Effects of Sodium Glucose Cotransporter 2 Inhibitors on Left Ventricular Diastolic Function in a Diabetic Rabbit Model

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ABSTRACT

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Sodium glucose cotransporter 2 (SGLT2) inhibitors represent a new class of diabetic medications. A recent trial reported that SGLT2 inhibitors reduce mortality and heart failure hospitalization in diabetic patients. However, mechanisms of the cardiovascular protective effects of SGLT2 inhibitors are not known. Considering that approximately 80% of diabetic patients have heart failure with preserved ejection fraction (EF), SGTL2 inhibitors might have beneficial effects on the diastolic function of the left ventricle (LV). In this study, we investigated whether targeting SGLT2 improves left ventricular diastolic function in a diabetic rabbit model. In addition, we sought to determine

if SGLT2 inhibitors reduce cardiac fibrosis by reducing profibrotic signaling protein expression.

Thirty male New Zealand rabbits were classified into 3 groups. Group 1 consisted of normal rabbits. Group 2 consisted of diabetic rabbits, while group 3 consisted of diabetic rabbits treated with SGLT2 inhibitor, dapagliflozin, for 8 weeks. At 8 weeks of follow-up, conventional echocardiography with speckle tracking analysis was performed. At the end of the experiments, all rabbits were sacrificed for immunohistochemistry staining. At the 8 week follow-up, blood fasting glucose was significantly higher in group 2 compared to groups 1 and 3. LV systolic parameters (LV ejection fraction and LV fractional shortening) and cardiac chamber sizes did not differ among the three groups. However, LV diastolic function, reflected by septal and lateral e' velocity, was significantly lower in group 2 compared to groups 1 and 3. LV global longitudinal strain was significantly lower and LV global circumferential strain was significantly higher in group 2 compared to groups 1 and 3. Group 2 revealed significantly increased cardiac fibrosis, which was accompanied by increased expression of the profibrotic proteins serum and glucocorticoid-regulated kinase 1 (SGK1) and epithelial sodium channel (ENaC). However, in group 3, fibrosis and profibrotic protein expression was significantly lower than in group 2.

Dapagliflozin attenuated LV diastolic dysfunction and subclinical LV systolic dysfunction, reflected by e' velocity and LV global longitudinal strain respectively. These findings suggest that dapagliflozin reduces the development

of cardiac fibrosis and expression of the profibrotic proteins, SGK1 and ENaC, in a diabetic rabbit model.

Keywords: diabetes mellitus, SGLT 2 inhibitor, left ventricular diastolic function

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1. INTRODUCTION

Patients with diabetes mellitus are world-widely increasing and are at high risk for development of heart failure and cardiovascular mortality. Previous studies have reported that 43% to 75% of diabetic patients have abnormal left ventricular (LV) diastolic function.[1-4] Population-based studies suggest that myocardial damage in diabetic patients affects diastolic function before the development of systolic dysfunction and heart failure with preserved ejection fraction (EF), which is prevalent in diabetic populations.[3,5-7] Although

the pathogenesis of LV diastolic dysfunction in diabetic subjects is not clearly understood, interstitial fibrosis and inflammation with oxidative stress are considered to cause LV diastolic dysfunction. [6,8,9]

Recent studies have reported that serum and glucocorticoid-regulated kinase 1 (SGK1) is highly expressed in the hearts of diabetic patients.[10,11] This protein is an emerging mediator of cardiac fibrosis that stimulates multiple downstream pathways. Epithelial sodium channel (ENaC) proteins, responsible for promoting fibrosis, are activated by SGK1 protein.[12] In a previous study using a murine model of transthoracic aortic constriction, SGK1 was rapidly activated and induced adverse ventricular remodeling, fibrosis, and increased size of cardiomyocytes, suggesting that SGK1 is a key mediator of cardiac remodeling.[11,13,14]

Sodium glucose cotransporter 2 (SGLT2) inhibitors represent a new class of drugs that lower blood glucose by inhibiting glucose reabsorption in the proximal renal tubule. SGLT2 inhibitors lower blood glucose via an insulin-independent mechanism and improve insulin sensitivity in animals and humans with diabetes mellitus.[15,16] In recent clinical trials, SGLT2 inhibitors improved blood glucose level and reduced rates of adverse cardiovascular outcomes compared to placebo in type 2 diabetic patients.[17] The difference between SGLT2 inhibitor and placebo was reflected by a significant reduction in cardiovascular death and readmission due to heart failure in patients receiving

SGLT2 inhibitors. Recent clinical studies and animal experiments have suggested various beneficial effects of SGLT2 inhibitors such as weight loss, increased fat oxidation, and hemodynamic unloading from reduced arterial stiffness. However, the underlying mechanisms remain to be elucidated.

