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Review

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SGLT -2 Inhibitors and the Inflammasome: What's Next in the 21st Century?

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Abstract: Nucleotide-binding domain like receptor protein 3 (NLRP3) inflammasome in the kidney and the heart are increasingly being suggested to play a key role in mediating inflammation. In the kidney, NLRP3 activation has been associated with the progression of diabetic kidney disease. In the heart, activation of the NLRP3 inflammasome has been related to enhanced release of interleukin-1β (IL-1β) and the subsequent induction of atherosclerosis and heart failure. Apart from their glucose-lowering effects, SGLT-2 Inhibitors have been documented to attenuate activation of the NLRP3, thus, resulting in the constellation of an anti-inflammatory milieu. In this review, we will focus on the interplay between SGLT-2 Inhibitors and the inflammasome in the kidney, the heart and the neurons in the context of diabetes mellitus and its complications.

Introduction

SGLT (sodium glucose co-transporters) inhibitors are transmembrane proteins that normally promote glucose reabsorption in the intestine and renal glomerulus through a sodium-dependent process that leads to concomitant sodium retention [1]. SGLT-1s are mainly expressed in the small intestine, in contrast to SGLT-2s, which are located mainly in the proximal renal tubule, specifically in the S1 and S2 segments, where they ensure the reabsorption of 90% of the glucose filtered into the renal glomerulus. The remaining 10% of the filtered glucose is reabsorbed with the help of SGLT-1 inhibitors, in the S3 segment of the renal tubule, where the available glucose in the lumen is in a smaller amount. Sodium-glucose co-transporter 2 (SGLT-2) inhibitors or gliflozins are a novel class of anti-diabetic drugs with a unique mechanism of action, as they promote the reduction of serum glucose, by inducing glycosuria in an insulin-independent manner. At the same time, they cause natriuresis, resulting in a reduction in blood pressure [2–4].

Phlorizin is the first natural SGLT1/2 inhibitor, first isolated in 1835, which belongs to the family of flavonoids. It was originally isolated from apple bark and was shown to cause glycosuria through non-selective action in SGLT-1 and SGLT-2 co-transporters, resulting in inhibition of intestinal and renal glucose reabsorption. Despite its beneficial effects on the complications of diabetes mellitus and the improvement of insulin resistance, it was eventually withdrawn, as it presented significant drawbacks, such as limited oral bioavailability, reduced intestinal absorption and rapid renal excretion [5–7]. Many years later, the scientific community in its quest to discover new therapeutic weapons in an effort to achieve glycemic control in diabetic patients, is bringing back gliflozins to the fore and creating newer synthetic SGLT inhibitors. Thus, in March 2013, the Food and Drug Administration (FDA) approved Canagliflozin, while a year later Dapagliflozin and Empagliflozin were also endorsed by the FDA [8]. Beyond their role in glycemic regulation, SGLT-2 inhibitors seem

to have remarkable pleiotropic effects, with multiple reports highlighting their potential anti-inflammatory action. According to more recent data, SGLT-2 inhibitors are no longer considered as pure anti-diabetic drugs, as they have now received an indication for administration to patients with heart failure and chronic kidney disease, regardless of the presence of diabetes mellitus [8].

NLR Family Pyrin Domain Containing 3 (NLRP3 inflammasome) is a protein complex of the innate immune system, with major triggers for its activation, infection and cell damage. Structurally, it consists of a sensor, NLRP3, expressed in cells of the immune system and especially in macrophages, an adapter (PYCARD, PYD and CARD Domain Containing) and finally an effector, caspase-1, which in turn cleaves Gasdermin D (GSDMD). This results in the formation of a pore in the cell membrane, leading to the leakage of intracellular components, including the mediators of the inflammatory response IL-1β and IL-18. GSDMD is the final driver of pyroptosis, a newer form of programmed cell death, as the inflammatory response to pathogens. While NLRP3 activation plays a central role in host defense against invading pathogens, its deleterious activation with consequent activation of pyroptosis appears to contribute significantly to the pathogenesis of many metabolic diseases, such as type 2 diabetes mellitus and chronic kidney disease [9–13]. In this article, we review the potential effects of SGLT2 inhibitors in inflammation, the potential actions of SGLTs in inflammation, emphasizing on their effect on NLRP3 function.

Inflammasome's Mechanism of action

Inflammation is a protective immune response to harmful stimuli, and can be caused by both infectious and non-infectious factors. The triggering of inflammation starts with the recognition of PAMPs and DAMPs. PAMPs (pathogen-associated molecular patterns) originate from microbial invaders, while DAMPs (danger-related molecular patterns) originate from damaged cells and tissues [14,15]. Furthermore, PAMPs and DAMPs are recognized by special receptors, known as Pattern Recognition Receptors (PRRs), which are mostly expressed on cells of innate immunity, such as macrophages, monocytes and dendritic cells. They belong to various protein groups and are subdivided into two main categories based on their location in the cell. TLRs (Toll-like receptors) and CTLs (C-type lectin receptors) are found in the cytoplasmic membrane, while NLRs (NOD-like receptors) and RLRs (RIG-I-like receptors) are located in the cytoplasm. TLRs mostly recognize elements of pathogenic bacteria, such as bacterial DNA, lipopolysaccharides (LPS), peptidoglycan and teichoic acid, in contrast with CTLs, which recognize fungi components, with β-D-glucan being a typical recognition target. On the other hand, RLRs act as sensory receptors of viruses' elements, such as the envelope membrane, and NLRs receptors recognize intermediate ingredients of cellular metabolism.

