



Review Article

SGLT2 Inhibitors Extra Glycemic Potential: A Possible Novel Approach to the Prevention of Atherosclerotic Events

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ABSTRACT

Introduction: Cardiovascular diseases are a major global health concern, with atherosclerosis being a significant contributor. Diabetes mellitus is a well-known risk factor for atherosclerosis, and SGLT2 inhibitors are more effective in reducing cardiovascular disease and mortality compared to other antihyperglycemic agents in patients with type 2 diabetes. Endothelial dysfunction, VSMC phenotypic alterations, inflammation, autophagy, oxidative stress, signaling hormones and dysbiosis are crucial factors and predictors of atherosclerosis and other cardiovascular events. SGLT2 inhibitors have been shown to alleviate these components in atherosclerotic models.

Material and Methods: To proceed we analyzed and took into account all publications, including clinical trials, animal studies, in vitro observations, reviews, and meta-analyses from various scientific databases.

Results: Evidently, SGLT2i attenuate the progression of atherosclerosis by repressing vascular inflammation, oxidative stress, endothelial dysfunction, mitigating vascular smooth muscle cells function, impairing foam cell formation, preventing platelet activation and restoration of normal autophagic function.

Conclusion: Based on these findings, it can be inferred that SGLT2 inhibitors exhibit anti-atherosclerotic characteristics and may have positive implications for the management of atherosclerosis, particularly in individuals with diabetic complications.

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INTRODUCTION

The prevalence of cardiovascular disease mortality across the globe has notably risen due to the ageing of the population, with atherosclerosis posing the most significant burden. This progressive inflammatory disease involves plaque accumulation in the arteries' walls, leading to cardiovascular diseases [1]. Diabetes mellitus is a well-known risk factor for atherosclerosis and its associated complications [2,10]. Sodium-glucose cotransporters 2 (SGLT2) are a class of glucose transporters existing in the proximal renal tubules and small intestinal mucosa [3,4]. SGLT2 inhibitors, including empagliflozin, canagliflozin, and dapagliflozin, are more effective in reducing

cardiovascular disease and mortality compared to other antihyperglycemic agents in patients with type 2 diabetes (DM2) [5-7,105].

A meta-analysis of different trials has shown that SGLT2 inhibitors positively reduce adverse atherosclerotic cardiovascular events in patients with preexisting atherosclerosis [8,22,24]. Sodium-glucose co-transport 2 inhibitors possess anti-atherosclerotic properties, as they alleviate inflammation, insulin resistance, vessel stress and metabolic lipid parameters, thereby hindering the development and progression of atherosclerosis [9,97]. Below we highlight in detail clinical and experimental evidence of SGLT2i atheroprotective mechanisms.

MATERIAL AND METHODS

To proceed we analyzed and took into account all publications, including clinical trials, animal studies, in vitro observations, reviews, and meta-analysis from various scientific databases.

RESULTS

1. Improving Endothelial Dysfunction

Endothelial dysfunction is a crucial factor and predictor of atherosclerosis and other cardiovascular events [10,12,16]. Endothelial activation, which is an essential pathway during atherosclerosis, occurs due to glycocalyx dysfunction and reduced cell contact, leading to weak junctions, promoting OX-LDL and triglyceride-rich lipoprotein absorption, uptake and oxidation in subendothelial cells [13]. Additionally, CD36 and LOX-1 regulate OX-LDLs' effect on endothelial cells, with CD36 being the primary binding receptor. Ox-LDL via CD36 inhibits macrophage migration, potentially causing atherosclerotic lesions. C-reactive protein-CD36 interaction reinforces ox-LDL uptake by macrophages [14]. Excess reactive oxygen species (ROS) damage DNA, lipids, and proteins, leading to structural protein changes and lipid peroxidation, posing significant risks for human aging and cardiovascular disease. In hyperglycaemic individuals, advanced glycosylated endpoints associate outer cellular receptors (RAGE) to activate various receptors e.g. ERK leading to a rise in ROS which depletes NADP⁺ and the glutathione systems [29]. Oxidative stress triggers the release of inflammatory mediators and inflammatory responses impairing cell signaling consequently leading to pathogenic conditions like atherosclerosis [112].

