Anti-inflammatory role of SGLT2 inhibitors as part of their anti-atherosclerotic activity: Data from basic science and clinical trials

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Atherosclerosis is a progressive inflammatory disease leading to mortality and morbidity in the civilized world. Atherosclerosis manifests as an accumulation of plaques in the intimal layer of the arterial wall that, by its subsequent erosion or rupture, triggers cardiovascular diseases. Diabetes mellitus is a well-known risk factor for atherosclerosis. Indeed, Type 2 diabetes mellitus patients have an increased risk of atherosclerosis and its associated-cardiovascular complications than non-diabetic patients. Sodium-glucose co-transport 2 inhibitors (SGLT2i), a novel anti-diabetic drugs, have a surprising advantage in cardiovascular effects, such as reducing cardiovascular death in a patient with or without diabetes. Numerous studies have shown that atherosclerosis is due to a significant inflammatory burden and that SGLT2i may play a role in inflammation. In fact, several experiment results have demonstrated that SGLT2i, with suppression of inflammatory mechanism, slows the progression of atherosclerosis. Therefore, SGLT2i may have a double benefit in terms of glycemic control and control of the atherosclerotic process at a myocardial and vascular level. This review elaborates on the anti-inflammatory effects of sodium-glucose co-transporter 2 inhibitors on atherosclerosis.

**KEYWORDS**

SGLT2 inhibitors (SGLT2i), SGLT2, atherosclerosis, atherosclerosis cardiovascular diseases, inflammation

**Introduction**

Atherosclerosis is a widespread chronic inflammatory disorder of large- and medium-caliber artery walls with a complex biochemical and cellular etiology (1). It is characterized by the accumulation of immune-system and endothelial cells, lipid particles, and extracellular matrix components in the sub-endothelial layer (2). In the
Atherosclerosis results from predisposing genetic factors and exposure to oxidative and inflammatory damage mediators. In addition, modifiable and non-modifiable risk factors, which include age, sex, cigarette smoking, unhealthy diet, physical inactivity, dyslipidemia, diabetes mellitus, hypertension, and obesity, contribute to its beginning and development (8). Diabetes mellitus is a well-known risk factor for atherosclerosis. Subjects affected by type 2 diabetes mellitus (T2DM) have an increased risk of atherosclerosis and its associated-cardiovascular complications than non-diabetic patients (9). To date, a new class of anti-hyperglycemic drugs, sodium-glucose co-transport 2 inhibitors (SGLT2i), represent a therapeutic novelty essential for their pleiotropic effects. In addition to glycemic control, SGLT2i is known for the cardio and nephron-protective role and, more recently, for the anti-inflammatory effect. Several experimental results have shown that SGLT2i, with suppression of inflammation, slows the progression of atherosclerosis (10).

This review reports experimental and clinical evidence to clarify the anti-inflammatory mechanisms underlying the anti-atherosclerotic effect of SGLT2i.

Methods

A literature search was conducted by searching updated and relevant publications on SGLT2i and atherosclerosis in databases, including PubMed and Google Scholar. During this research, keywords such as "SGLT2 inhibitor, empagliflozin, dapagliflozin, canagliflozin, ertugliflozin, ipragliflozin, luseogliflozin, satogliflozin, atherosclerosis, atherosclerosis cardiovascular diseases, inflammation, coronary heart disease, stroke, angina pectoris, myocardial infarction and peripheral artery disease, major adverse cardiac events (MACE), cardiovascular (CV) mortality" were used. In vitro and animal studies, clinical trials, reviews, meta-analyses, and guidelines were reviewed. We evaluated the anti-inflammatory effects of SGLT2i from experimental evidence (in vitro and animal models) and clinical trials. For the clinical trials, we considered the cardiovascular outcomes, including myocardial infarction, stroke, peripheral artery disease, cardiovascular death, or hospitalization for heart failure. Articles not in the English language or meeting abstracts were excluded.
Atherosclerosis: From inflammation to complications

Atherosclerosis disease can be schematized in three stages, starting from the endothelium activation/dysfunction and resulting in plaque formation (1).

In the initial stages of the lesion, endothelial dysfunction occurs under harmful stimuli such as hypertension, dyslipidemia, and disturbed shear stress resulting in a chronic inflammatory state. Parallel changes in endothelial permeability promote oxidation of low-density lipoproteins (LDL), followed by infiltration of monocytes in the intimal layer (11). Oxidized-LDL (ox-LDL) promotes damage-associated molecular patterns (DAMPs) secretion that initialize an innate immune response by Toll-like receptors (TLR). Ox-LDL accumulation induces the expression of adhesion molecules as vascular cell adhesion molecule 1 (VCAM-1) by endothelial cells, which recall other monocytes and leukocytes (12). In turn, monocytes transform themselves into activated macrophages altering the ratio between M1 and M2 macrophages. The imbalance of these polarized macrophages may be responsible for plaque development or regression. Ox-LDL, oxidized-LDL; ILs, interleukins; TNF-α, tumor necrosis factor-α; CCR2, C-C chemokine receptor type 2; NOS 2, nitric oxidase synthase 2; TGF-β, transforming growth factor beta; M-CSF, macrophage colony-stimulating factor.
and M2 macrophages. Specifically, based on the macrophage activation process, M1 macrophages are activated in response to TLR ligands, interferons, lipopolysaccharides (LPS), and lipoproteins and express the main pro-inflammatory molecules IL (interleukins)-1β, IL-6, and TNF-α (tumor necrosis factor-α) (13). Therefore, M1 macrophages play significant roles in maintaining chronic inflammation, forming foam cells, and plaque initiation and progression (14).