Intracellular (cytoplasmic) receptors (NLRs, RLRs) are part of a complex, called inflammasome, which is a key-parameter of non-specific immunity and acts as a "factory" for the production of pro-inflammatory cytokines (such as IL-1β), with pleiotropic effects on inflammation. The inflammasome is a polyprotein intracellular complex and consists of 3 parts; a protein-sensor, one or more protein-adaptors, which contain the caspase binding region, also known as the CARD region and pro-caspase-1. Caspases are a family of cysteine proteases that serve as main effectors during apoptosis to proteolytically dismantle most cellular structures. They act as specific molecules that are localized in the cytoplasm in the form of their inactive proenzymes, the pro-caspases. There are many types of inflammasome, although the best studied is the inflammasome 3 (NLRP3), which is the therapeutic target of SGLT-2 inhibitors. NLRP3 belongs to the NOD-like receptor family. In the case of NLRP3, the sensor protein is the NLRP3 receptor (LYR, NACHT and PYD domains), while the adaptor protein is ASC. Specifically, it contains a leucine-rich region (LRR), which recognizes and binds PAMPs or DAMPs, an intermediate nucleotide-binding domain (NACHT), which is responsible for polymerization and a pyrin domain (PYD), whose main role is the activation of caspase-1 through the CARD region [10–15].

Inflammasome formation and activation require two signals. The first one induces transcription and production of individual components, while the second one promotes polymerization and assembly into an active inflammasome. The first signal comes after the recognition and binding of

PAMPs and DAMPs to the respective receptors of the innate immune cells. Binding of PAMPs and DAMPs triggers end-to-end intracellular signal transduction pathways resulting in the activation of the cytoplasmic transcription factor NF-κB (Nuclear Factor-κB). NF-κB, in turn, induces transcription of the genes encoding pro-IL-18 and pro-IL-1β cytokines, which polymerize and activate the NLRP3 receptor. Then, secondary signals, such as potassium efflux and the production of oxygen free radicals, contribute to the polymerization and the connection of the remaining parts (ACS and procaspase-1), resulting in the formation and activation of the inflammasome. As a result, the production of active caspase-1, leads to hydrolysis of pro-IL-18 and pro-IL-1β to IL-18 and IL-1β, which are finally secreted into the environment [10–15].

IL-18 and IL-1β further stimulate non-specific and specific immunity. Also, the production of IL-18 and IL-1β is associated with the induction of insulin resistance in tissues, which plays a role in the treatment of infections. This local insulin resistance develops in order to conserve glucose and free fatty acids, essential energy substrates for immune cells to fight pathogen invasion. Caspase-1, resulting from inflammasome activation, along with caspase-11, hydrolyzes gasdermin-D, resulting in lysis pore formation and as a consequence the death of the infected cell, a process known as autophagy. As a sequelae, a release of DAMPs is observed, which in turn can strengthen immunity, as they can activate the inflammasome of the neighboring cells. Overall, the inflammasome is a major regulator of non-specific immunity and the goal of its activation is both the destruction of the infectious invader and the limitation of the tissue damage, through the production of interleukins and the routing of autophagy [15,16].

SGLT-2 inhibitors' Anti-inflammatory effects

In addition to their effect on glycemic regulation, numerous studies have revealed the cardioprotective and nephroprotective effect of SGLT inhibitors. SGLT-2 inhibitors display remarkable pleiotropic actions, targeting multiple mechanisms that go beyond the context of glucose homeostasis, although these are not completely understood to date. The favorable effects of this class of agents on weight loss through glucose excretion, blood pressure lowering through induced natriuresis, and mitochondrial function and biogenesis make these drugs a first-line therapeutic option in patients with cardiovascular disease. In addition, gliflozins appear to exhibit remarkable effects on mechanisms such as pyroptosis, autophagy, and oxidative stress, a finding that highlights their multisystem efficacy and their potential to target mechanisms at multiple pathophysiological levels [15–18].

Recent studies have shown that gliflozins exerts an anti-inflammatory activity, with a potential therapeutic role in the treatment of diseases, such as obesity, atherosclerosis and non-alcoholic steatohepatitis. In the abovementioned disorders, inflammation is well known to play a central pathophysiological role. In its initial stages, inflammation is a protective mechanism of the immune system and is mediated by molecules, which aim to heal the damage, through the clearance of harmful agents. Progressively, the beneficial role of inflammation is exhausted, it becomes chronic and the adverse effects on the body are reflected by causing tissue damage, as a vicious cycle is created, which perpetuates and at the same time worsens this damage. Inflammation appears to be a factor that accelerates atherosclerosis in diabetic patients with type II diabetes mellitus (T2DM), and thus it is believed that interventions that reduce inflammation may achieve beneficial effects in this group of patients. Relatively recently, a significant number of studies have described the anti-inflammatory effect of SGLT inhibitors, based on different models of experimental approach [16–19].

