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Full Paper

Pretreatment with KGA-2727, a selective SGLT1 inhibitor, is protective against myocardial infarction-induced ventricular remodeling and heart failure in mice

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ABSTRACT

Recent studies demonstrated that sodium-glucose co-transporter 1 (SGLT1) is associated with human ischemic cardiomyopathy. However, whether SGLT1 blockade is effective against ischemic cardiomyopathy is still uncertain. We examined the effects of KGA-2727, a selective SGLT1 inhibitor, on myocardial infarction (MI)-induced ischemic cardiomyopathy.

To create MI, left anterior descending coronary artery (LAD) ligation with or without KGA-2727 administration was performed in C57BL/6J mice. Four weeks after the operation, all mice were investigated.

Left ventricular fractional shortening (LVFS) was reduced and KGA-2727 significantly improved it in LAD-ligated MI mice. The cardiomyocyte diameter, and ANP, BNP, β-MHC, and IL-18 gene expressions significantly increased in LAD-ligated mouse left ventricles compared with those of sham-operated mouse left ventricles, and KGA-2727 inhibited increases in them. Myocardial fibrosis and upregulation of CTGF and MMP-3 gene expressions in the left ventricle were increased in LAD-ligated mice compared with sham-operated mice, and KGA-2727 decreased them in the LAD-ligated left ventricles. SGLT1 protein expression level was significantly higher in LAD-ligated compared with sham-operated mouse ventricles regardless of KGA-2727 treatment.

These results suggest that KGA-2727 pretreatment protects against MI-induced left ventricular remodeling through SGLT1 blockade and that it may become a new pharmacological therapy for ischemia-induced cardiomyopathy.

1. Introduction

Myocardial infarction (MI) causes cardiac remodeling, leading to heart failure (HF).[1,2] Although there are interventional and pharmacological therapies to mitigate initial cardiac damage during acute MI, current clinical pharmacological therapies are not sufficient to minimize subsequent cardiac remodeling that leads to HF. Therefore, identifying new targets to improve MI-induced HF are of marked interest. Recently, the expression of a novel cardiac sodium-glucose co-transporter 1 (SGLT1) was described, and SGLT1 gene expression was found to be elevated in hypertrophic, ischemic, and diabetic cardiomyopathy in humans.[3,4] Moreover, increases in SGLT1 protein expression caused cardiac remodeling such as hypertrophy and increased interstitial fibrosis in mice.[5] We recently demonstrated that chronic pressure overload-induced cardiomyopathy was improved in SGLT1 knock out mice,[6] suggesting that SGLT1 blockade can improve hypertrophic cardiomyopathy. However, the roles of SGLT1 activation in the development of ischemic cardiomyopathy are still controversial. In fact, phlorizin,

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a SGLT1 blocker, was demonstrated to impair left ventricular contractile recovery after the start of reperfusion and increase infarct size during ischemia-reperfusion in mouse hearts.[7] Moreover, phlorizin abrogated the beneficial effects of ischemic-preconditioning-induced cardio-protection in the ischemic-reperfusion rat model.[8] In contrast, Li et al[9] recently demonstrated that SGLT1 knock-down protected hearts from ischemia-reperfusion injury in mice. Therefore, in addition to the protective effects of SGLT1 blockade against the development of hypertrophic cardiomyopathy, the inhibition of SGLT1 may be effective for cardio-protection of the ischemic heart. However, whether SGLT1 blockade can inhibit the development of MI-induced HF is still uncertain. In this study, we investigated the effects of KGA-2727,[10] a selective SGLT1 blocker, on cardiac remodeling and HF induced by left anterior descending coronary artery (LAD) ligation in mice.

2. Materials and methods

The study was conducted in strict accordance with recommendations in the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health, and approved by the Animal Care Committee of Iwate Medical University. The protocol was approved by the Committee on the Ethics of Animal Experiments of Iwate Medical University (Permit Number: 27−008).

2.1. Experimental animals, left anterior descending coronary artery (LAD) ligation operation, and administration of KGA-2727, a selective SGLT1 inhibitor