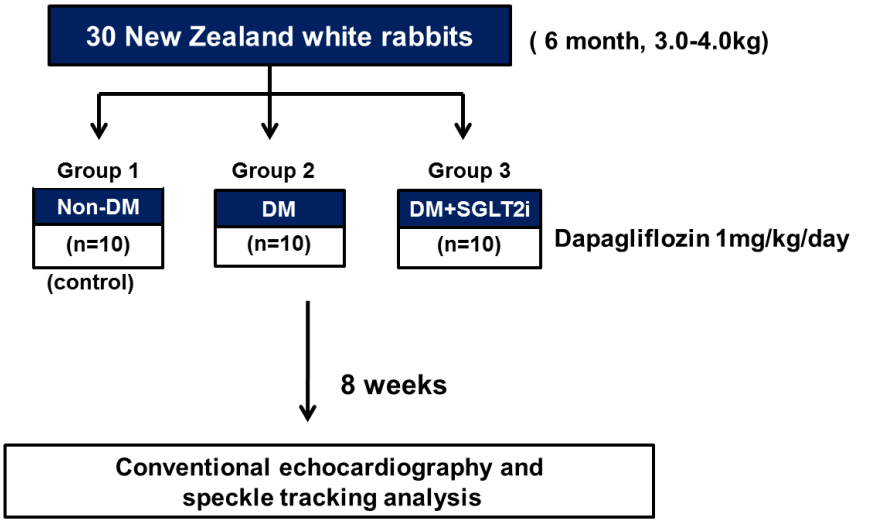
Considering that many diabetic patients develop diastolic dysfunction and heart failure with preserved EF, SGLT2 inhibitors might have a beneficial effect on diastolic function within the diabetic population. Dapagliflozin is an orally active, highly selective SGLT2 inhibitor.[18] In the present study, we hypothesized that dapagliflozin would have beneficial effects on left ventriclular diastolic function by reducing myocardial fibrosis and profibrotic signaling protein expression.

II. MATERIALS AND METHODS

1. Experimental animal model

A total of 30 male New Zealand white rabbits (6 months old, 3.0–4.0 kg) were used (Figure 1). Rabbits were grouped into three groups : healthy rabbits as controls (group 1, n=10), diabetic rabbits which received no oral hypoglycemic drug, except intermittent subcutaneous insulin (group 2, n=10), and diabetic rabbits which were treated with dapagliflozin (group 3, n=10). Diabetic condition was induced by intravenous treatment with alloxan at a dose of 150 mg/kg. Rabbits exhibiting blood glucose above 200 mg/dl were

diagnosed to be diabetic. The experimental protocol is shown in figure 1. All rabbits were fed with high cholesterol diet (2% cholesterol) for 8 weeks. For group 3, we mixed dapagliflozin in rabbit chow at a concentration calculated to deliver 1 mg/kg/day.



DM, diabetes mellitus; SGLT 2i, Sodium glucose cotransporter 2 inhibitors

Figure 1. Summary of the study protocol.

2. Blood chemistry

Plasma levels of glucose, total cholesterol, and triglycerides (TG) were measured in blood samples at baseline and at 8 weeks of follow-up. Blood samples were drawn from the ear veins of the rabbits.

3. Conventional echocardiography

Echocardiography was done after sedation with intra-muscular (IM) injection of 0.1 ml/kg Zoletil (Virbac, Australia) in prone. All images were obtained using a commercial ultrasound machine (Vivid 7 Dimension; GE Vingmed Ultrasound AS, Horten, Norway) with an S10 probe (2.5 megahertz). Images were acquired from apical three-chamber, four-chamber, and two-chamber views; and short-axis views of the mitral valves, papillary muscles, and apex.[19] The LV end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), septal and LV posterior wall thicknesses, and left atrial anteroposterior diameter (LAD) were measured from standard planes. The LV EF was calculated using the Teicholz formula.[20]

Pulsed Doppler echocardiography of the transmitral flow was performed. The sample volume was positioned at the level of the mitral tips in the apical four-chamber view. From the transmitral recording, the peak early (E) and late diastolic filling velocities were obtained. An apical four-chamber view was also used to obtain Doppler tissue imaging of the mitral annulus. Sample volumes were placed on the septal and lateral sides of the mitral annulus. Values

shown for systolic (S'), peak early (e'), and late diastolic annular velocities were obtained.

4. Speckle tracking strain analysis

Three consecutive cardiac cycles were recorded with a frame rate of 50–70 frames per second for analysis. Blinded off-line analyses of the short-axis views and apical long-axis views were performed using EchoPAC PC version 6.1.1 (GE Vingmed Ultrasound AS, Horten, Norway). LV global longitudinal strain was analyzed from the apical 4C view, while LV global circumferential strain and global radial strain were analyzed from the short-axis view at the papillary muscle level. [21]

5. Quantification of interstitial fibrosis and immunohistochemistry

Heart tissue was removed from rabbits and immediately frozen. From the level below the papillary muscels, 1mm-thick coronal slices of the heart were obtained. After fixing heart tissues in paraformaldehyde, and embedding in paraffin, heart tissues were all sectioned at 4 μm thickenss. Picrosirius Red and Masson's Trichrome were used to stain for collagens.