Inversely, M2 macrophages are associated with an anti-inflammatory phenotype being polarized in response to IL-4 and IL-13 and producing anti-inflammatory factors such as the IL-1 receptor agonist, transforming growth factor beta (TGF-β), and IL-10 (13, 15). Consequently, the imbalance of these polarized macrophages may be responsible for plaque development or regression (16) (Figure 1).

In addition, the lipids accumulation in macrophages, with associated foam cell formation, results in the activation of the nucleotide-binding domain-like receptor protein 3 (NLRP3) inflammasome complex, which promotes the release of pro-inflammatory cytokines contributing to the development and progression of atherosclerosis (17). Indeed, inflammasome activation causes the amplification of the inflammatory response by promoting the expression of adhesion molecules, a proliferation of vascular smooth muscle cells, and the activation of macrophages (18).

Moreover, in the atherogenesis process, oxidative stress and cellular senescence contribute to the maintenance of inflammation and endothelial dysfunction. Indeed, reactive oxygen species (ROS) induce both the synthesis of pro-inflammatory cytokines and stimulate the expression of adhesion molecules, thus allowing monocytes to transmigrate into the vessel wall (19). In addition, ROS can promote the expression of scavenger receptors on vascular smooth muscle cells, promoting lipid accumulation and transformation into foamy cells (17).

Senescence-inducing stress can result from various cardiovascular risk factors (20). Indeed, senescent cells exhibit a specific senescence-associated secretory phenotype (SASP), which consists of inflammatory cytokines, chemokines, growth factors, and proteases (21). The accumulation of senescent cells progressively results in chronic low-grade inflammation, termed “inflammaging” (21). Senescent endothelial cells show altered permeability and nitric oxide (NO) production at the vascular level, inducing endothelial dysfunction. Senescent vascular smooth muscle cells, on the other hand, show reduced proliferation and an increased tendency to apoptosis. Therefore, the accumulation of senescent cells in atherosclerotic lesions may promote plaque progression (22).

In the second stage, characterized by plaque progression, smooth muscle cells produce extracellular matrix components contributing to plaque thickening and progressive growth in the vessel’s lumen. In addition, macrophages and smooth muscle cells undergo apoptosis by going on to form a central lipid-rich core. Therefore, in the late stage, the plaque consists of a central lipid core and a fibrous cap (23). Pro-inflammatory cytokines, such as IL-1, IL-6, Interferons (IFN) gamma, metallocproteinases-2 and 9 (MMP-2 and MMP-9), and metalloproteinase inhibitors (TIMP-1, TIMP-2), are responsible for plaque erosion/rupture. Specifically, IL-6 plays a crucial role in inducing a prothrombotic state by positive regulation of plasminogen activator inhibitor type 1 (PAI-1) during the acute inflammation phase response and negative regulation of antithrombin and protein S (24). Moreover, IL-6 induces the up-regulation of adhesion molecules in the endothelial cells and increases vascular permeability and cellular dysfunction (25).

Therefore, it is possible to distinguish two types of plaques:

- Stable plaques, which possess a thick fibrous cap. It can regress with the proper lifestyle and/or drug therapy or may progress by occluding the vessel lumen.
- Vulnerable or unstable plaques are rich in macrophages and possess a thick lipid core and a thin fibrous cap. It is more likely to undergo rupture and complications (26).

As a result, the cardiac complications of atherosclerotic disease are related to (I) reduced blood flow due to an insufficient oxygen supply, such as angina pectoris; (II) plaque can occlude the vessel leading to ischemia of downstream tissues, for example, in myocardial infarction or stroke; (III) detachment of part of the thrombus, termed an embolus can result in occlusions of distal arteries as pulmonary embolism (7).

### SGLT2 inhibitors

The SGLT2i are a new category of anti-diabetic drugs, approved by the Food and Drug Administration (FDA), used to treat patients with type 2 diabetes (27). SGLT2i family includes dapagliflozin, empagliflozin, luseogliflozin, ipragliflozin, phosphor, and canagliflozin (28).

Sodium-glucose co-transport 2 inhibitors, targeting the major glucose transporter SGLT2 in the kidney, block glucose reabsorption in the proximal renal tubule, increasing glycoursia independently of insulin sensitivity and secretion (29). The SGLT2 inhibition reduces glucose reabsorption, promotes urinary glucose secretion, and causes a negative caloric balance (30).

Phlorizin was the first natural SGLT2i isolated from the root bark of apple trees. Phlorizin, binding the extracellular surface of SGLT1 and SGLT2 in the presence of Na^+., inhibits proteins in a reversible, competitive way (31). However, caused of its poor solubility in water and low absorption in the gastrointestinal tract, several molecules with a similar structure were subsequently developed (32). Many compounds with increased stability, bioavailability, and high selectivity for SGLT2 over SGLT1 have been...
Effects of SGLT2 inhibitors on inflammation in atherosclerosis

As described before, inflammation is a significant factor in vascular cell dysfunction, causing the development and progression of atherosclerosis in diabetes (45).

The atherosclerosis treatment from an inflammatory perspective could be a valid therapeutic strategy. Clinical trials have demonstrated that modulation of inflammation can prevent atherosclerosis and its complications. Recent studies have shown an improvement in inflammatory and oxidative status in subjects with T2DM treated with SGLT2i regardless of glycemic control (46). These data support the hypothesis that SGLT2i show cardio-protective effects action on inflammation.

Therefore, inflammation may be considered a mechanism by which SGLT2i can exert their protective effects against atherosclerosis. This role can be discussed regarding the impact of SGLT-2i on systemic inflammation and immune signaling pathways, including changes in local atherosclerotic tissues.

