Chapter 1

GENERAL INTRODUCTION AND OUTLINE OF THIS THESIS
GENERAL INTRODUCTION

Type 2 diabetes and chronic kidney disease (CKD) are global health burdens affecting about 6.3% and 9.3% of the world’s population, respectively. (1, 2) Although type 2 diabetes is the leading cause of CKD, both of these conditions are independent risk factors for kidney failure, cardiovascular disease, and death. (1, 3-6) An epidemiological study showed that individuals with type 2 diabetes and early CKD had 16 years shorter life expectancy than those without. (7) Thus, new treatments to improve the prognosis for people with diabetes and CKD are desired.

Sodium-glucose cotransporter2 (SGLT2) inhibitors were originally developed as glucose-lowering drugs. Early clinical trials of SGLT2 inhibitors successfully demonstrated their cardiovascular and kidney efficacy in patients with diabetes. (8-10) Subsequent trials expanded the clinical benefits of SGLT2 inhibitors to populations without diabetes. (11-13) However, the mechanisms by which SGLT2 inhibitors protect kidney and heart are not fully elucidated yet; but several potential mechanisms for the kidney protective effects are postulated and described below.

Physiology of SGLT and development of SGLT2 inhibitors

SGLT2 is located on the luminal side of the proximal tubule in the kidney, specifically in the S1 and S2 segments. In healthy individuals, SGLT2 reabsorbs 90-95% of glucose (160-180 g/day) filtered in glomeruli. (14, 15) The rest is reabsorbed by lower capacity transporter SGLT1 in the distal (S3) segments. (14) In patients with diabetes, the expression of SGLT2 can be upregulated to deal with increased glucose flow in pre-urine. (14-16)

Phlorizin is a non-selective SGLT inhibitor, which was originally isolated from the apple bark in the 1800s. (17, 18) Phlorizin induced glucosuria and natriuresis and exerted a blood glucose-lowering effect in animals and humans. (17) Its gastrointestinal side effects due to SGLT1 inhibition in the small intestine halted clinical development and stimulated the development of selective SGLT2 inhibitors. These selective SGLT2 inhibitors cause 50-60 g glucosuria per day in normal glucose tolerant individuals and lower hemoglobin A1c by approximately 0.5 to 0.8% in people with type 2 diabetes. (19, 20) Currently, available SGLT2 inhibitors in the United States, Europe and Japan include empagliflozin, canagliflozin, and dapagliflozin.

SGLT2 inhibitors and cardiovascular and renal outcome trials

As regulatory agents required to prove cardiovascular safety for newly approved glucose-lowering drugs, cardiovascular outcome trials of SGLT2 inhibitors were conducted in the 2010s (Table 1). (21) These multinational large placebo-controlled randomized trials, represented by the EMPA-REG OUTCOME trial and the CANVAS program, demonstrated
the risk reduction with SGLT2 inhibitors for major adverse cardiovascular events (MACE: death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke) and hospitalization due to heart failure in patients with type 2 diabetes at cardiovascular risk. (8, 22-24) Secondary analysis of these trials also reported a lower risk of kidney disease progression in patients treated with SGLT2 inhibitors, leading to the subsequent dedicated renal outcome trial. (25-27)

The CREDEENCE trial first assessed the kidney efficacy of canagliflozin in patients with type 2 diabetes and CKD defined as estimated glomerular filtration rate (eGFR) of 30 to <90 ml/min/1.73 m² and urine albumin-creatinine ratio (UACR) of >300 to 5000 mg/g. In this trial, canagliflozin lowered the risk of a composite of end-stage kidney disease, a doubling of the serum creatinine level, or death from renal or cardiovascular causes by 30% (HR, 0.70; 95% CI, 0.59 to 0.82; p<0.001) (9). Interestingly, these cardiovascular and kidney protective effect of SGLT2 inhibitor appeared independent of baseline hemoglobin A1c level. (33, 34) Indeed, the following DAPA-CKD trial expanded the kidney benefit of SGLT2 inhibitor to patients with CKD with and without type 2 diabetes. (11) More recently, the EMPA-KIDNEY trial further broadened the kidney protective effect of SGLT2 inhibitors to patients with lower eGFR and albuminuria. (12) Based on these findings, current guidelines recommend SGLT2 inhibitors to patients with and without diabetes at various levels of kidney and cardiovascular risk. (35-37)
### Table 1. Summary of cardiovascular and kidney outcome trials with SGLT2 inhibitors