Macrophages are immune cells that play a central role in the inflammatory process, as they contribute both to the defense against the invading microorganism and to the repair of damaged tissues. Traditionally, they are classified into two groups, the M1 subgroup (classically activated macrophages), which are stimulated by Th1 cytokines or LPS bacterial lipopolysaccharides and stimulate the production of proinflammatory cytokines, and the M2 subgroup (alternatively activated macrophages), which exert an anti-inflammatory effect after its activation by Th2 cytokines. Macrophage cells possess remarkable plasticity, which enables them to transform from one form to another, a process called polarization, in response to the respective stimulus of their

microenvironment. SGLT inhibitors appear to modulate the conversion of macrophages from one form to another in such a way as to favorably alter the inflammatory response [20]. Existing studies mainly concern the three main SGLT-2 inhibitors, dapagliflozin, canagliflozin and empagliflozin.

The main mechanisms through which gliflozins interfere with macrophage cells concern their ability to promote the conversion of M1 macrophages to M2, a property that is maintained even in hyperglycemic conditions, while they inhibit the LPS-induced secretion of pro-inflammatory cytokines (IL and TNF-a). Furthermore, SGLT inhibitors increase the expression of macrophage-mediated regulators of immune and inflammatory responses, with notable action on the TLR4 receptor and NF-kB. Their aforementioned actions have as a final consequence the limitation of the clinical effects of inflammation, such as atherosclerosis and fibrosis, both in the cardiovascular system and in the liver and kidneys. In particular, empagliflozin reduces the infiltration of atheromatous plaque by macrophage cells, by inhibiting the proliferation of macrophages in the plaque, thus contributing to its regression. In the liver, in addition to reducing tissue infiltration by macrophages, it activates their autophagy, through the activation of the mammalian target of rapamycin (mTOR) target. Finally, SGLT-2 inhibitors exert a favorable effect on adipose tissue as well, suppressing obesity-induced macrophage accumulation, while reducing the amount of adipose tissue and the size of adipocytes [15,20].

SGLT-2 and the heart

SGLT-2 inhibitors are glucose-sodium co-transporters and, thus, inhibition of their activity results in osmotic diuresis and natriuresis. The ensuing decrease in plasma volume has multiple benefits for the cardiovascular system, as it contributes to the reduction of stress on the cardiovascular wall, with a concomitant drop in blood pressure. Despite compensatory activation of the renin-angiotensin-aldosterone axis (RAAS), the reduction in plasma volume is long-lasting, likely due to continued glycosuria and the initial reduction in total body sodium content. In addition, the administration of an SGLT-2 inhibitor may enhance the action of diuretics in patients with heart failure, as it is believed that their action on the proximal tubule is not limited to SGLT-2 co-transporters, but may also inhibit the sodium-hydrogen exchanger (NHE3), which normally regulates the re-absorption of sodium bicarbonate. This protein is up-regulated in heart failure, with the main consequence being the reduced effect of diuretic therapy, through an increase in resistance to natriuresis. At the same time, it leads to cardiotoxicity, through changes in the intracellular space, with an increase in sodium and calcium. Inhibition of NHE in myocardial tissue, in a SGLT-2-independent manner, leads to limitation of hypertrophy and fibrosis in myocardial tissue [21].

According to Paulus and Tschope, systemic inflammation and oxidative stress play a key role in myocardial dysfunction. Inhibition of NHE1 appears to be the key point of empagliflozin's action, as it leads to a reduction of mitochondrial swelling, through inhibition of intracellular calcium entry and reduced release of ROS from myocardial cells. At the same time, the signaling pathway that serves the activation of AKT1/AKT2/Unit AKT318 is inhibited, preventing the stimulation of inducible NOS2 production, with the final result of reducing oxidative stress. The reduction in oxidative stress appears to be achieved through the inhibition of other signaling pathways, which normally serve the increased expression of NOS2 (NFAT1/NFAT2/NFAT3 pathway) and HDAC1 (histone deacetylase 1) by NF-κB downstream of AKT. Inactivation of NHE1 appears to show beneficial effects in myocardial extracellular matrix remodeling, cardiomyocyte stiffness and heart concentric hypertrophy, through multiple mechanisms. Inhibition of NHE1 also affects the function of the directly involved in HFpEF, NLRP3 inflammasome, and IL-1β, through inhibition of cathepsin B activation. In addition, the activation of TNF-α and ICAM1 molecules involved in the inflammatory process is inhibited, as the deactivation of NEH1 leads to the deactivation of their activators, specifically NFAT2 and NFAT3 [15,22,23].