The level of oxidative/nitrosative damage to myocardial proteins was assessed by 3-nitrotyrosine (3-NTY) residue analysis using an immunofluorescence technique.[22] LV tissue sections were also evaluated by immunofluorescence to detect SGK1 (1:100, #32374, Abcam) and ENaC (1:200, #77385, Abcam) expression. Average gray-scale intensity values were quantified within fixed region of interest rectangles.[12]

6. Statistical analysis

Statistical analyses were performed using SPSS v23.0 (SPSS Inc., Chicago, IL, USA). All data are expressed as mean ± SD. Continuous variables were compared using t-test (comparison of two groups) and ANOVA (comparison of three groups). If data distributions were skewed, a non-parametric test was used for comparison. A p-value <0.05 was considered statistically significant.

III. Results

1. Metabolic parameters

Baseline characteristics did not differ among the three groups. At 8 weeks of follow-up, body weights did not differ among groups 1, 2, and 3. Plasma cholesterol and triglycerides were similarly elevated in groups 2 and 3 (Table 1). Fasting blood glucose for group 2 was 425 ± 58 mg/dL, which was significantly higher than that of groups 1 and 3. Fasting blood glucose for group 3 was higher than that of group 1, although it was not significantly different.

Table 1. Parameters at baseline and at the 8 week follow-up.

	Group 1 (Control)	Group 2 (DM)	Group 3 (DM+SGLT2i)
Baseline			
Heart rate, bpm	200 ± 30	223 ± 51	226 ± 51
Body weight, kg	3.07 ± 0.07	3.05 ± 0.16	3.02 ± 0.17
Glucose (mg/dL)	128 ± 5	125 ± 6	120 ± 7
Total cholesterol (mg/dL)	76 ± 5	70 ± 4	72 ± 5
At 8 week follow-up			
Heart rate, bpm	200 ± 30	223 ± 51	226 ± 51
Body weight, kg	3.57 ± 0.07	3.27 ± 0.16	3.82 ± 0.17
Glucose (mg/dL)	130 ± 5†	425 ± 58*‡	212 ± 44†
Total Cholesterol (mg/dL)	76 ± 5†‡	145 ± 3*	148 ± 6*
Triglycerides (mg/dL)	27±2	78±15*	69±8*

*p<0.05 compared to Group 1,† p<0.05 compared to Group 2, ‡ p<0.05 compared to Group 3

DM, diabetes mellitus; SGLT 2i, Sodium glucose cotransporter 2 inhibitors

2. Echocardiographic parameters

There was no significant difference in systolic function parameters, ejection fraction (EF), or fractional shortening (FS) among the three groups. LV wall thickness and chamber sizes of LA and LV also showed no significant differences among the three groups. In group 2, septal e' and lateral e' velocities were significantly lower than those of groups 1 and 3 (Table 2). The E/septal e' ratio was significantly higher in group 2 compared to groups 1 and 3.

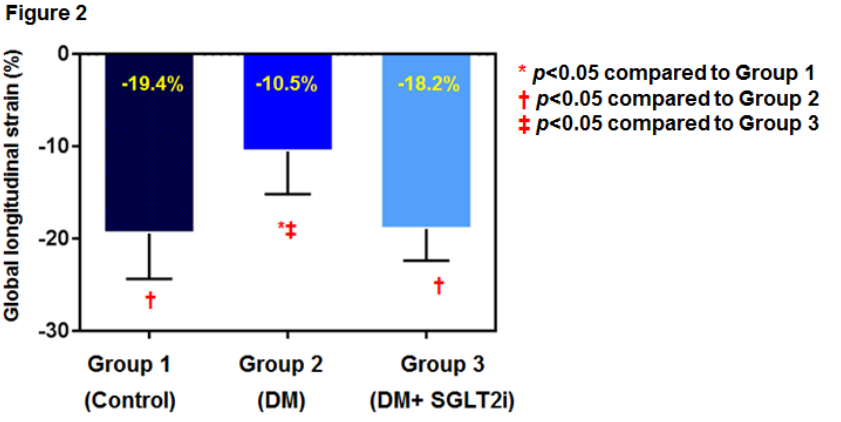
Table 2. Echocardiographic parameters at the 8 week follow-up.