Experimental evidence

Experimental evidence revealed that SGLT2i reduced the expression of circulating inflammatory molecules, such as TNF-α, monocyte chemoattractant protein 1 (MCP-1), platelet endothelial cell adhesion molecule-1 (PECAM-1), VCAM-1, intercellular adhesion molecule 1 (ICAM-1), IL-1β, and IL-6 (47–50). Several studies have described potential mechanisms by which SGLT2i inhibit the expression of inflammatory molecules. In cultured human endothelial cells, canagliflozin impeded the release of IL-6 and MCP-1 induced by IL-1β in an 5’ adenosine monophosphate-activated protein kinase (AMPK)-dependent manner (51).

Human umbilical vein endothelial cells (HUVECs) and macrophages exposed to dapagliflozin and LPS showed attenuate levels of LPS-induced TLR-4 expression, NF-κB phosphorylation, and miR-155 and elevated levels of miR-146a. Moreover, dapagliflozin shifted from inflammatory M1 macrophages toward M2-dominant macrophages (52).

In LPS-stimulated RAW 264.7 macrophages, empagliflozin in association with gemfibrozil and Dipetidyl peptidase-4 (DPP-IV) inhibitor reduced gene expression and pro-inflammatory cytokine and chemokine release through the IKK/NF-kB/JAK2 - STAT1/3, and MKK4/7 - JNK pathways (53).

Similar results were also obtained in mouse models. In nicotinamide and streptozotocin (NA/STZ)-treated ApoE KO mice, the treatment with luseogliflozin reduces the expression of inflammation-related genes, including F4/80, TNFα, IL-1β, IL-6, ICAM-1, PECAM-1, MMP2 and MMP9 (47).
TABLE 1  Experimental evidence of atheroprotective effects in animal models.

| Drugs               | Experimental model | Treatment                                                                 | Possible atheroprotective effects                                                                 | References |
|---------------------|--------------------|---------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|------------|
| Canaglifozin        | HUVECs             | 10 µmol/L Canaglifozin for 30 min                                         | Inhibition IL-1β-stimulated adhesion of pro-monocytic U937 cells and secretion of IL-6 and monocyte chemotaxtrant protein-1 (MCP-1) | (51)       |
| Dapaglifozin        | HUVECs             | Dapaglifozin (1 mg/kg/day) and lipopolysaccharide (LPS 20 ng/ml) for 24 h under normal (5.5 mmol/L, NG) or high glucose (25 mmol/L, HG) conditions | ▼ LPS-induced TLR-4 expression, NF-κB p65 phosphorylation, miR-155 and miR-146a Shift from M1 macrophages to M2-dominant macrophages | (52)       |
| Empaglifozin        | RAW 264.7 murine   | 40, 60, and 80 µM for 4 h                                                 | ▼ pro-inflammatory cytokine and chemokine                                                          | (53)       |
| Luseoglifozin       | NA/STZ-treated ApoE KO mice | Dose with maximal glucose-lowering efficacy for 1 week | TNFα, IL-1β, IL-6, ICAM-1, PECAM-1, MMP2, and MMP9                                      | (47)       |
| Dapaglifozin        | Type 2 diabetic (BTBR ob/ob) and wild-type (WT) mice | Dapaglifozin, or Dapaglifozin (1 mg/kg/day) + Saxagliptin (10 mg/kg/day) for 8 weeks | Inhibition ROS-NLRP3-caspase-1 pathway                                                            | (49, 54)   |
| Empaglifozin        | Diabetic ApoE –/– mice | (20 mg/kg/day) for 8 or 12 weeks                                          | ▼ CD68, MCP-1, ICAM-1, and TNF-α → suppressing the development and progression of atherosclerotic lesions | (60)       |
| Ipraglifozin        | NA/STZ-induced diabetic mice | 10 mg/kg/day for 10 weeks | ▼ ROS, TNF α, IL-6, CRP, MCP-1                                                             | (61)       |
| Empaglifozin        | (HFD)-induced obese C57BL/6J mice | HFD + Lo Empa, equivalent to 3 mg/kg bodyweight | ↑ FGF21                                                                                                | (60)       |
| Empaglifozin        | Apolipoprotein mice | 3 mg/kg per day                                                           | ▼ CCL-2, CCL-5, VEGF, MMP-2, MMP-9, p38 MAPK, and NF-κB                                       | (62)       |
| Dapaglifozin        | Non-diabetic male Wistar rats (200–250 g) | Dapaglifozin (0.1 mg/kg per day), phlorizin (0.4 g/kg per day), dapaglifozin + SII-201 (a STAT3 inhibitor), or phlorizin + SII-201 for 4 weeks | ▼ M1 by RONS-dependent STAT3-pathway                                                             | (65)       |
| Dapaglifozin        | Rabbit             | 1 mg/kg/day for 8 weeks                                                   | ▼ Expression of TLR4 and NF-κB                                                                   | (66)       |

CRP, C reactive protein; HDF, a high-fat diet; ICAM-1, intercellular cell adhesion molecule-1; LDLR, low-density lipoprotein receptor; IL-1ß, interleukin-1ß; IL-6, interleukin-6; MCP-1, monocyte chemotaxtrant protein-1; MMP, matrix metalloproteinase; NF-xB, nuclear factor-xB; NLRP3, nucleotide-binding domain-like receptor protein 3ROS, reactive oxygen species; TNF-α, tumor necrosis factor-α; VEGF, Vascular endothelial growth factor; VCAM-1, vascular cell adhesion molecule-1.

In STZ-treated ApoE –/– mice, dapaglifozin inhibited IL-1β and IL-18 secretion, blocking the ROS-NLRP3-caspase-1 pathway showing that NLRP3 inflammasome complex could be considered an SGLT2i target (49, 54).