One of the most characteristic side effects of SGLT2 inhibitors is the induction of euglycemic diabetic ketoacidosis. The observed increase in beta-hydroxybutyrate (β-OHB) levels can be fatal if not treated in time, but paradoxically can provide significant benefits. The above finding has been the subject of studies in recent years and according to existing evidence, it is attributed to the ability

of β-OHB to inhibit oxidative stress, while at the same time can protect the function of mitochondria and exert an anti-inflammatory effect, as it is an endogenous NLRP3 Inflammasome inhibitor. The increase in β-OHB levels by the administration of SGLT-2 inhibitors seems to be an alternative source of energy for the myocyte in conditions of heart failure, changing in a favorable way the energy environment of the cell, while promoting a reduction in the size of the heart infarct in ischemia-reperfusion injury (I/R injury). The above observation is in agreement with the findings of Horton et al, according to whom, in the final stages of heart failure, the utilization of β-OHB by the heart muscle is significantly increased [24].

The inhibitory effect of β-OHB on the NLRP3 inflammasome has been shown in experimental models in both the heart and the kidney. In 2015, Youm et al, showed that the adoption of the ketogenic diet can bring beneficial anti-inflammatory effects, through the inhibition of the NLRP3 inflammasome, which results from the observed increase in β-OHB levels. The proposed mechanisms, through which elevated levels of β-OHB paradoxically exert a beneficial effect on heart muscle through inhibition of NLRP3 action, are several and emerge from recent data in experimental models [25]. Bae et al showed that increased β-OHB levels can activate AMPK and thereby reduce endoplasmic reticulum stress and the subsequent increase in NLRP3 levels [26]. In addition, attenuation of NLRP3 activity and subsequent prevention of mitochondrial dysfunction through increased levels of β-OHB in HFpEF can markedly slow the progression of the disease, as recently found by Deng et al [27]. It is worth noting that it is still unknown whether elevated blood levels of β-OHB have the same hemodynamic effects among patients with HFrEF or HFpEF, an issue that is expected to be clarified in future studies [27]. Figure 1 depicts the main mechanisms of action of SGLT-2 inhibitors and the inflammasome in the heart, the kidney and the central nervous system (CNS) [Figure 1].
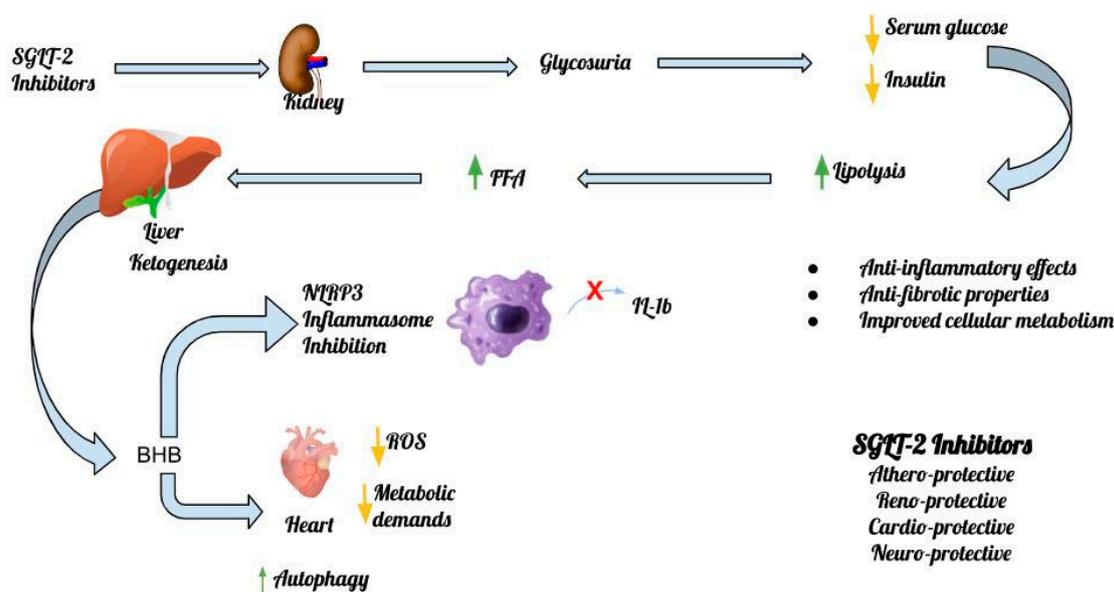

Figure 1. The main mechanisms of action of SGLT-2 Inhibitors and the Inflammasome.

A high-calorie diet appears to lead to NLRP3 activation in several organs, including the liver and kidney, with administration of the SGLT-2 inhibitor empagliflozin reducing this activation. In contrast, Liakos et al showed that hypercaloric diets do not show similar effects in myocardial tissue, as they do not lead to activation of the NLRP3 inflammasome in conditions of myocardial steatosis [28].

