Vascular failure and recent anti-diabetic drugs

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Abstract:

Type 2 diabetes mellitus (T2DM) is highly prevalent and is a critical risk factor for cardiovascular (CV) disease, increasing both morbidity and mortality. T2DM is one of the most important classical CV risk factors that promote atherosclerosis. Therefore, it is important for both patients with T2DM and their doctors to identify vascular dysfunction at an early stage of atherosclerosis. Recently, new therapies based on the actions of the incretin hormones and blockade of sodium glucose transporter (SGLT) 2 have become widely used, because they offer advantages over conventional treatments by achieving glycemic control and/or possible reducing CV risks. Many experimental studies have suggested that glucagon-like peptide (GLP)-1 and dipeptidyl peptidase (DPP)-4 inhibitors exert cardioprotective effects on atherosclerosis and cardiac dysfunction both *in vitro* and *in vivo*. However, thus far, there is little clinical evidence supporting the efficacy of incretin therapy in patients with CV disease. In contrast, the SGLT2 inhibitor empagliflozin achieved a remarkable reduction in CV-related mortality in a large clinical study. The present review focuses on the effects of GLP-1-related therapies and SGLT2 inhibitors on clinical indices of endothelial function.

Key words:

Vascular failure, Endothelial function, GLP-1, DPP-4 inhibitor, SGLT2 inhibitor

Introduction

Type 2 diabetes mellitus (T2DM) is one of the most important risk factors for the development of cardiovascular (CV) disease, because it promotes both systemic atherosclerosis and lifestyle-associated diseases. Cardiovascular complications are an important cause of morbidity and mortality in patients with diabetes, and endothelial dysfunction is an early indicator of developing cardiovascular complications.

Recently, several new classes of antidiabetic drugs, including glucagon-like peptide (GLP)-1, dipeptidyl peptidase (DPP)-4 inhibitors, and sodium glucose cotransporter 2 (SGLT2) inhibitors, which have different mechanisms of action from those of conventional antidiabetic agents, have become widely used. In this article, we review the effects of these agents, with a focus on the significance of endothelial function.

1. Endothelial function

1.1 Regulation of the vascular system

Blood vessels are composed of three layers of tissue and modulate vascular tone and blood flow by constricting or relaxing in response to physical, neurological, and chemical stimuli. The endothelium is a flat monolayer of cells that covers vascular lumina and plays a role as a barrier between blood and the vessel wall, preventing the aggregation of platelets and invasion of macrophages.

Vasodilator molecules are released in response to shear stress. Vascular endothelial cells can produce and secrete several vasodilators, including nitric oxide (NO), prostaglandin I₂, C-type natriuretic peptide, and endothelium-derived hyperpolarizing factor (EDHF). The cells also produce several vasoconstrictors, including endothelin, angiotensin II, prostaglandin H₂, and thromboxane A₂. NO in particular plays a significant role in atherosclerosis¹⁾. Normally, endothelial NO synthase (eNOS) is activated by shear stress caused by blood flow and various agonists that stimu-

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Table 1. Endothelial function

Position	Method	Stimulus	Advantage	Disadvantage
Brachial artery	SPG	ACh	High specificity	Invasive
Brachial artery	FMD	Reactive hyperemia	Non-invasive Simple	Low specificity
Finger	RH-PAT	Reactive hyperemia	Non-invasive Simple	Low specificity
Coronary artery	Flow-wire	ACh	High specificity	Invasive Complicated
Blood/Urine	Bioactive substance	None	Simple	Very low specificity Supportive tool

SPG, strain-gauge plethysmography; FMD, flow-mediated dilatation; RH-PAT, reactive hyperemia peripheral arterial tonometry; ACh, acetylcholine

late mechanically or bind with receptors on vascular endothelial cells. NO can be produced from L-arginine, and when released, it is transferred to vascular smooth muscle cells in the immediate vicinity by diffusion. It then activates soluble guanylate cyclase in the cell, relaxes vascular smooth muscle, and inhibits chemotaxis and platelet aggregation by increasing the amount of cyclic guanosine 3',5'-monophosphate (cGMP)¹⁻³.

Thus, normal vascular endothelium functions to regulate and maintain vascular homeostasis through multiple and complex physiological functions, including the release of vasoactive factors that regulate vascular tone, blood fluidity, and coagulation, while limiting vascular smooth muscle cell proliferation and inflammation³⁻⁵.