Experimental intervention with SGLT2i impedes oxidative stress and inhibits inflammatory cytokines (CD36), which are closely linked to endothelial dysfunction and atherosclerosis in animal models [11,12,17,18,29,30]. For instance, in the context of ferroptosis, an iron-dependent cell death results in glutathione peroxidase deficiency and reactive oxygen species (ROS) accumulation in cells responsible for the expansion of necrotic core and mtDNA damage in atherosclerosis, empagliflozin/EMPA promoted revascularization in diabetic mouse hindlimb ischemia by inhibiting ferroptosis which improved endothelial cell and cardiac activities [31-34]. In KK-Ay mice administered with empagliflozin (10 mg/kg/day) by oral gavage for 8 weeks, results showed that empagliflozin improved diabetic myocardial function, decreased myocardial oxidative stress, endothelial dysfunction and ameliorated myocardial fibrosis through inhibition of the transforming growth factor

β/Smad pathway and activation of Nrf2/ARE signaling [113].

Furthermore, empagliflozin (0.1–100 nM) prevented the senescence of ECs exposed to high glucose via NOX and cyclooxygenase-mediated ROS generation [35]. Empagliflozin downregulates miR-34a-5p in NAFLD-associated fibrosis and reduces miR-92 and mi-21 levels in HFpEF patients, improving endothelial activities [43,44]. However, further investigation is needed to determine miRNAs' potential as a treatment target in atherosclerosis. In other studies, iverapragliflozin and dapagliflozin were equally reported to alleviate endothelial dysfunction by impeding oxidative stress mechanisms in STZ diabetic mice and human cell lines [29,36,37].

2. Inflammation

Researchers initially believed atherosclerotic arteries were primarily composed of macrophages, but recent studies reveal that the human and mouse aorta contains the majority of known leukocytes [19]. Aging-accumulating pro-inflammatory cytokines like IL-1, IL-6, TNF-α, and NF-κB are strongly associated with atherosclerosis [19]. Data indicates that SGLT2i has significant anti-inflammatory effects in human and animal models. Empagliflozin and Canagliflozin decrease M1 polarized macrophage accumulation and shift macrophage phenotype towards anti-inflammatory M2 via AMPK activation cell types with or without LPS treatment [20,21]. In ApoE knockout mice, Ang II-induced abdominal aortic aneurysm was suppressed by empagliflozin in part by preventing p38 MAPK, NF-κB and NLRP3 activation in the aortas while decreasing macrophage infiltration within the lesion [26-28]. Diabetic mice receiving dapagliflozin showed down-regulation of lectin-like oxidized low-density lipoprotein receptor-1 and ACAT1 genes in peritoneal macrophages, while ATP-binding cassette transporter A1 expression was up-regulated. In the same vein, Leng et al. (2016) observed that dapagliflozin decreased the activation of the NLRP3, IL-1, and IL-18 inflammasomes in diabetic rodents [22].

High mobility group box 1 (HMGB1), a damage-associated molecular pattern released from deceased adipocytes and macrophage necrosis promotes Inflammation-driven atherosclerosis and vascular remodeling [32]. Ipragliflozin reduced its expression in abdominal PVAT of WD-fed mice [25]. In addition, Ipragliflozin suppressed macrophage infiltration in the abdominal PVAT of WD-fed mice. Luseogliflozin exposure also reduced the advancement of atherosclerosis in apoE KO mice, without impacting the serum lipid parameters. This effect was achieved through the modulation of inflammatory pathways mediated by aortic mRNA [27]. β-OHB, a ketone body,