Recent data advocate that the cardioprotective effect of SGLT-2 inhibitors could be attributed to their ability to stimulate autophagy, a process of maintaining cellular homeostasis under stress conditions. According to what we know today, the protective mechanism of autophagy is disturbed in heart failure, in such a way, that its enhancement appears favorable in the progression of the

disease. Through autophagy, the cell can remove waste and potentially toxic products of cellular stress, ensuring a more metabolically favorable environment. Autophagy can inhibit the action of NLRP3, as it removes endogenous activators of the inflammasome, such as dysfunctional mitochondria, which are a substrate for the occurrence of oxidative stress. Although the mechanism by which SGLT2 inhibitors stimulate autophagy is not entirely clear, it seems likely that they induce activation of adenosine monophosphate-activated protein kinase (AMPK), sirtuin-1 (SIRT1) and hypoxia-inducible factor (HIF-1α and HIF-2α). The activation of these molecules ensures a favorable metabolic balance for the myocardial cell, while at the same time it is related to the transcription of genes that ensure the oxygen supply to the myocyte, while it is hypothesized that the ketogenesis caused by SGL-2 inhibitors can itself induce the mechanism of autophagy, through its ability to activate the aforementioned molecules [29].

The vast majority of the available experimental data show that the cardioprotective properties of SGLT-2 inhibitors are induced, in part, by the inhibition of the NLRP3 inflammasome. However, in 2021 Gordon et al, published the results of an experimental study, which supports that the administration of empagliflozin for the period of 8 weeks did not ultimately lead to the inhibition of NLRP3 or IL-1β levels, suggesting the redirection to other pathophysiological mechanisms that could potentially explain the cardioprotective effects of SGLT2 inhibitors [30].

The Inflammasome and Chronic Kidney Disease (CKD)

The inflammasome has been demonstrated to be involved in the progression of diabetic kidney disease (DKD) [21,23]. This association has been confirmed by the finding of increased levels of IL-1β and Interleukin 18 (IL-18) in the serum and renal tissue among patients with DKD [31,32]. Furthermore, the activation of the NLRP3 inflammasome has been documented in renal infiltrating macrophages, podocytes, tubular epithelial cells as well as in mesangial cells [33–39]. In addition, confirmatory results of the involvement of the inflammasome in the progression of DKD have come from studies in which Inhibition or knockout of NLRP3 have been performed. For example, the NLRP3-specific inhibitor MCC950 has been demonstrated to ameliorate renal damage as well as fibrosis in diabetic mice (db/db mice) [40,41]. Also, knockout of the NLRP3 by Wu et al had a significant reno-protective effect in STZ-induced diabetic mice [42]. Apart from the NLRP3 inflammasome, the NLRP1 and NLRC4 inflammasome have also been activated in the kidneys of DKD mice. Their activation has been related to increased urinary albumin excretion and renal damage as well [43,44]. Furthermore, Luan et al. have demonstrated that the NLRC5 gene deficiency is associated with reductions in renal inflammation and damage, while they documented a delay in the progression of renal fibrosis in STZ-induced diabetic mice [45]. Other inflammasomes, such as the NLRP2, NLRP6, NLRP10, NLRP12, and AIM2 are also suggested to be implicated in the pathogenesis of non-diabetic kidney diseases [47–49]. Valiño-Rivas et al showed that the NLRP6 deficiency led to increased ischemia-reperfusion induced acute kidney injury [50]. In particular, the AIM2 activation has been related to the development of lupus nephritis and hepatitis B associated glomerulonephritis [51,52]. These data are suggestive of a plausible role of the inflammasome in the pathogenesis of chronic kidney disease in general and not only of DKD.

Based upon the above mentioned data, the inflammasome is documented to be implicated in DKD or/and non-diabetic CKD. Therefore, the use of SGLT2 Inhibitors, which have been documented to ameliorate renal damage, is increasingly being recognized as a potential target of the inflammasome in the kidney. Interestingly, empagliflozin has been shown to reduce obesity-related kidney disease in animal models. More specifically, Ye et al have demonstrated that empagliflozin by inhibiting the NLRP3 inflammasome, has resulted in protection against obesity related kidney disease. The exact mechanisms of this protection are not fully elucidated. However, empagliflozin is postulated to affect genes, which play a crucial role in inhibition of the NLRP3 inflammasome [53]. In addition, Benetti et al have also documented the inhibitory effects of empagliflozin upon the NLRP3 inflammasome and the subsequent mitigation of inflammatory responses in DKD in a mouse model of high fat high sugar fed diet [54]. Regarding dapagliflozin, Birnbaum et al have demonstrated an inhibitory effect on the inflammasome in an animal model of T2DM, which resulted

in attenuation of the renal damage [55]. Besides, only recently dapagliflozin has been shown to inhibit the NLRP3 inflammasome, thereby enhancing the anti-inflammatory and anti-fibrotic changes in DKD [56]. To our knowledge, there are no studies with other gliflozins until now, regarding other SGLT-2 inhibitors, the inhibition of the inflammasome and the reno-protective potential. Table 1 depicts major studies in animal models regarding glucose-lowering drugs and the inflammasome [Table 1].