	Group 1 (Control)	Group 2 (DM)	Group 3 (DM+SGLT2i)
LAD, mm	8.8 ± 0.9	9.9 ± 1.2	9.4 ± 0.9
LVEDD, mm	14.4 ± 2.3	14.6 ± 2.3	14.3 ± 2.6
LVESD, mm	9.2 ± 1.9	9.3 ± 1.4	9.4 ± 1.0
IVSd, mm	3.6 ± 0.7	3.5 ± 0.8	3.6 ± 0.8
PWd, mm	3.2 ± 0.4	3.5 ± 0.8	3.7 ± 0.5
LVEF (%)	69 ± 5	72 ± 2	73 ± 5
LVFS (%)	36 ± 5	38 ± 2	39 ± 4
E, cm/s	61 ± 12	69 ± 33	67 ± 16
A, cm/s	34 ± 10	59 ± 23	45 ± 18
Septal e', cm/s	9.6 ± 1.2†	5.6 ± 2.3*‡	9.6 ± 2.0†
Lateral e', cm/s	13.2 ± 3.2†	8.2 ± 6.1*‡	13.1 ± 1.6†
E/septal e'	6.6 ± 1.8†	12.9 ± 5.1*‡	7.2 ± 1.8†

*p<0.05 compared to Group 1,† p<0.05 compared to Group 2, ‡ p<0.05 compared to Group 3

LAD, left atrial dimension; LVEDD, left ventricular end diastolic dimension; LVESD, left ventricular end systolic dimension; IVSD, interventricular septal end diastole thickness; PWd, posterior wall thickness, end diastole; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; DM, diabetes mellitus; SGLT 2i, Sodium glucose cotransporter 2 inhibitors

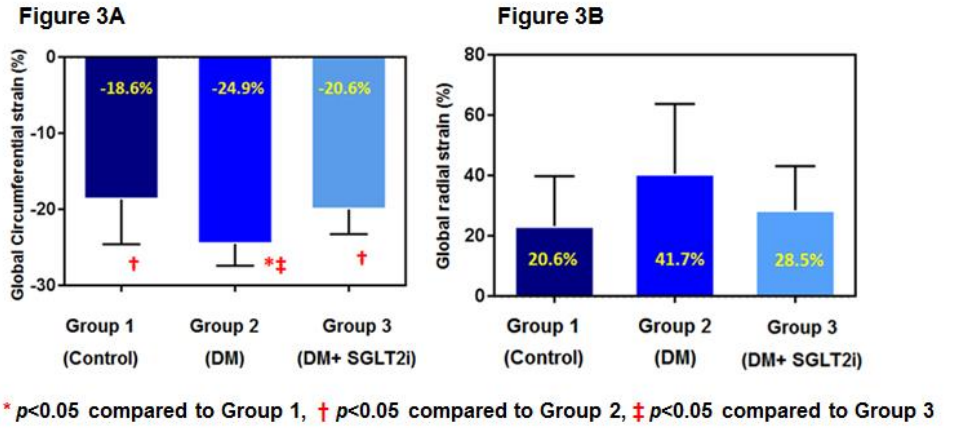
LV global longitudinal strain was significantly reduced in group 2 compared to groups 1 and 3 (absolute value: 10.5% (group 2) vs. 19.4% (group 1) and 18.2% (group 3), $p<0.05$) (Figure 2).



DM, diabetes mellitus; SGLT2i, sodium glucose cotransporter 2 inhibitors

Figure 2. Comparison of left ventricular global longitudinal strain at the 8 weeks of follow-up among the three groups.

LV global circumferential strain was significantly enhanced in group 2 (in a compensatory manner) compared to groups 1 and 3 (Figure 3A). The LV global radial strain did not show a significant difference among the three groups (Figure 3B).



* $p<0.05$ compared to Group 1, † $p<0.05$ compared to Group 2, ‡ $p<0.05$ compared to Group 3

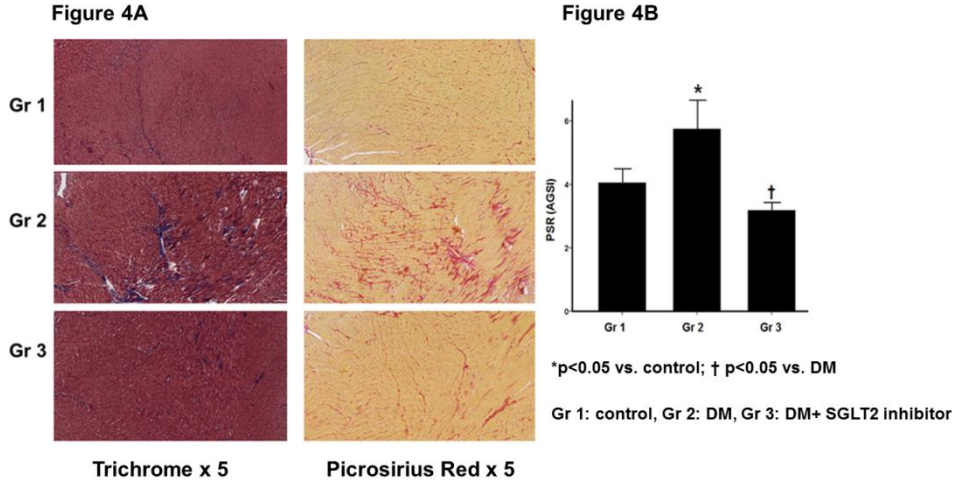
DM, diabetes mellitus; SGLT2i, sodium glucose cotransporter 2 inhibitors

Figure 3. Comparisons of global circumferential strain and global radial strain at 8 weeks of follow-up among the three groups.

