cofactors such as flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and tetrahydro-L-biopterin (BH4). In the process, NOS will transfer electrons from the co-substrate NADPH via FAD and FMN in the carboxy-terminal reductase domain to haem in the oxygenase domain. The oxygenase domain then binds to the essential cofactor BH4, molecular oxygen, and the substrate L-arginine.[26-27] In the oxygenase domain, electrons are used to activate molecular oxygen and oxidize L-arginine to L-citrulline and NO. The decrease in the amount of NO or the inactivation of NO will form a superoxide anion (O2-) which causes oxidative damage.[26]

Endothelial nitric oxide (eNOS) in type 2 diabetes mellitus and its association with vascular complications

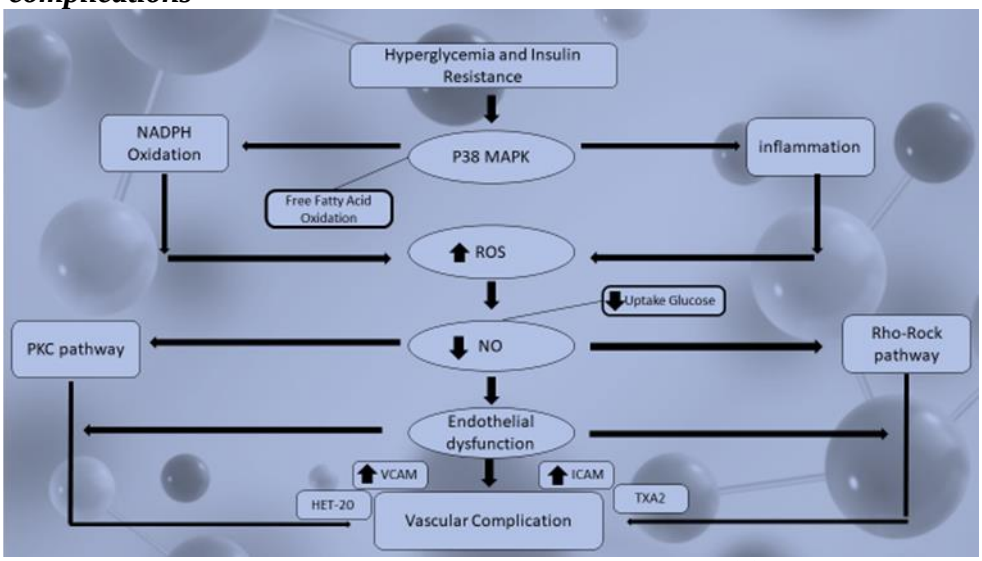

Figure 1: eNOS and its association with DMT2 Pathogenesis[9-12, 14-15]

The association between T2DM and vascular complications cannot be separated from an initial condition known as endothelium dysfunction. Normally, each cell has been designed in such a way to be able to withstand any changes that occur. However, when the adaptive capacity of a cell reaches its limit, homeostasis in the body can be disturbed causing morphological changes in the cell itself. The endothelium is a layer that lines the inner walls of blood vessels and plays a role in vascular homeostasis including vascular integrity, blood flow, cell adhesion, angiogenesis, vascular permeability, immune response, and metabolism.[28] In maintaining vascular homeostasis, the endothelium also releases several vasoactive mediators. The main mediator that plays an important role in NO or eNOS. Under physiological conditions, increased blood glucose will trigger the release of insulin by