<table>
<thead>
<tr>
<th>Study (reference)</th>
<th>Population</th>
<th>Intervention</th>
<th>Primary composite outcome</th>
<th>Hazard ratio (95% CI)</th>
</tr>
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<tbody>
<tr>
<td><strong>ASCVD trials</strong></td>
<td></td>
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<tr>
<td>EMPA-REGOutcome (24)</td>
<td>T2D and ASCVD</td>
<td>Empagliflozin</td>
<td>Nonfatal MI, nonfatal stroke, CV death</td>
<td>0.86 (0.74, 0.99)</td>
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<tr>
<td>CANVAS Program (8)</td>
<td>T2D and high CV risks</td>
<td>Canagliflozin</td>
<td>Nonfatal MI, nonfatal stroke, CV death</td>
<td>0.86 (0.75, 0.97)</td>
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<tr>
<td>DECLARE-TIMI 58 (23)</td>
<td>T2D and high CV risks</td>
<td>Dapagliflozin</td>
<td>Nonfatal MI, nonfatal stroke, CV death</td>
<td>0.93 (0.84, 1.03)</td>
</tr>
<tr>
<td>VERTIS-CV (22)</td>
<td>T2D and ASCVD</td>
<td>Ertugliflozin</td>
<td>Nonfatal MI, nonfatal stroke, CV death</td>
<td>0.97 (0.85, 1.11)</td>
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<td><strong>Heart failure trials</strong></td>
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<tr>
<td>DAPA-HF (13)</td>
<td>HF and reduced ejection fraction</td>
<td>Dapagliflozin</td>
<td>Worsening HF, CV death</td>
<td>0.74 (0.65, 0.85)</td>
</tr>
<tr>
<td>EMPEROR-Reduced (28)</td>
<td>HF and reduced ejection fraction</td>
<td>Empagliflozin</td>
<td>Hospitalization for HF, CV death</td>
<td>0.75 (0.65, 0.86)</td>
</tr>
<tr>
<td>EMPEROR-Preserved (29)</td>
<td>HF and preserved ejection fraction</td>
<td>Empagliflozin</td>
<td>Hospitalization for HF, CV death</td>
<td>0.79 (0.69, 0.90)</td>
</tr>
<tr>
<td>SOLOIST-WHF (30)</td>
<td>T2D and recent HF hospitalization</td>
<td>Sotagliflozin</td>
<td>Worsening HF, CV death</td>
<td>0.67 (0.52, 0.85)</td>
</tr>
<tr>
<td>SCORED (31)</td>
<td>T2D and CKD</td>
<td>Sotagliflozin</td>
<td>Worsening HF, CV death</td>
<td>0.74 (0.63, 0.88)</td>
</tr>
<tr>
<td>DELIVER (32)</td>
<td>HF and mildly reduced or preserved ejection fraction</td>
<td>Dapagliflozin</td>
<td>Worsening HF, CV death</td>
<td>0.82 (0.73, 0.92)</td>
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<td><strong>Kidney trials</strong></td>
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<tr>
<td>CREDENCE (9)</td>
<td>CKD and T2D</td>
<td>Canagliflozin</td>
<td>Doubling of sCr, ESKD, renal or CV death</td>
<td>0.70 (0.59, 0.82)</td>
</tr>
<tr>
<td>DAPA-CKD (11)</td>
<td>CKD with and without T2D</td>
<td>Dapagliflozin</td>
<td>≥50% eGFR decline, ESKD, renal or CV death</td>
<td>0.61 (0.51, 0.72)</td>
</tr>
<tr>
<td>EMPA-KIDNEY (12)</td>
<td>CKD with and without T2D</td>
<td>Empagliflozin</td>
<td>≥40% eGFR decline, ESKD, renal or CV death</td>
<td>0.72 (0.64, 0.82)</td>
</tr>
</tbody>
</table>

1. Dual SGLT1 and 2 inhibitor, 2 Defined as eGFR 30-90 ml/min/1.73m² and UACR 300-5000 mg/g, 4 Defined as eGFR 25-75 ml/min/1.73m² and UACR 200-5000 mg/g.