3A: LV global circumferential strain was significantly enhanced in group compared to groups 1 and 3. **3B:** LV global radial strain did not show a significant difference among the three groups.

3. Myocardial fibrosis and immunohistochemistry

Group 2 exhibited significantly increased myocardial fibrosis by picrosirius red staining compared to groups 1 and 3. A qualitative assessment by Masson's Trichrome stain also revealed prominent fibrosis in the myocardium of group 2 (Figure 4). Myocardial fibrosis by picrosirius red staining did not differ significantly between groups 1 and 3.

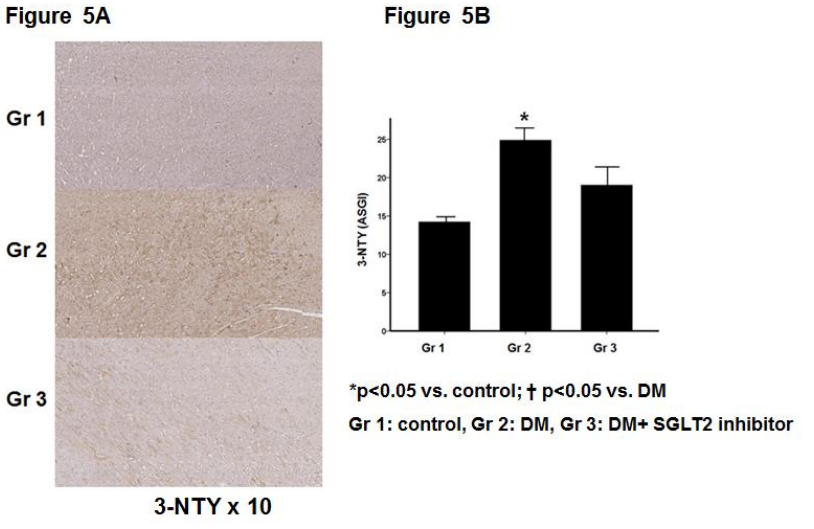


DM, diabetes mellitus; SGLT2i, sodium glucose cotransporter 2 inhibitors; AGSI, average grey scale intensity

Figure 4. Masson's trichrome and picrosirius red staining for determination of interstitial fibrosis (5x magnification).

4A: Myocardium of group 2 revealed greater trichrome and picroirius red staining than those of group 1 and 3, indicative of increased collagen deposition. **4B:** Average grey intensity for picrosirus red staining among three groups. Group 2 exhibited significantly increased myocardial fibrosis by picrosirius red staining compared to groups 1 and 3

Myocardial oxidative stress assessed by immunostaining for 3- NTY was significantly higher in group 2 compared to group 1. Myocardial oxidative stress tended to increase in group 3 but without statistical significance when compared to group 1 (p=0.09) and group 2 (p=0.12). (Figure 5).

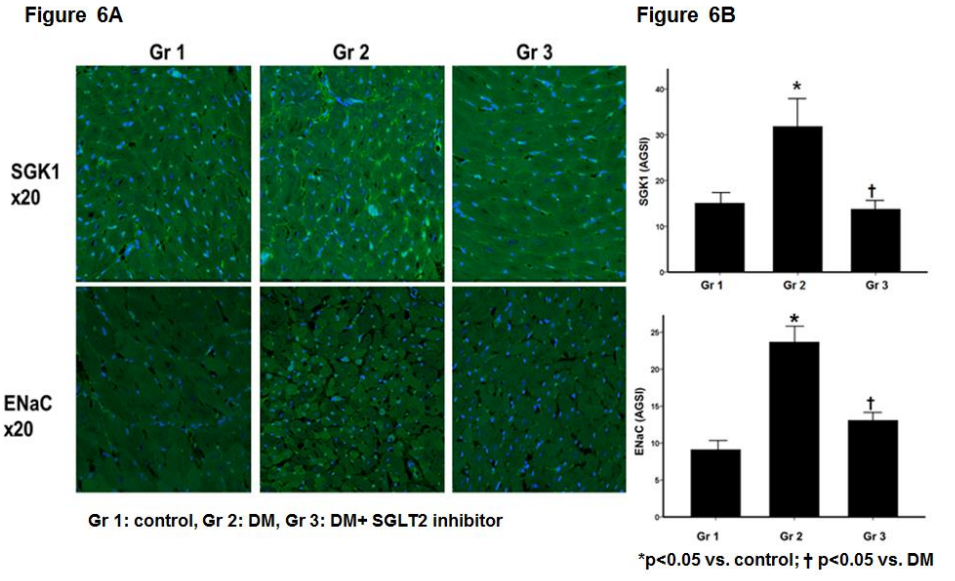


DM, diabetes mellitus; SGLT2, sodium glucose cotransporter 2; AGSI, average grey scale intensity; NTY, nitrotyrosine

Figure 5. 3-Nitrotyrosine immunostaining for determination of oxidative stress (10x magnification).