Table 1. List of main studies in animal models associating SGLT-2 Inhibitors with the NLRP3 Inflammasome.

Title of Study/Reference	Animal Model	Findings of the Study	Remarks
List of Studies with SGLT-2 Inhibitors			
SGLT inhibitor counteracts NLRP3 inflammasome via tubular metabolite itaconate in fibrosis kidney. Ke et al, 2022 [56].	Animal model regarding the anti-fibrotic and anti-inflammatory effects of DAPA on renal parameters, using the ischemia/reperfusion-induced fibrosis model.	1. DAPA interfered with the activation of mTOR and HIF-1α signaling, while restoring tubular cell-fatty acid oxidation. 2. NLRP3 inflammasome activation was ↓↓ by DAPA. 3. Immunomodulatory metabolite itaconate derived from the TCA cycle was ↑↑ 4. Administration of itaconate surrogate ↓↓ activation of NLRP3 inflammasome and ↓↓ kidney fibrosis.	5. The results of this study in animal models proved the interplay of metabolism and inflammation regarding DAPA beneficial effects on the kidney.
Empagliflozin attenuates obesity related kidney dysfunction and NLRP3 inflammasome activity through the HO-1 Adiponectin axis. Ye T et al, 2022 [53].	C57BL/6J mice were assigned to control, HFD groups, and EMPA (10 mg/kg) groups.	6. HFD mice had metabolic abnormalities and renal injury, as documented by ↑ UAE, ↑↑ lipids together with morphologic changes. 7. EMPA treatment improved metabolic disorders and lipotoxicity-induced renal injury.	8. EMPA improved obesity-related kidney disease by ↓↓ of NLRP3 inflammasome activity and upregulation of the HO-1-adiponectin axis. 9. The results are suggestive of a new mechanism for SGLT2 Inhibitors regarding renal protection in the context of obesity.
Empagliflozin protects against diet-induced NLRP3 inflammasome activation and lipid accumulation.	Male C57BL/6 mice were assigned to control, or a HFHS diet for 4 months. Over the last 2 months, subsets of male mice were also treated with EMPA (1-10 mg/kg).	10. EMPA resulted in body weight ↓↓ (P < 0.001 for the highest dose), while it ameliorated fasting glycemia and HOMA-IR. EMPA ↓↓ renal tubular damage and liver triglycerides level in a dose-dependent manner. EMPA	11. EMPA ↓↓ deleterious effects provoked by chronic exposure to HFHS diet. 12. EMPA treatment was related to

Benetti et al, 2016, [54].		also decreased cardiac lipid accumulation.	inhibition of the NLRP-3 inflammasome, suggesting that this inhibition may account for the therapeutic potential of EMPA.
List of Studies with combination of SGLT-2 Inhibitors and GLP-1 Analogs and/or DPP4 Inhibitors			
SGLT-2 inhibition with dapagliflozin reduced the activation of the NLRP3/ASC inflammasome and attenuates development of diabetic cardiomyopathy in mice with type 2 diabetes. Further augmentation of the effects with saxagliptin, a DPP4 inhibitor. Ye et al, 2017 [71].	Type 2 diabetic (BTBR ob/ob) and WT mice were assigned on vehicle, DAPA, or DAPA+SAXA for 8 weeks. Cardiofibroblasts from WT and BTBR hearts were incubated with DAPA and exposed to LPS.	13. LVEF was 81 ± 1% in the WT and 53 ± 1% in the T2DM mice. 14. DAPA and DAPA+SAXA ↑↑ LVEF to 68 ± 1 and 74.6 ± 1% in the BTBR mice (p < 0.001). 15. The mRNA levels of NALP3, ASC, IL-1β, IL-6, caspase-1, and TNFα were ↑↑ in the BTBR compared to the WT hearts. 16. DAPA and DAPA+SAXA ↓↓ these mRNA levels. Protein levels of NLRP3, TNFα, and caspase-1 were ↑↑ in the BTBR compared to the WT hearts. 17. DAPA, and even more DAPA+SAXA, ↓↓ the increase of the above parameters in the BTBR mice. 18. Collagen-1 and collagen-3 mRNA levels ↑↑ in the BTBR mice and these increases were diminished by DAPA and DAPA+SAXA. 19. This in vitro study showed that NALP3, ASC, IL-1β, and caspase-1 mRNA levels were ↑↑ in the BTBR cardiofibroblasts and furthermore ↓↓ with DAPA The effect was AMPK-dependent and SGLT1-independent.	✔ DAPA ↓↓ the activation of the inflammasome, fibrosis, and deterioration of LVEF in BTBR mice. ✔ The anti-inflammatory, anti-fibrotic effects are probably SGLT2- and glucose-lowering-independent. ✔ The effects on remodeling were ↑↑ when SAXA was added to DAPA. ✔ However, adding SAXA to DAPA did not result in a greater effect regarding myocardial fibrosis and collagen levels.
Combined SGLT2 and DPPA inhibition	Male BTBR ob/ob and wild-type (WT) mice	20. Serum BUN in the WT mice was 16.9 ± 0.8 mg/dl,	mRNA levels of NALP3, ASC, IL-