ASCVD, atherosclerotic cardiovascular disease; CI, confidence interval; CKD, chronic kidney disease; CV, cardiovascular; eGFR, estimated glomerular filtration rate; ESKD, end-stage kidney disease; HF, heart failure; MI, myocardial infarction; T2D, type 2 diabetes; sCr, serum creatinine; UACR, urinary albumin-to-creatinine ratio.
Mechanisms of kidney protection with SGLT2 inhibitors

Although kidney benefit of the SGLT2 inhibitors has been proven, the underlying mechanism has not been fully elucidated yet. Currently postulated mechanisms are roughly categorized into four domains: 1) hemodynamic changes, 2) improvement in metabolic parameters, 3) attenuation in inflammation, and 4) effects on hematological parameters (Figure 1).

Figure 1. Postulated mechanisms of kidney protection with SGLT2 inhibitors

Effects on hemodynamics

SGLT2 is implicated in the increased blood perfusion pressure within renal glomeruli in patients with diabetes. In a hyperglycemic situation, increased glucose and sodium reabsorption via SGLT1 and SGLT2 in the proximal tubule leads to decreased sodium and chloride concentration to the distal tubule and macula densa. Then, the juxtaglomerular apparatus at macula densa missense the drop in sodium concentration as a sign of volume depletion, activating tubular glomerular feedback (TGF). TGF causes dilation of the afferent arteriole and contraction of the efferent arteriole, followed by increased glomerular filtration pressure. Intra-glomerular hypertension is one of the critical pathways in kidney disease progression both in diabetic and non-diabetic CKD. (38-41)

Studies have shown that SGLT2 inhibition restored TGF and reduced intra-glomerular pressure in both rodents and patients with diabetes, although there might be some mechanistical difference between patients with type 1 and 2 diabetes. (42-44) Clinically, reduction in intra-glomerular pressure by SGLT2 inhibitors is observed as an acute and
reversible dip in eGFR. In the first week after initiation of the drug, eGFR decreased approximately 4 to 6 mL/min/1.73m² regardless of baseline kidney function. (45-47) This acute dip in eGFR was also seen in patients without diabetes and was associated with a modest reduction in albuminuria, an established surrogate endpoint for kidney disease progression. (48)

SGLT2 inhibitors also affect systemic hemodynamics, lowering systolic and diastolic blood pressure by approximately 4 and 2 mmHg, respectively. (49, 50) The mechanism for the reduction of blood pressure seems to be multifactorial. Previously, the natriuretic/osmotic-induced diuresis effect was thought to contribute to reductions in plasma volume and blood pressure. (51, 52) However, a recent randomized study with standardized salt intake showed that dapagliflozin did not increase urine volume and urinary sodium excretion, suggesting compensatory activation in the renin-angiotensin-aldosterone system and antidiuretic hormone. (53, 54) Apart from the change in plasma volume, several studies reported that reduction in blood pressure is attributed to the improvement of arterial stiffness and decreased vascular tone. (55, 56)

**Effects on metabolic parameters**

Constant urinary glucose loss with SGLT2 inhibition contributes to a reduction in body fat and weight loss of approximately 1-3 kg. (50, 57) Similarly, SGLT2 inhibitors reduced hemoglobin A1c by approximately 0.6% in patients with type 2 diabetes and preserved kidney function although the effect became smaller in patients with reduced kidney function. (50, 57)