5A: Myocardium of group 2 revealed greater 3-NTY staining than those of group 1 and 3, indicative of increased accumulation of nitro tyrosinolated protein damage. 5**B:**Average grey intensity for 3-NTY staining among three groups.

Myocardial SGK1 and ENaC proteins were significantly increased in group 2 compared to groups 1 and 3 when assessed by immunofluorescence techniques (Figure 6). They were not increased in group 3 when compared to group 1.



SGK1, serum and glucocorticoid- regulated kinase 1; ENaC, epithelial sodium channels; AGSI, average gray scale intensity; Gr, group; DM, diabetes mellitus; SGLT 2, Sodium glucose cotransporter 2

Figure 6. Eight weeks of dapagliflozin reduces expression of the profibrotic proteins, SGK1 and ENaC (20x magnification).

6A: Myocardium of group 2 revealed greater SGK1 and ENaC staining than those of group 1 and 3. **6B:** Average grey intensity for SGK1 and ENaC staining among three groups. Myocardial SGK1 and ENaC proteins were significantly increased in group 2 compared to groups 1 and 3.

IV. Discussion

Three important results from this study can be summarized as follows:

1) Dapagliflozin attenuated LV diastolic dysfunction in diabetic rabbits, reflected by improved LV filling pressure (E/e') and by longitudinal motion of

LV, assessed by septal e' velocity and lateral e' velocity.

2) Dapagliflozin also attenuated subclinical LV systolic dysfunction, reflected by preserved LV global longitudinal strain in diabetic rabbits.

3) The beneficial effect on diastolic dysfunction by dapagliflozin was related with attenuation of cardiac fibrosis and profibrotic protein expression (SGK1 and ENaC), suggesting a possible mechanism.

Diabetes mellitus is a major risk factor for cardiovascular disease.[5,7] It is well known from previous large-scale clinical trials that the presence of both diabetes mellitus and cardiovascular disease increases the risk of mortality.[23] Diabetic cardiomyopathy was first described in the 1970s.[24] This condition includes a broad range of diabetes-associated structural and functional irregularities of the myocardium.[6] It generally refers to myocardial dysfunction that is not directly caused by associated cardiovascular disease, such as coronary artery occlusive disease or hypertension. It is widely accepted that diabetes-associated functional and structural changes of the myocardium are augmented by co-existing cardiovascular diseases, which increase the possibility of progression to heart failure. The pathogenesis of diabetic cardiomyopathy has been a topic of active research for many decades. Many potential causes have been postulated to the underlying mechanism of diabetic cardiomyopathy, such as changes in myocardial structure, abnormal calcium signaling, and altered metabolism of myocardial cells. The pathophysiology also involves interstitial fibrosis and increased oxidative stress.

Regan et al. described a significantly increased collagen deposition around the intramural vessels and between the myocardial fibers of biopsies from familial diabetic patients in the 1970s.[25] Recent studies investigating collagen remodeling in the myocardium of diabetic patients showed that type III collagen (rather than type I or VI) was prominently increased in diabetic patients without clinically diagnosed hypertension or coronary artery occlusive disease.[9,26] Further studies revealed that diastolic dysfunction of the LV in diabetic patients without clinical evidence of cardiovascular disease correlated with type I pro-collagen.[27] These findings support the observation that myocardial fibrosis is an important structural change in diabetic cardiomyopathy.

Oxidative stress has been thought to play an important role in the development of diabetic cardiomyopathy. However, despite many studies, the ways in which reactive oxygen species contribute to the development of diabetic cardiomyopathy are not fully elucidated. Animal studies have shown that reactive oxygen species correlate with lipid overload and insulin resistance.[8,28,29] Boudina et al. suggested that impaired myocardial signaling promotes oxidative stress and causes mitochondrial uncoupling in a diabetic mouse model; the same study found that impaired insulin action caused mitochondrial dysfunction in the heart.[29] Reactive oxygen species interact with other molecules, such as nitric oxide, to form nitrotyrosine species. Nitrotyrosine species have been found to be significantly elevated in myocardial biopsies of diabetic patients.[30]

These structural changes of the myocardium in the diabetic heart lead to functional deterioration of the myocardium. Diastolic function is known to be impaired before the development of systolic dysfunction. Many investigators focused on detecting early signs of myocardial dysfunction, as an early diagnosis allows the use of therapeutic drugs to improve the patient's prognosis and broaden the understanding of the nature of the diabetic cardiomyopathy. In this study, we used both conventional echocardiographic parameters and speckle tracking analysis to investigate diastolic function in diabetic rabbits. Conventional echocardiography determines diastolic function by measuring mitral inflow velocity (E wave and A wave) and tissue Doppler (e' velocity of mitral annuli). The E/e' ratio is a widely accepted surrogate parameter for left ventricular diastolic pressure. Speckle tracking strain analysis measures deformation of the myocardium and is a novel technology that has made it possible to measure left ventricular diastolic function independent of loading conditions in an angle-independent manner.[31,32] LV global longitudinal strain reflects the longitudinal contraction of the myocardium, and its accuracy has been validated using tagged magnetic resonance imaging (MRI).[33] In the general population and in patients with heart failure, LV global longitudinal strain was shown to be a superior predictor of cardiac events and all-cause mortality compared to LV EF.[34]