reduces the activation of the NLRP3/ASC inflammasome and attenuates the development of diabetic nephropathy in mice with type 2 diabetes. Birnbaum et al, 2018 [55].	received vehicle, DAPA, or DAPA+SAXA for 8 weeks.	while it ↑↑ to 55.7 ± 2.8 mg/dl in the BTBR mice. DAPA alone ↓↓ BUN to 31.4 ± 1.2 mg/dl. A much greater decrease was even reported in the DAPA+SAXA combination (24.8 ± 0.8 mg/dl). 21. Serum creatinine was 0.16 ± 0.02 and 1.01 ± 0.04 mg/dl in the WT and BTBR mice, respectively. 22. Serum cystatin C was ↑ in the BTBR mice (3.9 ± 0.1 vs. 0.6 ± 0.2 ng/ml), when compared to WT mice. DAPA (2.4 ± 0.1) and DAPA+SAXA (1.4 ± 0.1) resulted in decreases in serum cystatic C levels. 23. Kidney weight was ↑↑ in the BTBR than in WT mice. DAPA ↓↓ the kidney/body weight ratio in the BTBR mice.	1β, IL-6, caspase-1, TNF-α, collagen-1, and collagen-3 significantly were ↑ in the kidneys of the BTBR, when compared to the WT mice. DAPA alone and to a greater extent, DAPA+SAXA, resulted in ↓↓ activation of the inflammasome. However, the combination of DAPA+SAXA did not result in greater attenuation of the collagen-1 and collagen-3 mRNA levels.
List of Studies with only DPP-4 Inhibitors and /or GLP-1 Analogs, without SGLT-2 Inhibitors			
DPP-4 inhibition by linagliptin prevents cardiac dysfunction and inflammation by targeting the NLRP3/ASC inflammasome. Birnbaum et al, 2019 [72].	LINA and GLP-1 receptor activation by exenatide followed by exendin-4 in an infusion pump (EX) on IS, post-infarction activation of the inflammasome and remodeling in WT and db/db diabetic mice. Mice underwent 30 min ischemia followed by 24 h reperfusion.	✔ LINA and EX ↓↓ IS in both the WT and the db/db mice. LINA and EX ↑↑ EF in both the WT and the db/db mice. mRNA levels of ASC, NALP3, IL-1β, IL-6, Collagen-1, and Collagen-3 ↑↑ in the db/db mice than in the WT mice. ✔ LINA more than EX ↓↓ the increase in ASC, NALP3, IL-1β, IL-6, Collagen-1 and Collagen-3, TNFα and IL-1β, and ↓↓ apoptosis, especially in the db/db mice.	✔ LINA and EX had similar effects on IS and post-infarction function, but LINA ↓↓ the activation of the inflammasome and the upregulation of collagen-1 and collagen-3 more than direct GLP-1 receptor activation.

Abbreviations: BUN: Blood Urea Nitrogen; DAPA: Dapagliflozin; EF: Ejection Fraction; EMPA: Empagliflozin; HFD:High Fat Diet; HFHS: High Fat High Sugar; HIF-1a: Hypoxia Inducible Factor 1a; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; IS: Infarct Size; LINA: Linagliptin; LPS: Lipopolysaccharide; LVEF: Left Ventricular Ejection Fraction; mTOR: mammalian Target Of Rapamycin; SAXA: Saxagliptin; TCA: Tricarboxylic Acid UAE: Urinary to Albumin Excretion; WT: Wild Type.

The Inflammasome and Central Nervous System

There is a growing interest regarding SGLT2 inhibitors and their neuro-protective potential. SGLT2 inhibitors are lipid-soluble molecules, which have the capacity to cross the blood-brain barrier. A brain-to-serum ratio of the area under the curve has been reported to be 0.3 for canagliflozin and dapagliflozin and up to 0.5 for empagliflozin [57]. As SGLT1 and SGLT2 receptors are expressed

in the brain, SGLT2 inhibitors are capable of directly affecting their target. Brain expression of SGLT2 is lower than SGLT1, and it occurs mainly in the microvessels of the blood-brain barrier as well as in the amygdala, hypothalamus, periaqueductal gray, and the dorsomedial medulla [58,59]. It is noteworthy that the brain locations, where SGLTs have been found, are suggested to be responsible for learning process, food intake, energy and glucose homeostasis. Moreover, these regions have been implicated in central cardiovascular and autonomic regulation as well [59,60]. It is possible that SGLT2 receptors also exert a cardioprotective effect through central mechanisms by directly influencing cardiovascular regulation and autonomic pathways, including the paraventricular nucleus of the hypothalamus, the nucleus of the solitary tract, and the periaqueductal gray [61].