Continuous glucosuria also promotes metabolic alterations toward the fasting state. The metabolic shift is characterized by the increase in glucogenesis, lipolysis, and ketogenesis, partly due to the increased glucagon secretion. (58, 59) Increased free fatty acid and ketone bodies may serve as additional efficient energy sources to the kidney and heart, attenuating hypoxia and restoring mitochondrial function. (59-61) Some studies also reported that increased ketone bodies by SGLT2 inhibitors were associated with its anti-inflammation properties, which are discussed in the next section. (59, 62)

**Anti-inflammatory effect**

Chronic inflammation is involved in the pathogenesis and progression of kidney disease in patients with diabetic and non-diabetic CKD. (63-65) In experimental studies, numerous stimuli characteristic of diabetes induce the production of proinflammatory mediators in the kidney, such as tumor necrosis factor (TNF)-α, Interleukin (IL)-1 and -6, nuclear factor kappa B (NF-κB) and monocyte chemoattractant protein-1 (MCP-1). (63, 66) In CKD patients with and without diabetes, circulating inflammatory markers of TNF receptor
(TNFR)-1 and -2 were robustly associated with kidney disease progression. (67, 68) In addition, urinary proteins, including albumin, were also reported to trigger inflammation and cause kidney injury. (69-71) These data suggest that chronic inflammation in patients with diabetes or CKD can be a potential therapeutic target. (65, 72)

SGLT2 inhibitors have been reported to ameliorate inflammation. In animal models of diabetes, SGLT2 inhibitors decreased kidney mRNA expression of various inflammatory cytokines, including NF-κB, MCP-1, and IL-6, and macrophage infiltration. (73, 74) In addition, SGLT2 inhibition also reduced markers of kidney injury and fibrosis, such as kidney injury molecule (KIM)-1, fibronectin, and type IV collagen. (75, 76) In the post-hoc analysis of the CANVAS trial, canagliflozin, compared to placebo, reduced plasma concentration of TNFR-1, TNFR-2 and KIM-1 in patients with type 2 diabetes at high risk of cardiovascular disease. (77) Moreover, reductions in TNFR-1 and -2 were independently associated with a lower risk of subsequent kidney events, suggesting the clinical relevance of the anti-inflammatory property of SGLT2 inhibitors. (77) Similar effects of empagliflozin on circulating TNFR-1 and KIM-1 were recently confirmed in patients with heart failure. (78)

**Effects on hematological parameters**

In clinical trials conducted in patients with type 2 diabetes and with heart failure, SGLT2 inhibitors, compared to placebo, modestly increased hematocrit and hemoglobin by 1.5-4.0 % and 4.5-8.0 g/L. (79-81) Increased red blood cells may augment oxygen delivery to kidney and heart and contribute to the clinical benefit of SGLT2 inhibitors. (82) Mediation analysis of cardiovascular-renal outcome trials highlighted increases in hematocrit and hemoglobin as the most important mediators of SGLT2 inhibitor’s salutary effects. (83-85)

The increase in hematocrit and hemoglobin with SGLT2 inhibitors could be partially attributed to their diuretic effect. However, some evidence suggests that SGLT2 inhibitors increase the production of erythrocytes in the bone marrow. First, after initiation of SGLT2 inhibitors, a transient increase in erythropoietin (EPO) concentration, a hormone that promotes the differentiation of erythroblasts into red blood cells, and reticulocyte count, an indicator of erythropoiesis activity, have been observed. (86-88) It is hypothesized that SGLT2 inhibitors increase EPO via its effects on oxygen consumption in the kidney and on non-hypoxia related cellular signals in kidney and liver. (89) Second, in patients with heart failure or type 2 diabetes, SGLT2 inhibitors reduced circulating transferrin saturation (TSAT), ferritin, and hepcidin levels. (88, 90, 91) These effects on iron parameters suggest that SGLT2 inhibitors might improve iron homeostasis and promote hematopoiesis, although data in patients with CKD are sparse.
AIMS AND OUTLINE OF THIS THESIS

As described above, the mechanism by which SGLT2 inhibitors confer cardiovascular and kidney protection is multifactorial and incompletely understood. Although we categorized postulated mechanisms into four domains (Figure 1), the extent to which each effect contributes to the clinical benefit of SGLT2 inhibitors may vary by patients and underlying disease, given that patients reacted differently to SGLT2 inhibitors. (92, 93) Thus, elucidating the working mechanism of SGLT2 inhibition may help clinicians select patients more likely to benefit from the drug.