1.2 The concept of vascular failure

Clinically, abnormalities of vascular endothelium are critical in relation to atherosclerosis, hypertension, and diabetes. Oxidative stress, inflammation, obesity, hyperlipidemia, and insulin resistance are major contributors to endothelial dysfunction in cardiovascular disorders. Endothelial dysfunction is strongly associated with aging, atherosclerosis, diabetes, hyperlipidemia, hypertension, and obesity⁶.

Imbalances in the production of endothelium-derived relaxing and contracting factors are important contributors to endothelial dysfunction¹. Decreased synthesis and/or increased degradation of NO can contribute to impairment of its bioavailability as a consequence of enhanced production of reactive oxygen species (ROS)^{7,8}.

Tetrahydrobiopterin (BH4) binds to NOS as a cofactor and suppresses superoxide production⁹. However, insufficient BH4 directs NOS to produce superoxide rather than NO¹⁰, and increased oxidative stress can oxidize BH4, resulting in the uncoupling of eNOS and reduced NO production^{11,12}. Moreover, the absence of BH4 increases oxidative stress through transfer of electrons to molecular oxygen, forming oxidant species that further consume NO and increase oxidative stress^{7,11,12}. Given that superoxide converts NO into peroxynitrite, generates hydroxyl radicals, and injures cells, the balance of production and the existence of NO, superoxide, and related factors in vascular endothelial cells play important roles in vascular endothelial injury.

Elevated levels of asymmetric dimethylarginine (ADMA) may reduce NO production, because ADMA is an endogenous inhibitor of eNOS through competition with L-arginine⁷. Endothelial dysfunction can affect vasoconstrictor levels, including endothelin-1 and angiotensin II, and other vasodilators, including endothelial-derived hyperpolarizing factor (EDHF) and prostacyclin^{7,8}. Therefore, increased ROS, inflammatory, and vasomotor factors and deficiency of NO bioavailability are hallmarks of endothelial dysfunction¹³. Dysfunctional endothelial cells may permit increases in leukocyte adhesion, permeability, inflammatory cell infiltration, and lipid deposition, thereby aggravating atherosclerotic plaque formation^{14,15}. In addition, the proliferation, migration, and apoptosis of endothelial cells are closely related to endothelial dysfunction in cardiovascular disorders^{16,17}. Because atherosclerosis is characterized as a response to chronic multifactorial injury and inflammation, which leads to the formation of atheromatous or fibrous plaques, vascular endothelial dysfunction occurs in the early stages of the disease and is closely related to it mechanistically.

In addition to endothelial dysfunction, smooth muscle dysfunction, metabolic abnormalities of the vessel wall including inflammation, oxidative stress, and neurohormonal imbalance occur at various stages of atherosclerosis. The concept of "vascular failure" is defined as the integration of these vascular abnormalities¹⁸. Vascular failure is not an anatomical disease but a syndrome of abnormal vascular function beyond endothelial dysfunction, because the vasculature is not merely a system of conduits, but an endocrine organ that regulates the total blood supply to end-organs by contracting and relaxing. Vascular failure encompasses several divergent concepts from risk factors to established atherosclerotic disease with arterial stenosis, and further to calcification of the vessel wall or serious vascular events including plaque rupture and thrombo-embolic occlusion. Therefore, active intervention to correct risk factors is important to treat the condition. Comprehension and therapeutic strategies against vascular failure may be expected to prevent and treat vascular lesions.

1.3 Measurement of endothelial function and its clinical utility

Table 1 lists the methods available for evaluating endothelial function. Indirect measures in the peripheral circulation assess the vasodilatory responses of conduit and resistance arteries to stimuli that increase NO release^{2,7)}. Endothelial function can be assessed by invasive and non-invasive methods. Invasive angiographic quantification of the changes in vascular diameter in response to intra-coronary infusion of muscarinic receptor agonists such as acetylcholine (ACh) is used to evaluate endothelium-dependent vasodilation¹⁹⁾. In contrast, endothelium-independent relaxation is measured using an NO donor such as sodium nitroprusside or nitroglycerin, because NO directly acts on vascular smooth muscle cells. Invasive venous occlusion strain-gauge plethysmography (SPG) can be used to evaluate blood flow changes in the forearm circulation following hyperemia or after intra-arterial infusion of ACh^{20,21)}. However, invasive methods are technically difficult and cannot be used routinely.

Thereafter, flow-mediated dilatation (FMD) is measured in the brachial artery, using high-resolution ultrasonography as a non-invasive measure of endothelium-dependent function^{22,23)}. FMD quantifies the transient changes that occur in the diameter of the brachial artery in response to shear stress generated by hyperemia following an induced period of local ischemia. FMD is calculated as the percentage change in brachial artery diameter from baseline in response to the increase in blood flow. Non-invasive ultrasound FMD of the brachial artery is now widely used, although results vary owing to technical issues, and the procedure is not easy to standardize among institutions.