Two mechanisms by which empaglifozin inhibited NLRP3 activation were identified, one is dependent on β-hydroxybutyrate (BHB), and the other is Ca+ dependent. Specifically, ex vivo experiments with macrophages demonstrated that empaglifozin inhibited NLRP3, increasing BHB levels and reducing glucose, uric acid, and insulin (55). Empaglifozin reduced Ca+ levels by attenuating Na+ intracellular accumulation, causing the NLRP3 inhibition and improving functional recovery (56).

Moreover, it was demonstrated that also dapaglifozin reduced the production of NLRP3 protein by activating mTOR Complex 2 (mTORC2), leading to the activation of AMPK and Forkhead box 3 (FOXO3) (57, 58).

Considerable evidence showed that SGLT2i ameliorated endothelial dysfunction and improved endothelium-dependent vasodilation. In particular, in diabetic ApoE –/– mice,
empagliflozin improved endothelial function by reducing CD68, MCP-1, ICAM-1, and TNF-α, suppressing the development and progression of atherosclerotic lesions (59). In a High-fat diet (HFD)-induced obese C57BL/6j MICE, empagliflozin increased plasma levels of Fibroblast Growth Factor 21 (FGF21), protecting the cells from damage caused by atherosclerosis-associated oxidative stress (60). Ipragliflozin reduced oxidative stress markers, thiobarbituric acid reactive substances (TBARS), and inflammation proteins (CRP, TNF-α, IL-6, and MCP-1) in streptozotocin-nicotinamide-induced diabetic mice (61). In abdominal aortic aneurysm induced by Angiotensin II infusion in apolipoprotein mice, the treatment with empagliflozin inhibited leukocyte-endothelial cell interactions, macrophages infiltration, and secretion of pro-inflammatory markers such as chemokine (C-C motif) ligand 2 (CCL-2), CCL-5, Vascular Endothelial Growth Factor (VEGF), MMP-2, MMP-9, p38 mitogen-activated protein kinase (MAPK), and NF-κB (62).

Evidence revealed that SGLT2i attenuates macrophage infiltration and inflammation, foam cell formation, and M1 polarization, crucial steps in the development of atherosclerosis. It was demonstrated that empagliflozin reduced macrophage infiltration and CD36 gene expression resulting in the loss of macrophage capability to scavenger ox-LDL and foam cell formation in db/db mice (63). A similar result was obtained in the streptozotocin (STZ)-induced diabetic model, where the macrophages proliferation and leukocyte adhesion were significantly decreased in empagliflozin and dapagliflozin-treated mice with a subsequent reduction in the plaque size (51, 64).

Moreover, dapagliflozin reduced macrophage infiltration and induced M2 polarization with a concomitant decrease in M1 through a mechanism depending on a RONS-dependent STAT3 pathway. These data demonstrated that dapagliflozin induced the production of anti-inflammatory factors participating in inflammation prevention and tissue repair (65).

Similar results were demonstrated in white rabbits, where dapagliflozin increased M2 macrophages and inhibited the expression of TLR4 and NF-κB (66).

However, the exact mechanism by which SGLT2i can induce M2 polarization and reduce macrophage infiltration is not fully elucidated, and many possible hypotheses are formulated. Indeed, reducing glucose levels, the principal energy source of macrophages, might have a role (35) (Figure 1; Table 1).

Clinical evidence

Several clinical studies have been conducted to evaluate the changes in the main inflammatory and oxidative stress biomarkers to evaluate the potential role of SGLT2i in protecting against atherosclerosis. The inflammatory markers implicated and therefore assessed in the atherosclerosis process are CRP, IL-6, and TNF-α.

In a single-center, open-label, randomized, prospective study, the administration of empagliflozin (10 mg/day for 12 months) to 51 diabetic patients induced a significant reduction of blood hs-CRP levels associated with baseline and placebo (−74.4% vs. placebo and −55.6% vs. baseline) (67).

Similarly, the CANOSSA trial, a prospective and open-label study that enrolled 35 patients with diabetes mellitus and stable chronic heart, demonstrated that the canagliflozin administration (100 mg/day for 12 months) induced a significant decrease in hs-CRP after 3, 6, and 12 months compared with baseline (3 months: $p = 0.002$, 6 months: $p = 0.001$, 12 months: $p = 0.007$) (68).

A comparative efficacy study between canagliflozin and empagliflozin was also conducted on 32 diabetic patients to evaluate the effect on inflammatory cytokines. The results suggest that treatment with empagliflozin 10 mg/day for 6 months is more effective in reducing inflammatory cytokines IL-6 ($p = 0.002$ vs. $p = 0.27$) and TNF-alpha ($p = 0.002$ vs. $p = 0.29$), while canagliflozin was more effective in reducing HbA1c (69).

DEFENSE STUDY, a prospective, randomized, open-label, blinded-endpoint, parallel-group, evaluated dapagliflozin’s effectiveness on vascular endothelial function and glycemic control in subjects affected by T2DM early-stage. Dapagliflozin, in association with metformin for 16 weeks, improved endothelial function and significantly decreased urine 8-OHdG/creatinine, a marker of oxidative stress, compared to the only metformin group ($p < 0.001$) (70).

Moreover, atherosclerotic plaques of diabetic patients treated with canagliflozin displayed increased SIRT 6 expression and lower oxidative stress and inflammation markers (71) (Table 2).

Effects of SGLT2i on atherosclerosis cardiovascular diseases and cardiovascular mortality

Atherosclerotic cardiovascular diseases (ASCVD) represent a very important cause of death and disability, especially in subjects affected by T2DM. The main manifestations of ASCVD are coronary heart disease, ischemic stroke, peripheral artery disease, and heart failure (73).