SGLT2 inhibitors represent a new class of anti-diabetic agents. Inhibitors of the sodium glucose cotransporter 2 protein reduce rates of hyperglycemia in

diabetic patients by decreasing renal glucose reabsorption, which in turn increases urinary glucose excretion.[35] Recent clinical trial (EMPA-REG OUTCOME) results showed the cardio-protective effects of empagliflozin, an SGLT2 inhibitor. Among patients with type 2 diabetes at high risk for cardiovascular events, those receiving empagliflozin had a lower rate of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke than patients receiving a placebo.[17] Patients in the empagliflozin group also had a more significant reduction in hospitalization for heart failure than patients receiving the placebo. Interestingly, this occurred without dramatic improvements in glycemic, lipid, or blood pressure metrics. These findings suggest that the cardio-protective effects of empagliflozin are multifactorial. Because the cardio-protective effects of SGLT2 inhibitors have only recently been recognized, their underlying mechanisms remain unknown. We explored a pathway that could potentially contribute to the cardio-protective benefits of SGLT2 inhibitors. We hypothesized that treatment with dapagliflozin would attenuate LV diastolic dysfunction responsible of heart failure with preserved EF in a diabetic rabbit model. We sought to investigate possible underlying mechanisms related to diabetic cardiomyopathy, such as fibrosis and oxidative stress.

Our results showed that dapagliflozin attenuated diastolic dysfunction in diabetic rabbits. The dapagliflozin-treated group showed significantly higher septal and lateral e' velocities and improved LV filling pressure (reflected by

E/e') compared to the other groups. Conventional parameters of systolic function, such as EF and FS, did not differ among the three groups. This finding is consistent with previous clinical findings, as heart failure with preserved EF is more common in diabetic cardiomyopathy than is heart failure with reduced EF. LV global longitudinal strain was impaired in the diabetic rabbits, but dapagliflozin attenuated this impairment. LV global circumferential strain was significantly higher in the diabetic rabbits than in the normal and SGLT2 inhibitor-treated groups. This compensatory enhancement of global circumferential strain (to compensate for the decreased global longitudinal strain) has also been observed in patients with heart failure with preserved EF.

The results of the conventional and speckle tracking echocardiography procedures showed that dapagliflozin attenuated diastolic dysfunction and subclinical LV systolic dysfunction, as reflected by LV global longitudinal strain. This finding may offer clues to explain the cardio-protective effects observed in the previous EMPA-REG OUTOME trial. It is noteworthy that, in this study, cardiac fibrosis and profibrotic protein expression reduced with attenuation of diastolic dysfunction.

As mentioned above, interstitial fibrosis is one of the conditions that contribute to diastolic dysfunction and heart failure symptoms in diabetic patients. Emerging evidence supports a role for SGK1 and ENaC in promoting fibrosis in human and murine heart disease. Recent studies have shown that SGK1 is highly expressed in pathophysiological settings including obesity,

heart disease, and diabetes mellitus.[10] SGK1 regulates the expression of a number of ion channel proteins, including ENaC, which is known to be upregulated in tissues in the setting of obesity and diabetes mellitus.[11] Recent studies have demonstrated that rapidly activated SGK1 is associated with adverse ventricular remodeling (including fibrosis, increased myocardial cell size, and LV hypertrophy) in both a murine model of transthoracic aortic constriction and a diabetic mouse model.[13, 17] From our results, cardiac fibrosis and profibrotic protein (SGK1 and ENaC) expression were significantly increased in diabetic rabbits compared to the control and dapagliflozin-treated groups. Dapagliflozin significantly reduced cardiac fibrosis and profibrotic proteins by 8 weeks of treatment.

There was markedly increased oxidative stress in group 2 compared to group 1 ($p = 0.02$), reflected by increased 3-NTY immunofluorescence. Oxidative stress tended to increase in group 3, but without statistical significance, when compared to group1 ($p=0.09$) or group 2 ($p=0.12$). Increased 3-NTY staining reflects increased oxidative myocardial injury from hyperglycemia. It is interesting that, even though the mean fasting blood glucose level differed significantly between group 2 and group 3 (425 mg/dL vs. 212 mg/dL, $p<0.05$), oxidative myocardial injury by 3-NTY staining did not show significant differences between the two group 2 and group 3. This suggests that lowering glucose effect by SGLT 2 inhibitors might not contribute to the beneficial effects on oxidative injury of myocardium.