For the assessment of pre-clinical disease, procedures for measuring endothelial function that are simple, non-invasive, reliable, reproducible, and inexpensive are desirable. Recently, reactive hyperemia peripheral arterial tonometry (RH-PAT) has become commercially available to evaluate endothelial dysfunction^{24,25)}. It is an automated, quantitative, non-invasive, clinical procedure for the digital measurement of hyperemic responsiveness to assess peripheral vasodilator function. RH-PAT correlates positively with FMD and inversely with various cardiovascular risk factors and has been shown to have good reproducibility^{26,27)}.

1.4 Predictive value of endothelial dysfunction

Endothelial dysfunction not only represents the present status of vascular function but also acts as an independent predictor of future cardiovascular events^{28,29)}. Endothelial dysfunction is thought to be reversible and useful as a screening test for pre-atherosclerosis changes in the vessel wall. Risk of future cardiovascular disease can be assessed by screening for endothelial dysfunction, enabling early intervention to prevent the development of complications. As therapeutic interventions, drug and supplemental therapy, and modifications of lifestyle habits are available, treatment of vascular endothelial dysfunction is of potential value in the primary

prevention of atherosclerosis. Therefore, it is important to undertake intensive screening for endothelial dysfunction in the early stages of arteriosclerosis.

2. Diabetes and endothelial dysfunction

2.1 Incretin-related agents on endothelial function

In vitro experiments

Several studies *in vitro* have found that GLP-1 attenuated tumor necrosis factor- α (TNF- α)-mediated induction of plasminogen activator inhibitor-1 expression, advanced glycation end-product (AGE)-induced upregulation of vascular cell adhesion molecule (VCAM)-1, and ROS-induced protein kinase A activation-mediated cell senescence all upregulate the expression of eNOS in human umbilical vein endothelial cells (HUVEC)³⁰⁻³³⁾. In cardiac microvascular endothelial cells, glycototoxicity-induced ROS production and apoptosis were decreased by GLP-1 with an increase in cAMP/PKA activity³⁴⁾. On the other hand, GLP-1 promoted angiogenesis in a dose-dependent manner and restored oxidized LDL-induced loss of cell viability accompanied by a decrease in intracellular NO activity^{35,36)}. GLP-1 also improved the proliferation and differentiation of endothelial progenitor cells by increasing vascular endothelial growth factor (VEGF) generation³⁷⁾. The proliferation of human coronary artery endothelial cells was promoted by exendin-4, a GLP-1R agonist, through eNOS-, PKA- and PI3K/Akt-dependent pathways via the GLP-1 receptor³⁸⁾. Exendin-4 also restored eNOS-induced ROS production due to lipotoxicity and protected against lipoapoptosis³⁹⁾. Liraglutide, a GLP-1 analogue, prevented the onset of glycototoxicity-induced endoplasmic reticulum stress in HUVEC, inhibited TNF- α -induced intracellular adhesion molecule (ICAM)-1 and VCAM-1 expression via GLP-1R, attenuated ROS production, and increased the expression of anti-oxidant enzymes in HUVEC⁴⁰⁻⁴²⁾.

Because DPP-4 inhibitors act by inhibiting the degradation of GLP-1, thereby maintaining the plasma level of active GLP-1, sitagliptin augments the protective effects of GLP-1 on the eNOS mRNA level in AGEs-exposed HUVEC while suppressing the receptor for AGE expression and subsequent ROS generation⁴³⁾. Alogliptin-induced vascular relaxation via NO and EDHF-mediated mechanisms, as well as increased NO production with eNOS phosphorylation in HUVEC, may be mediated by GLP-1R-independent mechanisms⁴⁴⁾.