Fig. 1. Effects of SGLT2 Inhibitors on Atherogenic Factors. (BNDF; Brain-derived Neurotropic Factor , MAO; Monoamine Oxidase, RASS; Renin-Angiotensin System , ACHE ; Acetylcholine Esterase , RVLN ; Restoral Ventrolateral Medulla)

is a potent inhibitor of the NLRP3 inflammasome. It effectively reduces the production of IL-1 β and IL-18 in human monocytes by inhibiting the formation of NLRP3 inflammatory vesicles [59]. SGLT2 inhibitors effectively suppress the activation of NLRP3 inflammasome and the release of IL-1 β in human macrophages by elevating blood β -OHB levels and reducing serum insulin, glucose, and uric acid levels in diabetes mice [60]. Other SGLT2 inhibitors have demonstrated comparable anti-inflammatory effects in atherosclerosis animals [12,51].

3. Vascular Smooth Muscle Cells (VSMC)

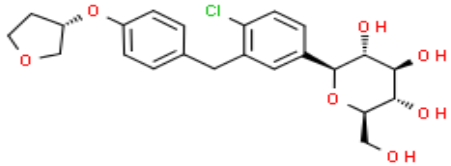
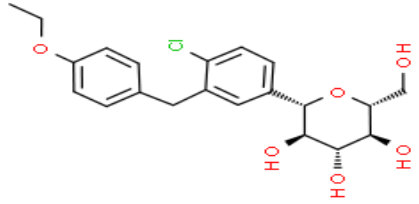
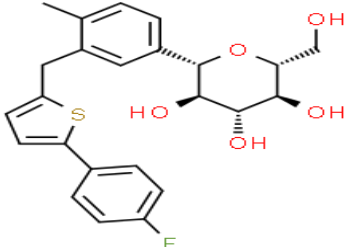
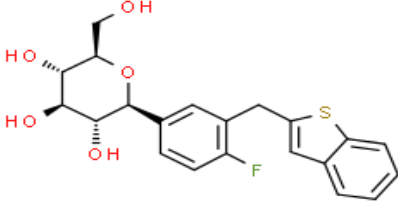
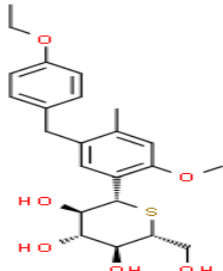
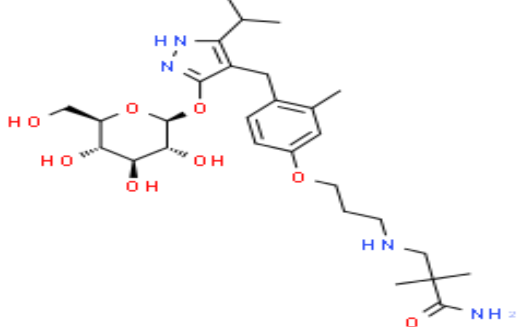
Research on the composition of atherosclerotic plaques in autopsies of humans and animals indicates that vascular smooth muscle cells (VSMCs) play a significant role in plaque formation at all stages by undergoing phenotypic switching and forming fibrous caps [38,39]. Therefore, inhibiting the phenotypic switching of VSMCs could be beneficial for managing advanced atherosclerosis. A study on rat aortic smooth muscle cells (SMCs) revealed that Canagliflozin inhibited the migration and proliferation of vascular SMCs, leading to reduced DNA synthesis and arrest in the G0/G1 phase.

Additionally, it increased HO-1 activity, distinguishing its effects from empagliflozin or dapagliflozin, suggesting that off-target effects of

SGLT2 inhibitors extend beyond glycaemic and inflammation modulation [40]. Similarly, it was observed that the inhibitory effect of canagliflozin on SMC development is likely specific to this compound, as empagliflozin and dapagliflozin did not affect SMC proliferation. However, a recent study by Wei-Jan et al. (2023) demonstrated that empagliflozin also inhibits VSMC proliferation and migration by suppressing PDGF-BB-related signaling in PVAT, which aligns with similar findings by Mori et al. in WD-fed mice treated with Ipragliflozin for 10 weeks [41,45].