Coronary artery disease

Coronary artery disease (CAD) is the most common atherosclerotic vascular disease, and it includes two main clinical phenotypes: stable/unstable angina and acute MI (AMI). Data regarding MI in patients treated with SGLT2i are conflicting. In the EMPA-REG OUTCOME study and CANVAS study, no
HF hospitalizations independently of diabetes (approved SGLT2 inhibitors for treating HFrEF and preventing Heart failure study showed a significant reduction in hospitalizations for 21 and safety of SGLT2i in patients with AMI (Dapagliflozin Prospective, open-label, blinded endpoint, randomized)

| Drugs     | Type of study                          | Characteristics of patients (number) | Treatment                                                                 | Possible atheroprotective effects | References |
|-----------|----------------------------------------|-------------------------------------|---------------------------------------------------------------------------|----------------------------------|------------|
| Empaglifozin | Prospective, open-label observational   | T2DM (15)                           | 24-week empaglifozin 10 mg vs. baseline                                    | ↓ CRP/hs-CRP                     | (52)       |
| Canaglifozin | Prospective, open-label, randomized controlled trial | T2DM, HF (35)                     | 12 month CRP canaglifozin 100 mg vs. baseline                             | ↓ CRP/hs-CRP                     | (53)       |
| Dapaglifozin | Prospective, open-label, blinded endpoint, randomized | T2DM (72)                           | 16 week dapaglifozin 5 mg/day + Metformin 750 mg/day vs. Metformin 1,500 mg/day | ↓ 8-OHdG                         | (55)       |

CRP, c-reactive protein; TNF α, tumor factor alpha necrosis; IL6, interleukin-6; hsCRP, high-sensitivity c-reactive protein; 8-OHdG, 8-Hydroxy-2-deoxyguanosine; T2DM, type 2 diabetes mellitus.

The effects of early SGLT2i treatment in patients with recent AMI were not well-studied. Early SGLT2i treatment might improve cardiovascular outcomes through its beneficial effects on endothelial function, neurohormonal activation, cardiomyocyte necrosis, and reperfusion injury. Three studies are evaluating the efficacy and safety of SGLT2i in patients with AMI (EMMY (Impact of EMPagliflozin on Cardiac Function and Biomarkers of Heart Failure in Patients With Acute Myocardial Infarction) trial (NCT03087773), DAPA-MI (Dapagliflozin Effects on Cardiovascular Events in Patients With an Acute Heart Attack) (NCT04564742), EMPACT-MI (A Streamlined, Multicenter, Randomized, Parallel Group, Double-blind Placebo-controlled Superiority Trial to Evaluate the Effect of EMPAgliflozin on Hospitalization for Heart Failure and Mortality in Patients With Acute Myocardial Infarction) trial (NCT04509674). HF in patients treated with empagliflozin and canagliflozin, respectively, vs. placebo (RR 0.65; 95% CI 0.50–0.85) (RR 0.67; 95% CI 0.52–0.87) (75, 84). DECLARE-TIMI 58 showed a reduced hospitalization rate for HF compared to the placebo group (RR 0.73; 95% CI 0.61–0.88) (85).

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Heart failure

The recent European clinical guidelines on HF have approved SGLT2 inhibitors for treating HFrEF and preventing HF hospitalizations independently of diabetes (37, 72, 81–83). In particular, EMPA-REG OUTCOME and the CANVAS study showed a significant reduction in hospitalizations for HF in patients treated with empagliflozin and canagliflozin, respectively, vs. placebo (RR 0.65; 95% CI 0.50–0.85) (RR 0.67; 95% CI 0.52–0.87) (75, 84). DECLARE-TIMI 58 showed a reduced hospitalization rate for HF compared to the placebo group (RR 0.73; 95% CI 0.61–0.88) (85).

However, the CVD-REAL and the CVD-REAL2 studies showed a lower risk of stroke associated with SGLT2i therapy (RR 0.83; 95% CI 0.71–0.97) (RR 0.68; 95% CI 0.55–0.84) (76, 77).

Stroke

Data regarding the risk of stroke in patients treated with SGLT2i are conflicting. The EMPAREG OUTCOME study did not show a significant reduction in the incidence of stroke in patients treated with empagliflozin compared to the placebo group (RR 1.18; 95% CI 0.89–1.56) (89). The same result was found in CANVAS and DECLARE-TIMI 58 studies (RR 0.9; 95% CI 0.71–1.15 and RR 1.01; 95% CI 0.84–1.21, respectively) (75, 85).

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However, the analysis conducted by these studies did not consider the SGLT2i effect on different types of stroke
Peripheral artery disease

Peripheral artery disease (PAD) is a manifestation of systemic atherosclerosis, characterized by progressive narrowing to occlusion of arterial vessels in the limbs, resulting in ulceration and subsequent gangrene and amputation.

Peripheral artery disease increases the risk of coronary artery and cerebrovascular disease and is an independent predictor of CVD death (91). The EMPAREG study, although it did not consider PAD as a primary and secondary endpoint, revealed that Empagliflozin resulted in significant benefits in subjects with PAD, which no reported amputations.

However, the CANAVAS and CANAVAS-R studies reported that amputation risk was twice in the treated group compared to the placebo group (6.3 cases per 1,000 patients per year) (75). Although the molecular mechanisms responsible for this have not yet been elucidated, the EMA has reported the potential increased risk of lower limb amputation (affecting mainly the toes) in patients taking SGLT2 inhibitors.