This thesis aimed to assess the effects of SGLT2 inhibitors on hematopoiesis (Part I) and inflammation (Part II). We were also object to provide new insights into the pathophysiology of cardiovascular and kidney disease. We addressed these goals using data and biosamples collected from clinical trial participants with type 2 diabetes and/or CKD.

Part I: SGLT 2 inhibition and hematopoiesis

Chapter 2 is a secondary analysis of the DAPA-CKD trial. In this study, we investigated the beneficial effect of dapagliflozin on anemia: hemoglobin, hematocrit, and incident/prevention of anemia, in patients with CKD with and without diabetes. We also studied the association between anemia and cardiovascular and kidney events. Anti-erythropoietin receptor antibodies have been recently reported as a novel biomarker associated with kidney events and anemia in patients with diabetes. (94, 95) However, these studies are limited for its small population with a single ethnicity (94) and its case-control study design (95). In Chapter 3, we assessed the association of anti-erythropoietin receptor antibodies with cardiovascular and kidney events and mortality in the CREDEENCE trial. We also tested the possible modification of the canagliflozin’s effect on anemia by anti-erythropoietin receptor antibodies.

To get more mechanistic insight into the SGLT2 inhibitors’ effect on anemia, we assessed the effect of SGLT2 inhibitors on markers related to iron metabolism and hematopoiesis in chapters 4 and 5. In Chapter 4, we evaluated the effect of canagliflozin on iron markers in patients with type 2 diabetes and CKD using serum samples at baseline and year 1 from the CREDEENCE trial. We also assessed the associations of iron deficiency with the efficacy of canagliflozin on cardiovascular and kidney disease and anemia. Chapter 5 evaluated the shorter effect of 24 weeks of dapagliflozin treatment on markers of erythropoiesis, iron metabolism and inflammation in patients with type 2 diabetes and CKD. We additionally described the correlations between iron metabolism and inflammation markers.
Part II: SGLT 2 inhibition and inflammation

Systemic inflammation plays a crucial role in the cardiovascular and kidney disease progression in patients with diabetes. (63, 64) Chapter 6, a secondary analysis of the CANVAS trial, demonstrated the association between circulating IL-6, a key inflammation modulator, and cardiovascular and kidney events in patients with type 2 diabetes at high cardiovascular risk. We further evaluated the effect of canagliflozin on IL-6 over time.

SGLT2 inhibitors reduce albuminuria, probably via reduced glomerular filtration pressure. (48, 96) Urinary albumin is reported to cause intrarenal inflammation and consequent kidney injury. (70, 97) In Chapter 7, we assessed how much the reduction in albuminuria and kidney inflammation by canagliflozin contributes to decrease in kidney damage. In this post hoc analysis of the CANVAS trial, urinary MCP-1 and KIM-1 were used as proxies for inflammation and kidney damage, respectively.

Patients with type 2 diabetes often suffer from concomitant Non-alcoholic fatty liver (NAFLD). (98) NAFLD is characterized by lipid accumulation within hepatocytes and varying extent of liver inflammation, which eventually leads to liver fibrosis and cirrhosis. (98) Since metabolic dysregulation such as hyperlipidemia and insulin resistance play a vital role in the pathogenesis of NAFLD (98), we evaluated the effect of canagliflozin on three markers of liver steatosis and fibrosis: hepatic steatosis index, NAFLD fibrosis score, and fibrosis-4 index, in Chapter 8.

In the last Chapter 9, we summarized the results of this thesis and discussed the research questions to be addressed in future studies.
REFERENCES


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General introduction and outline of this thesis


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Part I

SGLT 2 INHIBITION AND HEMATOPOIESIS