In vivo or ex vivo experiments

Exendin-4 increased NO production, improved endothelium-dependent vasodilation, and reduced the expression of NF- κ B via the cAMP or AMPK-eNOS pathways in aortas isolated from obese rats⁴⁵⁾. Exendin-4 also inhibited monocyte adhesion and attenuated the formation of atherosclerotic lesions in apolipoprotein E-deficient (ApoE^{-/-}) mice⁴⁶⁾. Liraglutide improved endothelial function via GLP-

Table 2. Characteristics of studies included in this review

Author	Subjects	Methods	Medication (n)	Control (n)	Duration	Result
GLP-1 related drug						
Tesaro M et al. (50)	MetS	SPG	GLP-1 (5)	Saline	30min	Improved (conditional)
Basu A et al. (51)	T2DM	SPG	GLP-1	Placebo	240min	Improved
Nystrom T et al. (52)	T2DM	FMD	GLP-1	Saline	115min	Improved
Koska J et al. (53)	T2DM	RH-PAT	Exendin-4	Saline	210min	Improved
Ceriello A et al. (54)	T2DM	FMD	GLP-1 (12)	Saline (12)	2h	Improved
Irace C et al. (55)	T2DM	FMD	Exendin-4 (10)	Glimepiride (10)	16w	Improved
Ha SJ et al. (56)	Volunteer	FMD	Exenatide (20)	Placebo (20)	30min	Improved
Kelly AS et al. (57)	IGT	PAT	Exendin-4	Metformin	6m	No change
Hopkins ND et al. (58)	Obese T2DM	FMD	Exendin-4 (n=9) Liraglutide (n=2)	None (0)	6m	No change
Nomoto H et al. (59)	T2DM	FMD	Liraglutide (16)	Glargine (15)	14w	No change
DPP-4 inhibitor						
Ayaori M et al. (60)	T2DM	FMD	Sitagliptin (66) Alogliptin (42)	Voglibose (24)	6w	Worsened
Maruhashi T et al. (61)	T2DM	FMD	Sitagliptin (17)	Conventional therapy (18)	2y	No change
Ida S et al. (62)	T2DM	FMD	Trelagliptin (27)	None (0)	12w	No change
Kubota Y et al. (63)	T2DM	FMD	Sitagliptin (40)	None (0)	12w	Improved
Noda Y et al. (64)	Healthy volunteer	FMD	Alogliptin	Placebo	1w	Improved
van Poppel PC et al. (65)	T2DM	SPG	Vildagliptin	Acarbose	4w	Improved
Nakamura K et al. (66)	T2DM	FMD	Sitagliptin (31)	Voglibose (35)	12w	Improved (in both groups) No difference between two groups
Hashikata T et al. (67)	T2DM	RH-PAT	Tenagliptin (31)	None (0)	3m	Improved

MetS, metabolic syndrome; T2DM, type 2 diabetes mellitus; IGT, impaired glucose tolerance; SPG, strain-gauge plethysmography; GLP-1, glucagon-like peptide-1; FMD, flow-mediated dilatation; RH-PAT, reactive hyperemia peripheral arterial tonometry

1R, increased the eNOS level, and reduced ICAM-1 expression in the aortic endothelium in mice⁴¹. Liraglutide also inhibited the progression of atherosclerotic plaques, and improved plaque stability in ApoE^{-/-} mice, although the extent of endothelial vasodilation induced by ACh was unchanged⁴⁷. Sitagliptin protected the endothelial function of the renal artery in spontaneously hypertensive rats, and exenatide ameliorated endothelial dysfunction in renal arteries from hypertensive patients in an *ex vivo* study⁴⁸. However, it has been reported that triglyceride-induced endothelial dysfunction was not restored by exendin-4 from rat conduit arteries in *ex vivo*⁴⁹.

Clinical data

Table 2 lists the effects of GLP-1 and GLP-1-related drugs on endothelial function. GLP-1 treatment enhanced endothelium-dependent and endothelium-independent responses to ACh and sodium nitroprusside (SNP) during infusion of insulin in patients with metabolic syndrome by SPG⁵⁰; several studies have indicated that GLP-1 analogues improved endothelial function⁵¹⁻⁵⁶. However, Kelly et al. demonstrated that exendin-4 did not improve the responses of RH-PAT compared to metformin in patients with impaired glucose tolerance⁵⁷. In addition, exendin-4 and liraglutide did not improve the FMD responses in patients with T2DM^{58,59}. Surprisingly, Ayaori et al. reported that sitagliptin and alogliptin led to a deterioration in the FMD response in patients with T2DM⁶⁰. Two papers reported that

sitagliptin and trelagliptin did not improve FMD or RH-PAT responses in patients with T2DM when given for three months^{61,62}. However, sitagliptin, alogliptin, vildagliptin, and tenagliptin improved endothelial dysfunction in most studies⁶³⁻⁶⁷. We reported that long-term therapy with sitagliptin failed to improve FMD relative to conventional treatment⁶¹. Adding sitagliptin to conventional antihyperglycemic drugs for two years in patients with T2DM did not alter endothelial function measured by FMD. Antihyperglycemic agents including metformin, pioglitazone, and an α -glucosidase inhibitor have been found to improve glycemic control in patients with T2DM^{61,66,68-70}. Based on lowering glucose levels with anti-diabetic drugs, reduction of hyperglycemia may improve endothelial dysfunction clinically. DPP-4 inhibitors may also improve endothelial dysfunction, but possibly not more than conventional agents.