Major adverse cardiac events

The most important cardiovascular trials for SGLT2 evaluated the incidence of major adverse cardiac events (MACEs), such as cardiovascular death, non-fatal myocardial infarction, and non-fatal stroke. A meta-analysis of 6 placebo-controlled clinical outcomes trials (EMPA-REG OUTCOME, CANVAS, DECLARE-TIMI 58, CREDENCE, VERTIS CV) (85, 86, 92, 93), including a total of 46,969 patients (66.2% with prevalent ASCVD) suggests that SGLT2 inhibitors significantly reduced the risk of MACE (HR, 0.90; 95% CI, 0.85–0.95); the presence or absence of ASCVD did not modify the treatment outcome on MACE (HR, 0.89; 95% CI, 0.83–1.07) (94).

Cardiovascular mortality

Four large cardiovascular outcome studies have recently been completed: EMPAREG OUTCOME, CANVAS, DECLARE-TIMI, and VERTIS CV (85, 86, 92, 93). These studies showed a reduction in overall and cardiovascular mortality (CV Mortality), which was significant in the EMPA-REG and CANVAS studies but not in DECLARE-TIMI 58. Specifically, the CV mortality rate was lower in the EMPA-REG OUTCOME study, in patients treated with SGLT2i compared to placebo (RR 0.62; 95% CI 0.49–0.77) (89). CV mortality rate was not significantly reduced in CANVAS, DECLARETIMI 58 and VERTIS-CV (RR 0.87; 95% CI 0.72–1.06 and RR 0.98; 95% CI 0.82–1.17, HR 0.89 95% CI 0.73–1.05, respectively) (75, 95). A recent meta-analysis showed no significant effect on CV mortality associated with SGLT2i treatment (except Empagliflozin) compared to placebo (OR 0.87; 95% CI 0.63–1.21) (96) (Table 3).

From clinical impact to future prospective

Sodium-glucose co-transport 2 inhibitors exert anti-atherosclerotic properties attenuating inflammatory factors, alleviating inflammation, mitigating insulin resistance, and reducing stress on vessels, inhibiting atherosclerosis development and progression (46). Recent clinical trials have analyzed the landmark cardiovascular outcomes, showing that SGLT2i may reduce MI, heart failure and HF hospitalization, MACE, and cardiovascular death in subjects affected by T2DM (81, 86–89). Nevertheless, data regarding MI, stroke, and PAD are conflicting, probably because the analyzed population is heterogeneous, the different stroke subtypes were not considered, different molecules were analyzed, and the follow-up time is short. Therefore, observational studies of high quality, with an adequate number of events and follow-up times, should be conducted to examine the potential role of SGLT2i in subclinical atherosclerosis and ASCVD events in subjects affected and not by T2DM. Despite conflicting data, SGLT2i represents a promising drug class; important innovation is reported in European guidelines on diagnosing and treating heart failure. SGLT2i is used as a class I recommendation in treating HFrEF (72).

Moreover, cardiovascular safety studies conducted with SGLT2i suggested that subjects affected by T2DM could benefit from SGLT2i treatments with positive effects on cardiovascular events (97). Unfortunately, only a small number of subjects affected by T2DM are treated with SGLT2i, whereas their widespread use would reduce deaths and hospitalizations each year. We think using these drugs will lead to a global evaluation of patients regarding glycemic control and cardiac function. Moreover, its use will have a substantial cost reduction and hospitalization advantage.
TABLE 3 Cardiovascular outcomes of SGLT2 inhibitors trials.

|                | EMPA-REG | CANVAS | CREDENCE | DECLARE-TIMI | DAPA–HF | VERTIS-CV |
|----------------|----------|--------|----------|--------------|---------|-----------|
| **References** | (74)     | (60)   | (77)     | (69)         | (66)    | (78)      |
| **Drugs**      | Empagliflozin | Canagliflozin | Canagliflozin | Dapagliflozin | Dapagliflozin | Ertugliflozin |
| **Study population** | T2DM patients with CVD | T2DM patients with CVD or CV risk factors | T2DM patients with CKD | T2DM patients with ASCVD or CV risk factors | Patients with rHF | T2DM patients with ASCVD |
| **Number of patients** | 7,020 | 10,142 | 4,401 | 17,150 | 4,744 | 8,246 |
| **Median follow-up** | 3.1 years | 2.4 years | 2.6 years | 4.2 years | 18.2 months | 3.5 years |
| **CV outcomes** | | | | | | |
| MACE           | 0.86 (0.74–0.99) | 0.86 (0.75–0.97) | 0.80 (0.67–0.95) | 0.93 (0.84–1.03) | – | 0.97 (0.85–1.11) |
| CV death       | 0.62 (0.49–0.77) | 0.87 (0.72–1.06) | – | 0.98 (0.82–1.17) | 0.82 (0.69–0.98) | 0.92 (0.77–1.11) |
| CV death or HHF| 0.66 (0.55–0.79) | – | – | 0.83 (0.73–0.95) | 0.75 (0.65–0.85) | – |

ASCVD, atherosclerotic cardiovascular diseases; CKD, chronic kidney disease; CV, cardiovascular; CVD, cardiovascular diseases; HF, heart failure; HHF, hospitalization for heart failure; MACE, major adverse cardiovascular events: including death from cardiovascular causes, non-fatal MI, and non-fatal stroke; T2DM, type 2 diabetes mellitus.

Conclusions

Atherosclerosis is a chronic inflammatory disorder representing the major potential risk factor of CVDs. The progress in managing the atherosclerosis complications has extended life, but many individuals still present impaired cardiac function, straining healthcare systems and resources (7). In this review, we emphasize SGLT2i by providing updated information about its implication in atherosclerosis and ASCVD. SGLT2i exerts anti-atherosclerotic properties attenuating inflammatory factors and reducing MI, heart failure MACE in subjects affected by T2DM.