V. CONCLUSION

Dapagliflozin attenuated LV diastolic dysfunction, as reflected by septal e' velociy, lateral e' velocity, and E/e'. Dapagliflozin also had beneficial effects on subclinical LV systolic dysfunction, as reflected by LV global longitudinal strain. These findings were accompanied by reduced cardiac fibrosis and reduced expression of the profibrotic proteins SGK1 and ENaC in a diabetic rabbit model. Our results support other recent studies showing the cardiovascular benefits of SGLT2 inhibitors regarding reducing heart failure related hospitalization in diabetic patients. Our study highlights the potential cardiovascular benefits of using dapagliflozin in diabetic patients for the treatment of diastolic dysfunction and diabetic cardiomyopathy.

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ABSTRACT IN KOREAN

토끼 당뇨모델에서 Sodium Glucose Cotransporter 2 억제제가 좌심실 이완기능에 미치는 영향

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최근 새로운 개념의 경구 혈당강하제인 Sodium glucose cotransporter2 (SGLT2) 억제제가 개발되었다. SGLT2 억제제는 신세뇨관에서 포도당이 재흡수 되는 것을 억제하여 소변으로 포도당이 배출되는 기전을 가지는 약제이다. 당뇨병 환자에게 SGLT2 억제제 투약시의 효과를 연구한 임상 연구에서 SGLT2 억제제가 심장혈관 질환과 관련된 사망과 심부전으로 입원할 위험을 의미가 있게 감소시키는 효과가 보고되었다. SGLT2 억제제가 다른 당뇨병 약제와 다르게

심혈관계 질환에 긍정적인 영향을 미치는 기전에 대해서 여러 가설이 제시되었으나, 아직 정확한 기전은 연구가 되어 있지 않다.

당뇨병 환자들이 좌심실의 이완기능장애로 인한 심부전에 대부분 이환되어 있으며, SGLT2 억제제 투약이 심부전으로 인한 입원을 의미가 있게 감소시켰다는 사실에 근거하여, 본 연구에서는 SGLT2 억제제가 당뇨병 환자의 좌심실 이완기능 장애를 호전시키고, 좌심실 이완기능장애의 원인이 되는 심근 섬유화의 발생을 억제한다는 가설을 세웠다. 이의 기전으로 SGLT2 억제제가 심근 섬유화 및 섬유화 촉발 단백질의 발현이 억제함을 규명하고자 하였다. 30마리의 뉴질랜드산 수컷 토끼를 각각 10 마리씩 정상군, 당뇨병군, 당뇨병 + SGLT2 억제제 투약군으로 배정을 하였다. 당뇨병군과 당뇨병 + SLGT2 억제제 투약군에 배정된 토끼는 알록산을 투약하여 당뇨병를 유발하였다. 8주후 세 군의 심장초음파와 심근조직의 변형을 측정하는 strain 영상분석을 시행하였다. 이후 토끼의 심근의 조직학적 분석을 시행하였고, 심근 섬유화와 심근 섬유화를 촉발하는 것으로 최근에 연구가 되고 있는 serum- and glucocorticoid-regulated- kinase1 (SGK1) 와 Epithelial sodium channels (ENaC)의 발현을 세 군의 심장조직의 면역화학염색으로 비교하였다. 8주후 3군의 체중은 큰 차이가 없었고, 당뇨병군의 혈당이 유의미하게 높았다.

심장의 수축능력을 나타내는 지표들은 세 군간 큰

차이는 없었으나, 심장의 좌심실 이완기능을 나타내는 지표인 septal e' velocity와 lateral e' velocity는 당뇨병군에서 의미있게 떨어져 있었다. 이에 반해 당뇨병 + SGLT2 억제제 투약군은 좌심실 이완기능이 대조군과 큰 차이가 없었다. 또한 당뇨병군에서는 좌심실의 global longitudinal strain이 의미있게 감소되어 있고, 이를 보완하기 위하여 global circumferential strain이 의미가 있게 증가되어 있었다. 당뇨병 + SGLT2 억제제 투약군과 대조군에서는 global longitudinal strain이 큰 차이가 없었다. 심근 섬유화 정도는 당뇨병군에서 의미가 있게 증가하였으며, 심근 섬유화를 촉발하는 인자인 SGK1과 ENaC의 발현도 의미있게 증가하여 있었다. 이에 반해 당뇨병 + SGLT2 억제제 투약군에는 섬유화 정도도 대조군과 큰 차이가 없었고, SGK1과 ENaC의 발현도 증가하지 않았다.

이러한 결과를 바탕으로 SGLT2 억제제는 당뇨병 토끼 모델에서 심근의 섬유화 억제와 섬유화로 유발되는 좌심실의 이완기능 장애의 발현을 억제한다는 결론을 내릴 수 있다.

핵심되는 말 : 좌심실 이완기능, 당뇨, SGLT2 억제제