NLRP3 inflammasome is activated not only by microorganisms, but also in cases of chronic inflammatory diseases, such as atherosclerosis and Alzheimer's disease [62,63]. NLRP3 contributes to the inflammatory process in atherosclerosis,   as its activation has been demonstrated in arterial walls, as a result of lipoprotein accumulation [64]. Indeed, in an animal model of atherosclerosis, the inhibition of the NLRP3 inflammasome by MCC950 led to a significant decrease in atherosclerotic lesions [65]. SGLT2 inhibitors are suggested to ameliorate atherosclerosis and cognitive dysfunction by inhibiting the NLRP3 inflammasome. Kim et al used empagliflozin or glimepiride, a sulfonylurea, among 61 patients with T2DM and high CVD risk and measured several metabolic parameters after 30 days of treatment [18]. They documented that empagliflozin resulted in a more significant reduction in the release of IL-1β and serum insulin levels together with increased serum levels of BHB, when compared to sulfonylureas. Furthermore, they performed an ex vivo study with human macrophages that confirmed the inhibitory effects of empagliflozin on the NLRP3 inflammasome [18].

Indeed, in an animal model of diabetes mellitus and Alzheimer's disease, empagliflozin was documented to improve both cerebral microvascular impairment and cognitive dysfunction, too [66]. More specifically, empagliflozin was shown to reduce thinning of the cortex, hemorrhages and microglia burden as well as senile plaques in this mouse model of T2DM and Alzheimer's disease [67]. In Alzheimer's disease, NLRP3 inflammasome has been postulated to interconnect systemic inflammation with neuro-inflammation by impairing the removal of amyloid-beta via the microglia [68]. This effect may be clinically significant, as, in another study, the inhibition of NLRP3 by OLT1177 significantly improved cognitive impairment in an animal model of Alzheimer's disease [68]. Apart from animal models, Wu et al have very recently published an intriguing study in Diabetes Care. Among 106,903 individuals that were enrolled in this study, SGLT-2 inhibitors compared with DPP-4 inhibitors were associated with a decreased risk of dementia (14.2/1,000 person-years; aHR 0.80 [95% CI 0.71-0.89]). Regarding SGLT-2 inhibitors, dapagliflozin showed the lowest risk (aHR 0.67 [95% CI 0.53-0.84]), followed by empagliflozin (aHR 0.78 [95% CI 0.69-0.89]), whereas no relationship between canagliflozin and dementia was noted (aHR 0.96 [95% CI 0.80-1.16]) [69]. Therefore, nowadays, there is limited evidence for a truly neuroprotective role of SGLT-2 inhibitors in the context of cognitive impairment and especially diabetes. However, further large-scale studies are needed to support this notion and the study by Wu et al may be just the beginning.

Conclusion

The inflammasome, especially NLRP3 inflammasome, seems to play a crucial role in mediating inflammation among patients with diabetes and heart failure, CKD and cognitive impairment. SGLT-2 inhibitors, apart from their glucose-lowering effects are postulated to mitigate activation of the inflammasome and thus, create an anti-inflammatory milieu. As chronic low-grade inflammation   is the cornerstone of diabetes, heart failure, CKD and cognitive impairment, it seems likely that SGLT-2 inhibitors may exert beneficial effects on the abovementioned clinical entities. In fact, SGLT-2 inhibitors have been demonstrated to exhibit their therapeutic potential among patients with heart failure and CKD, even without diabetes. Their utility regarding cognitive impairment is still under investigation. Nowadays, there is growing evidence for the pleiotropic effects of SGLT-2 inhibitors [70]. These pleiotropic effects are largely attributed to their attenuation of inflammasome activation. This attenuation as well as other mechanisms of action of this advantageous class of glucose-lowering

drugs will be further elucidated in the 21st century. Overall, their utilization among patients with diabetes and heart failure, CKD or even cognitive impairment should be a fact, not a fiction.

Abbreviations: AMPK: Adenosine Monophosphate-activated Protein Kinase; CVD: β-HOB: beta hydroxybutyrate; Cardiovascular Disease; CKD: Chronic Kidney Disease; CNS: Central Nervous System; DAMPs: Danger related molecular patterns; DKD: Diabetic Kidney Disease; DPP4-Inhibitors: Dipeptidyl Peptidase 4 Inhibitors; FDA: Food and Drug Administration; GSDMD: Gasdermin D; HFpEF: Heart Failure with preserved Ejection Fraction; HFrEF: Heart Failure with reduced Ejection Fraction; HIF: Hypoxia Inducible Factor; IL: Interleukin; LPS: Lipopolysaccharide; mTOR: mammalian Target Of Rapamycin NLRP3: Nucleotide-binding domain like receptor protein 3; PAMPs: Pathogen associated molecular patterns; SIRT1: Sirtuin 1; SGLT: Sodium Glucose Co-transporters; NHE3: sodium-hydrogen exchanger; TLRs: Toll Like Receptors; T2DM: Type 2 Diabetes Mellitus; TNF-α: Tumor Necrosis Factor alpha.

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