Therefore, in conclusion, SGLT2 inhibitors can represent up-and-coming therapeutic drugs with pleiotropic effects in terms of metabolic control and reduction of associated cardiovascular complications.

Author contributions

LSc, VC, and MB: concept and design. LSc, VC, FT, RF, APe, PG, MF, APu, AD, LSp, RM, and MB: drafting of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding

This study was funded by PON Ricerca e Innovazione 2014–2020 ARS01_01270.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Jebari-Bendaiman S, Galicia-Garcia U, Larrea-Sebal A, Olaetxea JR, Alloza I, Vandenbroeck K, et al. Pathophysiology of atherosclerosis. Int J Mol Sci. (2022) 23:3346. doi: 10.3390/ijms23063346
2. Emini Veseli B, Perrotta P, De Meyer GRA, Roth L, Van der Donckt C, Martinet W, et al. Animal models of atherosclerosis. Eur J Pharmacol. (2017) 816:3–13. doi: 10.1016/j.ejphar.2017.05.010
3. Prenyiuk A, Grechko AV, Poggio P, Myasoedova VA, Alfieri V, Orekhov AN. The diabetes mellitus-atherosclerosis connection: the role of lipid and glucose metabolism and chronic inflammation. Int J Mol Sci. (2020) 21:1835. doi: 10.3390/ijms21051835
4. Crowther MA. Pathogenesis of atherosclerosis. Hematology Am Soc Hematol Educ Program. (2003) 436–41. doi: 10.1182/asheducation-2005.1.436

Frontiers in Cardiovascular Medicine 10 frontiersin.org
Biochim Biophys Acta Mol Basis Dis. (2020) 26. doi: 10.1016/j.bbadis.2020.11.010.24

Circ Res. (2019) 5:338–49. doi: 10.1038/s41591-019-0552-7.26

Frontiers in Cardiovascular Medicine

28. Shaffner J, Chen B, Malhotra DK, Dworkin LD, Gong R. Therapeutic targeting of SGLT2: a new era in the treatment of diabetes and diabetic kidney disease. Front Endocrinol. (2021) 12:749010. doi: 10.3389/fendo.2021.749010.29

Wilcox CS. Antihypertensive and renal mechanisms of SGLT2 (Sodium-Glucose Linked Transporter 2) inhibitors. Hypertension. (2020) 75:894–901. doi: 10.1161/HYPTENSIONAHA.119.11684.30

Valton V, Thomson SC. Targeting renal glucose reabsorption to treat hypeglycaemia: the pleotropic effects of SGLT2 inhibition. Diabetologia. (2017) 60:215–25. doi: 10.1007/s00125-016-4157-3.31

Wright EM. SGLT2 inhibitors: physiology and pharmacology. Kidney360. (2021) 2:2027-37. doi: 10.34067/KID.000277201.32

Kuhrasag RP, Kulkarni AA, Chouthe RS, Pathan SK, Une HD, Reddy GR, et al. SGLT2 inhibitors as anti-diabetic agents: a comprehensive review. RSC Adv. (2020) 10:1733–56. doi: 10.1039/c9ra08766k.33

Cowie MR, Fisher MA. SGLT2 inhibitors: mechanisms of cardiovascular benefit beyond glycaemic control. Nat Rev Cardiol. (2020) 17:761–72. doi: 10.1038/s41574-020-0406-8.34

Cinti F, Moffa S, Impromta E, Cefalo CM, Sun VA, Sorice GP, et al. Spotlight on etrugirolitin and its potential in the treatment of type 2 diabetes: evidence to date. Drug Des Devel Ther. (2017) 11:2905–19. doi: 10.2147/DDDT.S114932.35

Bonora RM, Avogaro A, Fadini GP. Extraglycemic effects of SGLT2 inhibitors: a review of the evidence. Diabetes Metab Syndr Obes. (2020) 13:161–74. doi: 10.2147/DMSO.S233538.36

Provenzano M, Pelle MC, Zaffina L, Tassone B, Puja R, Ricchio M, et al. Sodium-glucose co-transporter-2 inhibitors and nephropathies: a review. Diabetes Metab Syndr Obes. (2020) 13:161–74. doi: 10.2147/DMSO.S233538.37

Pepachud GD, Verma S. Mechanisms of cardiovascular benefits of sodium glucose co-transporter 2 (SGLT2) inhibitors: a state-of-the-art review. JACC Basic Transl Sci. (2020) 5:632–44. doi: 10.1016/j.jactbs.2020.02.004.38

Tamargo J. Sodium-glucose co-transporter 2 inhibitors in heart failure: potential mechanisms of action, adverse effects and future developments. Eur J Cardiol. (2019) 14:23–32. doi: 10.15402/eurjdic.2019.34.2.39

Paolizzo P, Bergamaschi I, Santulli G, Gallinoro E, Cesaero A, Gagnano F, et al. Infarct size, inflammatory burden, and admission hyperglycemia in diabetic patients with acute myocardial infarction treated with SGLT2-inhibitors: a multicenter international registry. Cardiovasc Diabetol. (2022) 21:77. doi: 10.1186/s12933-022-01506-8.40

Sardu C, Barbieri M, Santamaria M, Giordano V, Sacra C, Paolizzo P, et al. Multipolar pacing by cardiac resynchronization therapy with a defibrillator treatment in type 2 diabetes mellitus failing heart patients: impact on responders rate, and clinical outcomes. Cardiovasc Diabetol. (2017) 16:75. doi: 10.1186/s12933-017-0554-2.41

Hodrea I, Saeed A, Molnar A, Finta H, Barzci A, Wagner JI, et al. SGLT2 inhibitor dapagliflozin prevents atherosclerotic and cardiac complications in experimental type 1 diabetes. PLoS ONE. (2019) 17:e0263285. doi: 10.1371/journal.pone.0263285.42