Growth factors promote VSMC proliferation in the intima-media during atherosclerosis. Soares et al. observed a significant reduction in cofilin and F-actin expression in VSMCs from coronary and mesentery arteries of old-aged mice treated with empagliflozin for 6 weeks. F-actin and cofilin play a role in the contractile movements of VSMCs [42]. Another study demonstrated that empagliflozin potentially inhibits interleukin-17A-induced proliferation human aortic smooth muscle cells by targeting TRAF3IP2/ROS/NLRP3/Caspase-1-dependent IL-1 β and IL-18 secretion [46]. Blocking TRAF3IP2 in ApoE knockout mice impeded atherosclerosis [47]. However, without excluding different experimental designs, it is speculated that empagliflozin and dapagliflozin may require high dosages to exhibit a moderate decrease in VSMC activity.

Table 1. Sodium Glucose Transporters 2 Inhibitors

	<p>Empagliflozin</p>
	<p>Dapagliflozin</p>
	<p>Canagliflozin</p>
	<p>Ipragliflozin</p>
	<p>Luseogliflozin</p>
	<p>Mizagliflozin</p>

4. Autophagy

Autophagy, a process that breaks down organelles and eliminates pathogens, is associated with diabetes and cardiovascular complications [48,49]. Autophagy plays a crucial role in atherosclerosis by preserving endothelial functions and protecting against plaque formation. Imbalances in autophagy can lead to inflammation, oxidative stress, and cell death [50]. Recent research suggests that modulating autophagy could be a potential therapeutic target for atherosclerosis prevention and treatment. The modulation of autophagy by SGLT2 inhibitors is closely related to nutrient and oxygen deprivation in the heart and involves the activation of AMPK, SIRT1, HIF-1 α and HIF-2 α [59]. Empagliflozin and dapagliflozin have been shown to rectify autophagy deficiency in diabetic or obese rodent models by activating nutrient-sensing pathways such as the AMPK/mTOR signaling pathway [51–53].

Canagliflozin activated AMPK, effectively reducing the inflammatory response while elevating autophagic flux in immune cells [54]. Similarly, in preliminary diabetic models, empagliflozin restored autophagy in the heart by activating AMPK [55]. In animal models with myocardial infarction, both with and without diabetes, empagliflozin-induced SGLT2 inhibition resulted in decreased infarct size and myocardial fibrosis, mediated by the prevention of autophagic flow in cardiomyocytes [57]. Autophagy also breaks down the NLRP3 inflammasome system, inhibiting the expression of pro-inflammatory markers [59]. Dapagliflozin restored impaired autophagy and suppressed inflammation in HK-2 cells treated with high glucose [52]. Henceforth, these inhibitory mechanisms could help manage atherosclerosis.

5. Autonomous Nervous System

The nervous system uses the lamina adventitia, the outside connective tissue of arteries, as its primary route to peripheral tissues during atherosclerosis, indicating a potential relationship between these two physiological elements [107]. Furthermore, accumulating evidence also propounds the significance of the autonomic nervous system in atherosclerosis [61]. In addition to causing hypertension, persistent high sympathetic activity damages the vascular system [111].