pancreatic beta cells. The release of insulin will activate eNOS so that NO production will increase. Increased production of eNOS will cause blood vessels to dilate thereby helping glucose uptake by peripheral tissues.[29] T2DM, which is characterized by insulin resistance and hyperglycemia, affects NO production by the endothelium.[30] This state activates a pathway known as P38MAPK. The activation of this pathway has an impact on the state of hyperinsulinemia and atherogenesis.[28,31] This situation will trigger an increase in free fatty acids and pro-inflammatory cytokines that trigger an increase in reactive oxygen species (ROS).[32] The inflammatory pro-cytokines involved consist of IL-1beta and IL-6. A case-control study conducted by European Prospective Investigation showed that there was an increase in the levels of IL-1beta and IL-6 in patients with T2DM.[8] The increase of these two inflammatory pro-cytokines is associated with inflammation in the pathogenesis of T2DM. In addition to IL-1beta and IL-6, tumor necrosis factor-α (TNF-α) also plays a role in regulating immune cells and inflammatory pro-cytokines. An increase in TNF- was found in hyperglycemic states followed by an increase in IL-6.[11] Research has shown that increased ROS in DM is associated with activation of the NADPH-dependent pathway that increases the amount of oxidative stress in the body.[33] The reduction in NO also induces the activity of endothelium-derived hyperpolarizing factors such as cyclooxygenase (COX-2). In addition, this state also activates TXA2 and an increase in 20-HETE levels.[14-15] An increase in 20-HETE will inhibit the PIK/Akt pathway, including binding between insulin and insulin receptor substrate 1 (IRS-1). The reduced NO production by eNOS also increases advanced glycation end-products (AGEs) and NFkB and other adhesion molecules such as VCAM-1 AND ICAM-1.[34] This increase in adhesion molecules affects the adhesion of leukocytes to the endothelium surface which will have an impact on vascular complications. Activation of the p38 MAPK pathway will also activate other pathways such as the protein kinase C pathway and the Rho-associated protein kinase (ROCK) pathway.[35-36] The mechanism or description of the p38 MAPK pathway can be seen in Figure 1.

eNOS Uncoupling in Diabetes mellitus

The association of vascular complications in DM is also associated with a mechanism known as eNOS uncoupling. This event is associated with a decrease in eNOS in both T1DM and T2DM DM. Normally; eNOS is activated together with several cofactors and substrates such as tetrahydrobiopterin (BH4), L-arginine, and eNOS S-glutathionylation as well as molecular oxygen. These cofactors and substrates play an important role in producing NO. Disruption of these molecules will cause eNOS uncoupling which effects on increases ROS and endothelium dysfunction.[26,37-38] Uncoupling of eNOS has been found in various DM research models. It is associated with deficiencies of eNOS cofactors such as BH4 deficiency, L-arginine deficiency, and eNOS S-glutathionylation deficiency.[39-40]

BH4 deficiency in T2DM occurs due to two mechanisms. First, BH4 deficiency occurs due to increased blood sugar so that the MAPK or p38 MAPK pathway is activated which results in the activation of protein kinase C (PKC). In addition, this mechanism is accompanied by an increase in ROS due to the high activation of NADPH oxidase.[40-41] Second, this situation is related to the reduced biosynthesis of BH4. Research shows that the increased production of ROS in DM causes the activation of prosthetic degradation of the GCH1 enzyme. This enzyme plays a role in the synthesis of BH4.[42] The increase in ROS was followed by an increase in several compounds that inhibit the eNOS cofactor in producing NO such as superoxide (SO) and peroxynitrite which can oxidize BH4 to BH2.[43] The oxidation of BH4 to BH2 causes BH2 to bind to eNOS so that NO production

is disrupted. Nitric oxide (NO) could not be produced but NO would be replaced by SO production which has an impact on increasing oxidative stress. Research on fructose-induced DM rats showed that BH4 levels in the rat aorta decreased. On the other hand, there was an increase in BH2 levels in these mice.[44] The relationship between BH4 and NO was further strengthened by studies showing that endothelial dysfunction in DM can be reduced by ex vivo incubation of BH4.[45] Apart from BH4 deficiency, reduced NO production may also be due to L-arginine substrate deficiency associated with arginase induction. Arginase induction in DM occurs due to hyperglycemia, which then activates arginase I through the activation of the RhoA-Rock pathway, which affects leukocyte adhesion through activation of the ICAM-1 molecule, increased endothelial permeability, and inactivation of eNOS which causes microvascular damage so that diabetic retinopathy can end.[46] This situation is supported by studies in streptozotocin-induced mice which showed that there was excessive activation of arginase I in the coronary arteries and activation of arginine II in the mesenteric arteries. The presence of arginase activation in T1DM and T2DM suggests that reduced NO production reduces vascular vasodilation and contributes to endothelium dysfunction.[47]