Salvatore T, Galiero R, Caturano A, Rinaldi L, Di Martino A, Albanese G, et al. An overview of the cardiorenal protective mechanisms of SGLT2 inhibitors. Int J Mol Sci. (2022) 23:3651. doi: 10.3390/ijms23073651.43

Tsai KF, Chen YL, Chiou TT, Chu TH, Li LC, Ng HY, et al. Emerge nce of SGLT2 inhibitors as powerful antioxidants in human diseases. Antioxidants. (2021) 10:1166. doi: 10.3390/antiox10081166.44

Nedosugova LV, Markina YV, Bochkareva LA, Kuzina IA, Petunina ME, et al. Sodium glucose co-transporter 2 (SGLT2) inhibitors: a state-of-the-art review. RSC Adv. (2020) 10:1733–56. doi: 10.1039/c9ra08766k.45

Oguntibeju OO. Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. Int J Physiol Pathophysiol Pharmacol. (2019) 11:45–63.46

Nakatsu Y, Kokubo H, Bumdelber F, Yoshizumi M, Yamamoto T, Matsunaga Y, et al. The SGLT2 inhibitor luseogliflozin rapidly normalizes aortic mRNA levels of inflammation-related but not lipid-metabolism-related genes and suppresses atherosclerosis in diabetic ApoE−/− mice. Int J Mol Sci. (2017) 18:17040. doi: 10.3390/ijms18071704.47

Dimitriadis GK, Nasiri-Ansari N, Agrogianis G, Kostakis ID, Randeva MS, Nikiteas N, et al. Empagliflozin improves primary haemodynamic parameters and attenuates the development of atherosclerosis in high fat fed fed APOE knockout mice. Mol Cell Endocrinol. (2019) 494:110487. doi: 10.1016/j.mce.2019.110487.
85. Wiviott SD, Raz I, Bonaca MP, Mosenzon O, Kato ET, Cahn A, et al. Dapagliflozin and cardiovascular outcomes in type 2 diabetes. *N Engl J Med.* (2019) 380:347–57. doi: 10.1056/NEJMoa1812389

86. Cannon CP, Pratley R, Dagogo-Jack S, Mancuso J, Huyck S, Masukiewicz U, et al. Cardiovascular outcomes with etrugliflozin in type 2 diabetes. *N Engl J Med.* (2020) 383:1425–35. doi: 10.1056/NEJMoa2004967

87. Packer M, Anker SD, Butler J, Filippatos G, Ferreira JP, Pocock SJ, et al. Empagliflozin in patients with heart failure, reduced ejection fraction, and volume overload: EMPEROIR-reduced trial. *J Am Coll Cardiol.* (2021) 77:1381–92. doi: 10.1016/j.jacc.2021.01.033

88. Zannad F, Ferreira JP, Pocock SJ, Anker SD, Butler J, Filippatos G, et al. SGLT2 inhibitors in patients with heart failure with reduced ejection fraction: a meta-analysis of the EMPEROIR-reduced and DAPA-HF trials. *Lancet.* (2020) 396:819–29. doi: 10.1016/S0140-6736(20)31824-9

89. Zinman B, Lachin JM, Inzucchi SE. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med.* (2016) 374:1094. doi: 10.1056/NEJMoa1600827

90. Tsai WH, Chuang SM, Liu SC, Lee CC, Chien MN, Leung CH, et al. Effects of SGLT2 inhibitors on stroke and its subtypes in patients with type 2 diabetes: a systematic review and meta-analysis. *Sci Rep.* (2021) 11:15364. doi: 10.1038/s41598-021-94945-4

91. Mohler ER 3rd. Peripheral arterial disease: identification and implications. *Arch Intern Med.* (2003) 163:2306–14. doi: 10.1001/archinte.163.19.2306

92. Scheen AJ. [EMPA-REG OUTCOME: empagliflozin reduces mortality in patients with type 2 diabetes at high cardiovascular risk]. *Rev Med Liege.* (2015) 70:583–9.

93. Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erdogan N, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N Engl J Med.* (2017) 377:644–57. doi: 10.1056/NEJMoa1611925

94. McGuire DK, Shih WJ, Cosentino F, Charbonnel B, Cherney DZI, Dagogo-Jack S, et al. Association of SGLT2 inhibitors with cardiovascular and kidney outcomes in patients with type 2 diabetes: a meta-analysis. *JAMA Cardiol.* (2021) 6:148–58. doi: 10.1001/jamacardio.2020.4511

95. Cosentino F, Cannon CP, Cherney DZI, Masukiewicz U, Pratley R, Dagogo-Jack S, et al. Efficacy of ertugliflozin on heart failure-related events in patients with type 2 diabetes mellitus and established atherosclerotic cardiovascular disease: results of the VERTIS CV trial. *Circulation.* (2020) 142:2205–15. doi: 10.1161/CIRCULATIONAHA.120.050255

96. Sinha B, Ghosal S. Meta-analyses of the effects of DPP-4 inhibitors, SGLT2 inhibitors and GLP1 receptor analogues on cardiovascular death, myocardial infarction, stroke and hospitalization for heart failure. *Diabetes Res Clin Pract.* (2019) 150:8–16. doi: 10.1016/j.diabres.2019.02.014

97. Nashawi M, Ahmed MS, Amin T, Ahualfoul M, Chilton R. Cardiovascular benefits from SGLT2 inhibition in type 2 diabetes mellitus patients is not impaired with phosphate flux related to pharmacotherapy. *World J Cardiol.* (2021) 13:676–94. doi: 10.4330/wjc.v13.i12.676