Interestingly, the cardiovascular benefits of SGLT2 inhibition are believed to be caused by the interaction between the sympathetic nervous system and SGLT2 regulation [62]. Here, we highlight the effect of SGLT2 inhibitors on catecholamines, CNS, and acetylcholine on the progression of atherosclerosis. Catecholamines generally autoxidize into highly reactive prooxidant species, including amino chromes, o-semiquinones,

OHI, and O₂, promoting lipid peroxidation and cellular necrosis. Catecholamine MAO activation in the plasma membrane generates hydrogen peroxide (H₂O₂) and aldehydes, which opens up the mitochondrial permeability transition pore (MPTP) to promote oxidative stress-induced mitochondrial DNA damage, cardiomyocyte death, inflammation, and ROS generation [63]. These factors are sought to aggravate atherosclerosis. On the other hand, the cholinergic anti-inflammatory pathway appears to act by posttranscriptional suppression of synthesis and release of the inflammatory cytokines TNF- α , IL-6, and IL-1 β induced by pro-inflammatory stimuli (e.g., exogenous lipopolysaccharide-induced endotoxemia) in vivo and in vitro via α 7 nicotinic acetylcholine receptor (α 7nAChR) [63,64,68,106].

To further study outcomes of sympathetic and atherosclerosis association, Li and colleagues designed a model in which they observed the effects of renal denervation (RDN) and atherosclerosis progression in ApoE deficient (ApoE^{-/-}) mice. RDN reduced Atherosclerosis, EC mitochondrial oxidative stress and inflammation by impairing MAO-A [93]. MAO-A activation impairs mitochondrial homeostasis, resulting in ROS accumulation and NF- κ B activation, thereby enhancing the expression of atherogenic and pro-inflammatory molecules in ECs. It suppresses mitochondrial function regulator PGC-1 α , an enzyme responsible for collagen synthesis, which could cause plaque instability [93]. In a rat model of cognitive impairment induced by scopolamine, canagliflozin, similar to galantamine, decreased AChE activity and increased acetylcholine M1 receptor (M1 mAChR) and monoamines levels [69]. With documented anti-inflammatory actions of acetylcholine, SGLT2 inhibitors like canagliflozin equally show cardioprotective activities in T2DM patients with a risk of atherosclerosis [69].

As earlier noted, β -OHB (a ketone body) is an NLRP3 inflammasome inhibitor that can reduce NLRP3 inflammation. Empagliflozin and dapagliflozin are known to increase the production of this ketone body in humans [66]. β -OHB binds to FFAR3 and blocks it, suppressing sympathetic nervous system activities leading to lowered norepinephrine liberation from nerve endings [67]. Dapagliflozin was found to have sympatholytic action in a murine model of hypertension, reducing TH expression and norepinephrine levels in renal tissue, demonstrating its ability to downregulate TH and impede catecholamine production in adrenal glands [62]. Empagliflozin, equally elevated cerebral BDNF (Brain-derived neurotrophic factor) levels in db/db mice. BDNF's atheroprotective effects are linked with its anti-inflammatory properties, stimulating M2 macrophage polarization via STAT3 [70].

In another study, Liu and colleagues observed EMPA atheroprotective activities by potentially modulating sympathetic pathways. Norepinephrine, neuropeptide Y, serum lipid profiles, RAAS and inflammatory indicators were significantly reduced in mice exposed to EMPA treatment [91]. The rostral ventrolateral medulla (RVLM) is a crucial brain node that generates sympathetic neuronal activity (SNA) by directing catecholaminergic projections to sympathetic preganglionic neurons in the spinal cord [71]. The RVLM of hypertensive rodent models consistently shows signs of increased oxidative stress (SH rat, high-salt diet, SHR-stroke prone, 2K1C, angiotensin perfusion, high-fat diet, low dose LPS infusion, CIH) [71].

For instance, Wu et al. infusion (2012) of *Escherichia coli* lipopolysaccharide (LPS) into the peritoneal cavity of normotensive rats induced systematic inflammation. This activated RVLM microglia, facilitating COX-2-dependent neuroinflammation and increased O_2^- production. The overall effect was neurogenic hypertension and possibly atherosclerosis accompanied by augmented TNF- α levels, IL-1 β , IL-6, or iNOS in RVLM [74]. Histological analysis confirmed the existence of SGLT2s and SGLT1s in the RVLM neurons [72]. Recently, Oshima et al. (2024) analyzed three SGLT2 inhibitors (Mizagliflozin, Canagliflozin and Dapagliflozin) and concluded that they exerted antihypertensive activity by suppressing the activities of the RVLM neurons, leading to reduced sympathetic activities [73].