It is generally accepted that correcting blood sugar may improve endothelial dysfunction in T2DM. However, its effectiveness beyond the glycemic control is still debated as all studies included fewer than 50 patients, and some of the trials were non-randomized. Therefore, a carefully designed large randomized trial will be required to clearly define the impact of these agents on endothelial function.

2.2 SGLT2 inhibitors and endothelial function

Preclinical studies

New anti-diabetic agents target sodium-glucose cotransporter 2 (SGLT2), the main glucose transporter in the kid-

ney, which is located in the S1 and S2 segments of the proximal tubule and responsible for reabsorption of >90% of glucose in primary urine. SGLT2 inhibitors lower blood glucose by inhibiting the reabsorption of filtered glucose, thereby increasing urinary glucose excretion, which is independent of insulin secretion and action⁷¹. Generally, SGLT2 inhibitors achieve an emission of 60-100 g/day glucose into urine, equivalent to a caloric loss of 240-400 kcal/day, which can ameliorate systemic glucotoxicity and insulin resistance⁷². In addition to the glycemic pathway, SGLT2 inhibitors are associated with non-glycemic effects, including hemodynamic, metabolic, renal, and neurohormonal effects that decrease blood pressure, weight, visceral adiposity, hyperinsulinemia, arterial stiffness, albuminuria, serum uric acid levels, and oxidative stress⁷³.

SGLT2 inhibitors increase urinary glucose excretion and reduce the serum glucose level through energy loss, and can lead to improved metabolism throughout the body. There are few data on pharmacological cardiovascular effects *in vitro*. Empagliflozin reduced atherosclerosis *in vivo* by decreasing inflammation and insulin resistance in ApoE^{-/-} mice⁷⁴. Canagliflozin attenuated obesity-induced inflammation in mice⁷⁵. Ipragliflozin ameliorated impaired phosphorylation of Akt and eNOS, reduced ROS generation, monocyte chemotactic protein (MCP)-1, VCAM-1 and ICAM-1, and ameliorated endothelial dysfunction in diabetic mice⁷⁶. Oelze et al. reported that empagliflozin improved endothelium-dependent relaxation in diabetic rats⁷⁷. Thus, on the basis of experimental data, SGLT2 inhibitors may ameliorate endothelial dysfunction through the correction of glucose and other metabolic abnormalities.

Clinical study

Currently, the SGLT2 inhibitors including canagliflozin, dapagliflozin, empagliflozin, ipragliflozin, luseogliflozin, and tofogliflozin are available clinically in Japan. Recently, the EMPA-REG OUTCOME trial showed that empagliflozin greatly improved clinical outcomes in diabetic patients with a high risk of CV⁷⁸. Therefore, it may be possible to determine whether other SGLT2 inhibitors can also improve endothelial function. However, there are no pertinent clinical data currently available regarding endothelial function and we are planning to undertake such studies. The results of our studies will be available in 2018.

3. Limitations

Our study has a limitation. It is a comprehensive review and not a systematic review according to PRISMA guidelines.

4. Conclusions

Vascular failure and subsequent cardiovascular disease are often fatal, and early prevention of cardiovascular complications by ensuring strict glucose control is essential in pa-

tients with T2DM. The current findings add to a growing body of evidence that we might be entering a new era of cardiovascular diabetology with new anti-diabetic drugs. On the other hand, several studies contradicted the beneficial effects on the vasculature of incretin-related agents. For the time being, a definite relationship between treatment with gliptin or gliflozins and endothelial function remains uncertain and needs to be proven. Future large-scale clinical studies and their sub-analyses will provide evidence on whether treatment with gliptins or gliflozins provides clinical benefits for vascular protection beyond glycemic control in patients with T2DM at increased risk of cardiovascular disease.

Conflicts of Interest

JO has no conflicts of interest regarding the content of the manuscript. KN has received honorariums from Boehringer Ingelheim, Daiichi Sankyo, Astellas, MSD, Takeda, Mitsubishi Tanabe, and Sanofi; research grants from Sanwa Kagaku Kenkyusho, Astellas, Takeda, Boehringer Ingelheim, Bayer, Teijin, and Mitsubishi Tanabe.

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