eNOS as a therapeutic target in diabetes mellitus

eNOS can be used as glycemic control in patients with T2DM. Glycemic control in T2DM is important as therapy for the underlying disease and its complications, eNOS can be modulated by oral antidiabetic drugs such as sulfonylurea and acarbose. Aydin et al study showed that NO levels in T2DM patients, when given oral antidiabetic drugs such as sulfonylurea, were significantly higher than the control group. This shows that NO modulation also has a certain level of contention which is still a matter of controversy.[18] Other studies with metformin have shown that metformin modulates or affects NO production in T2DM patients. Metformin can increase endothelial precursor cells (EPCs) which is correlated with an increase in NO and a decrease in superoxide (SO). An in vivo study in DM rats by Han et al who was given metformin showed that there was an increase in intracellular NO levels in the bone marrow EPCs of T2DM rats compared to the control group, namely non-diabetic rats ($P<0.001$). In addition, this study also showed that the metformin group significantly ($P<0.05$) decreased intracellular superoxide levels in bone marrow EPCs of T2DM mice.[19] Olive oil intervention also affected modulating NO in eNOS. Giving olive oil to ECV304 cells that have been modified into endothelial cells with low blood sugar and intracellular NO levels showed an increase in NO levels after being given olive oil polyphenol extract. The increase in NO level in this intervention was associated with an increase in calcium ions induced by acetylcholine. The increase in eNOS is thought to be related to the activation of the PI3/Akt pathway as a substitute for MAPK. The activation of this pathway is associated with a decrease in the levels of ROS present followed by glucose uptake in peripheral tissues.[49]

eNOS Targets on Macrovascular Complications of Diabetes Mellitus

Coronary artery disease (CAD) is one of the macrovascular complications of diabetes mellitus. Coronary artery disease itself is one of the most common causes of death in the world that can arise due to a combination of genetic and environmental factors. Most patients with CAD themselves experience endothelial dysfunction at an early stage where the main feature of endothelial dysfunction is an impaired amount of nitric oxide (NO). Nitric oxide acts as a vasodilator of blood vessels, the availability of NO itself is influenced by the presence of endothelial nitric oxide (eNOS). In a case-control study in a population in Tunisia, it was found that the 894G>T

mutation was associated as a risk factor for CAD.[50] On the other hand. Other studies have shown that there is an association between CAD and eNOS 4b/a polymorphism in African populations.[51] Other studies have also shown that the presence of -786T>C polymorphism in the promoter region of eNOS increases the risk of developing CAD.[52]

In the South Korean population, eNOS gene polymorphisms, especially type -786T>C, 4b and 894G>T, are known to be risk factors for the incidence of ischemic stroke in patients with type 2 diabetes mellitus in South Korea.[53] Studies in diabetic rats have shown that there is a decrease in the amount of phosphorylation of eNOS which is also associated with impaired vascular reactions, hypertension, and greater stroke size in Middle Cerebral Artery occlusion.[54] Further studies have shown that the eNOS 894G>T mutation plays a role in increasing the risk of ischemic stroke.[55]

Peripheral arterial disease (PAD) is a manifestation of the presence of systemic atherosclerosis that occurs at a different location from coronary arterial disease (CAD). PAD is associated with an increased risk of cardiovascular and cerebrovascular disorders. Studies show that in smokers the eNOS-786T>C mutation increases, thereby increasing the incidence of PAD.[56] Follow-up studies also showed that the rs 1799983 polymorphism in the eNOS gene was significantly associated with the presence of PAD.[57]

eNOS Targets on Microvascular Complications of Diabetes Mellitus

Diabetic nephropathy (DN) is a major cause of End-Stage Renal Disease (ESRD) and is also one of the main complaints of patients with type 2 diabetes mellitus. in the eNOS gene.[58] A similar study was also conducted in the Czech Republic where the results obtained are consistent with the results obtained by previous studies.[59] A study in a population in Tunisia showed that patients with diabetic retinopathy (DR) had polymorphism 4b/4a and -786T/C significantly.[60] Other studies have shown that polymorphisms in eNOS G894T increase the likelihood of DR by increasing the pressure on the retina.[61] Decreased AMPK-eNOS bioavailability triggers the development of diabetic peripheral neuropathy (DPN) by increasing the rate of apoptosis and decreasing the rate of autophagy due to oxidative stress.[62] In addition, in another study, patients with the genotype 'aa' in eNOS type 27VNTR (a/b) had a high risk of developing DPN.[63]

CONCLUSION

Seeing the vital role of eNOS in the process of vascular endothelial dysfunction, eNOS is a promising potential as a therapeutic target in the treatment of vascular complications of diabetes mellitus. Several studies have shown that increased eNOS can affect oxidative stress, glycemic control, and vascular complications.

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