6. Gut Microbiome

The gut microbiota governs a range of physiological and immunological responses to sustain human health. Dysbiosis can heighten susceptibility to diseases and impact the pharmacokinetic or pharmacodynamic effects of medications [75]. Animal studies demonstrate bacterial infection's relevance in atherosclerosis pathogenesis [82]. Direct infection of the blood vessel walls results in lesion-prone areas, whereas indirect infection at distant sites stimulates the immune system, raising systemic inflammatory levels and aggravating atherosclerosis [81]. Microbial compositional alterations have been found in individuals with cardiovascular disease risk factors and other metabolic abnormalities [77]. For instance, obese individuals generally have a less diverse gut microbiota, and some studies have observed reduced levels of the bacterial phyla Bacteroidetes [76]. Additionally, bacterial DNA has been detected in atherosclerotic plaque samples from individuals [77,80,90].

Furthermore, TMAO-induced gut microbiome reprogramming is a typical example that serves as a prognostic index in cardiovascular diseases and

senescence [78]. TMAO can release inflammatory factors like caspase-1 and IL-1 β through the ROS-TXNIP-NLRP3 signaling pathway and inhibit the SIRT3-SOD2-mitochondrial ROS signaling pathway, leading to endothelial cell injury and atherosclerosis development [79]. Secondly, SCFA produced by the gut microbiome also provides an intestinal mucosal barrier and prevents LPS-induced inflammatory responses in atherosclerotic models [83].

Empagliflozin elevated levels of short-chain fatty acid-producing bacteria, such as species from *Roseburia*, *Eubacterium*, and *Faecalibacterium*, and reduced those of several harmful bacteria, including *Escherichia-Shigella*, *Bilophila*, and *Hungatella* in 76 treatment-naïve patients with T2DM and risk factors for CVD [84]. Similarly, in HFD/STZ diabetic neuropathy mice, Empagliflozin Improved T2DM-related DN by modifying the gut microbiota, explicitly reducing LPS-releasing bacteria and augmenting SCFA-producing bacteria according to Deng et al. (2022) [85]. Contrary to existing studies, Azizi et al. analyzed TMAO levels in response to 26-week empagliflozin treatment following an AMI compared to the standard post-MI treatment. The average increase in TMAO levels over time (interaction = 0.007) was significantly higher in the Empagliflozin compared to the Placebo group [87]. Dapagliflozin's further treatment enhanced arterial stiffness, endothelial dysfunction, and VSMC function in type 2 male diabetic mice [88]. Despite authors noting an alteration in gut microbiota composition, the extent to which this mechanism provided cardioprotection was unknown as the fecal samples for analysis were collected at the end of the study. Canagliflozin and sotagliflozin equally up-regulate the production of SCFA, but like other SGLT2is, no effect has been reported on TMAO metabolism. Only SGL5213—a novel and potent intestinal SGLT1 inhibitor—ameliorated kidney function and reduced gut-derived uremic toxins (phenyl sulfate and trimethylamine-N-oxide), improving renal fibrosis and overall inflammation [86]. Collectively, these improvements present essential mechanisms underlying the cardiovascular benefits of SGLT2i treatment.

CONCLUSION

In conclusion, SGLT2 inhibitors have shown pharmacological advantages in improving endothelial dysfunction, reducing inflammation, inhibiting vascular smooth muscle cell proliferation, inducing autophagy, Gut Microbiome attenuation and modulating the autonomous and central nervous system, all of which contribute to the prevention and treatment of atherosclerosis. Though beyond the scope of this review, SGLT2 inhibitors modulate various metabolic lipid profiles that contribute to atherosclerosis [91].

Generally, SGLT2i gut microbiome regulation focuses mainly on SCFA and less on metabolites like TMAO. TMAO inhibition was only noted in combined SGLT2i/SGLT1i treatment models [86]. Additionally, hydrogen sulphide was experimentally shown to downregulate SGLT2 similarly to EMPA treatment, reducing senescence in endothelial cells via PPAR δ /SGLT2/STAT3 [93,94]. Hence, with senescence-induced inflammation strongly linked to atherosclerosis [95], could the combined treatment of current SGLT2i and H₂S provide synergic beneficial atheroprotective and other cardiovascular effects? Epigenetics, through DNA methylation, histone modifications, and non-coding RNA regulation, mainly regulates the expression of genes involved in oxidative stress, inflammation, and atherosclerosis [97-101].

Diabetic patients, as in atherosclerosis, show decreased DNA methylation in similar genes [102]. Specifically, in human aortic endothelial cells (TeloHAEC), high glucose (HG) is associated with significant demethylation in the promoter region of nuclear factor- κ B (NF- κ B), superoxide dismutase 2 (SOD2), and Sirtuin (SIRT) 6 leading to their detrimental expression [102]. Similarly, atherogenic enzymes and receptors, including superoxide dismutase, endothelial nitric oxide synthase, and estrogen receptors, are hypomethylated [103]. This overshadows their atheroprotective mechanisms. For instance, human ventricular cardiac myoblasts AC16 exposed to hyperglycemia for 7 days of treatment induced significant demethylation in the promoter regions of NF- κ B and SOD2 with a consequent high level in mRNA expression of both genes. The observed DNA demethylation was mediated by increased TET2 expression and binding to the CpGs island in the promoter regions of analyzed genes. EMPA treatment prevented the HG-induced demethylation changes by reducing TET2 binding to the investigated promoter region and counteracted the altered gene expression. In AC16 cells exposed to HG, TET2 binding to CpG island in NF- κ B and SOD2 promoter region was higher compared to cells exposed to NG concentration. Co-treatment with EMPA reduced the TET2 occupancy compared to cells exposed to HG concentration, reducing oxidative stress, inflammation and improved cell viability [104].

Another essential feature in SGLT2i atheroprotective mechanisms is their overall effect on the NRF2 component. Generally, NRF2 has shown both pro and atheroprotective outcomes in various models [107]. However, NRF2 as a target of SGLT2i has not been thoroughly examined in atherosclerosis. Could this be a foe or friend? [109] For instance, Overexpression of Nrf2 in renal proximal tubular Cells Stimulated Sodium-Glucose Cotransporter 2 expression and exacerbated dysglycemia and Kidney Injury in

Diabetic Mice [108]. Bearing that SGLT2 inhibition experimentally alleviates oxidative stress and inflammatory and fibrotic pathways that contribute to diabetes, renal injuries and atherosclerosis, could the NRF2/SGLT2i pathway be the answer? [108]. Supporting this claim, in both Parkinson's disease, diabetic mice and cancer models, SGLT2i suppressed NADPH oxidase 4 (NOX4) while enhancing Nrf2-heme oxygenase-1 (HO-1)-mediated oxidative stress response by targeting ROS-dependent AKT/GSK-3 β /NF- κ B, DJ-1/Nrf2 and AMPK pathways [110]. Put together, this suggests that NRF2 could be a potential therapeutic target in atherosclerosis therapy. Finally, we invite extensive research on SGLT2 inhibitors in cardiovascular disease management.

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Abbreviation (SGLT2i; Sodium-Glucose Transporter Inhibitors, PVAT; Perivascular Adipose Tissue, PDGF; Platelet-Derived Growth Factor, AMPK; AMP-activated protein kinase, B-OHB; Beta-hydroxybutyrate, HFD/STZ; High-fat diet/Streptozotocin, TMAO; Trimethylamine N-oxide, SCFA; Short Chain Fatty Acids, HIF-1/2; Hypoxia Inducible Factor 1/2).

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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