C57BL/6J male mice (9 weeks old, 25−30-g body weight) were purchased from Japan SLC. To create myocardial infarction (MI), left anterior descending coronary artery (LAD) ligation was performed as described elsewhere.[11] Briefly, mice were anesthetized with isoflurane and intubated with a 22-gauge polyethylene catheter. After mice were ventilated with a rodent ventilator (MiniVent Type845 Harvard Apparatus, Holliston, MA, USA), the chest was opened at the left fourth intercostal space and the LAD was ligated with 8-0 nylon sutures. After LAD ligation, the chest was closed with negative pressure in the chest cavity using 5-0 nylon sutures. In sham-operated mice, the same procedure was performed except for the LAD ligation. KGA-2727 (100 mg/ml based on inhibitor constant of KGA-2727 for SGLT1 (Ki = 48 nM)) was administered for 5 weeks using an osmotic mini pump (Alzet 2006 (0.15 μL/h, 200 μL)) implanted subcutaneously one week before LAD ligation (Fig. 1A). Four weeks after LAD ligation, all experiments were performed in all mice. Sodium pentobarbital (30 mg/kg) applied intraperitoneally and/or isoflurane inhalation was used to anesthetize all animals. The adequacy of anesthesia was monitored based on the heart rate, the degree of motion of the sternum, and movement of the extremities. Toe pinch reflex test was also performed to check the adequate information about sufficient or insufficient depth of anesthesia. Before the heart was excised, a blood sample was taken and the blood glucose level was measured using animal glucometer (LAB Gluco, Fore Care Tokyo Co., Ltd). We also measured mean plasma KGA-2727 concentration (302 ± 38 nM), suggesting that the dosage of KGA-2727 used in this study is enough to inhibit SGLT1.

2.2. Echocardiography

During isoflurane anesthesia, the cardiac contractile function was assessed with echocardiography (ProSound 70 Hitachi Aroka, Tokyo, Japan) in sham-operated (control), LAD-ligated (MI), and MI plus KGA-2727-treated (MI + KGA) mice (n = 8 for each), as previously described.[12] Hearts were viewed at the level of the papillary muscles along the short axis. M-mode tracings with the average of three consecutive beats were used to measure the left ventricular end-diastolic dimension (LVEDd) and end-systolic dimension (LVESd). Left ventricular fractional shortening (LVFS) was calculated as follows: (LVEDd - LVESd)/LVEDd × 100%.

2.3. Histology

Hearts were quickly excised after a midline sternal incision. The hearts were fixed with a 10% solution of formalin in phosphate-buffered saline at 24 °C for more than 24 h, embedded in paraffin, and then cut serially from the apex to base. Six sections were stained with hematoxylin/eosin or Masson's trichrome for histopathological analysis. Transverse sections were captured digitally, and the cross-sectional diameter of at least 50 cardiomyocytes was measured at site 1 (non-infarcted area) and site 2 (infarcted area) of each section using the image analyzing software of BZ-X Analyzer (KEYENCE Osaka, Japan) (Fig. 2A). To assess the degree of fibrosis, digital microscopic images were taken from the sections stained with Masson's trichrome using light microscopy with BZ-X Analyzer software. Measurements were performed involving sites 1 and 2 of each section in each preparation (Fig. 3A).[6,13] The fibrosis fraction was obtained by calculating the ratio of total connective tissue area to the total myocardial area from 3 images in each preparation.

Immunohistochemistry was performed for the localization of SGLT1. For antigen retrieval, sections were dipped in antigen activator reagent, pH = 9, boiled for 10 min in a microwave oven (50 Hz), and then left until the reagent reached room temperature. Then, specimens were blocked with blocking reagent for one hour. After this step, incubation of the primary antibody (Rabbit antibody to SLC5A, Osenses, Pty., Ltd., Australia) was carried out in a humidity box at 4 °C overnight. The next day, they were rinsed in TBS four times for 10 min each and incubated with the secondary antibody for 1 h. After rinsing, mounting was conducted with Fluoromout Plus™ (Diagnostic BioSystems Inc., USA). Images were obtained using a confocal laser microscope (EZ-C1, Nikon, Japan). The images of immunofluoroscene were analyzed using the image analyzing software “Image J (ver.1.51s)”. Quantitative analysis was conducted by calculating the ratio of the SGLT1-stained area to total myocardial area in each preparation.

2.4. Quantification of mRNA by real-time PCR

Total RNA was prepared from the control, MI, and MI + KGA mouse left ventricles (n = 6 for each) with ReliaPrep™ RNA Tissue Miniprep System (Promega, Madison, WI, USA) according to the manufacturer's instructions. Five hundred nanograms of total RNA was used as a template for reverse transcription with the SuperScript® III First-Strand synthesis system (Invitrogen, Carlsbad, CA, USA). Real-time reverse transcription-polymerase chain reaction (RT-PCR) analysis was performed with an ABI Step One Real-Time PCR System using Fast SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA) to detect atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), β-myosin heavy chain (β-MHC), interleukin-1β (IL-1β), interleukin-18 (IL-18), connective tissue growth factor (CTGF), collagen type 1 (collagen 1), matrix metalloproteinase 2 (MMP-2), matrix metalloproteinase 3 (MMP-3), glucose transporter 1 (GLUT1), GLUT4, GLUT8, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The expression of each gene was normalized to that of GAPDH mRNA because the expression of GAPDH mRNA was constant among groups.

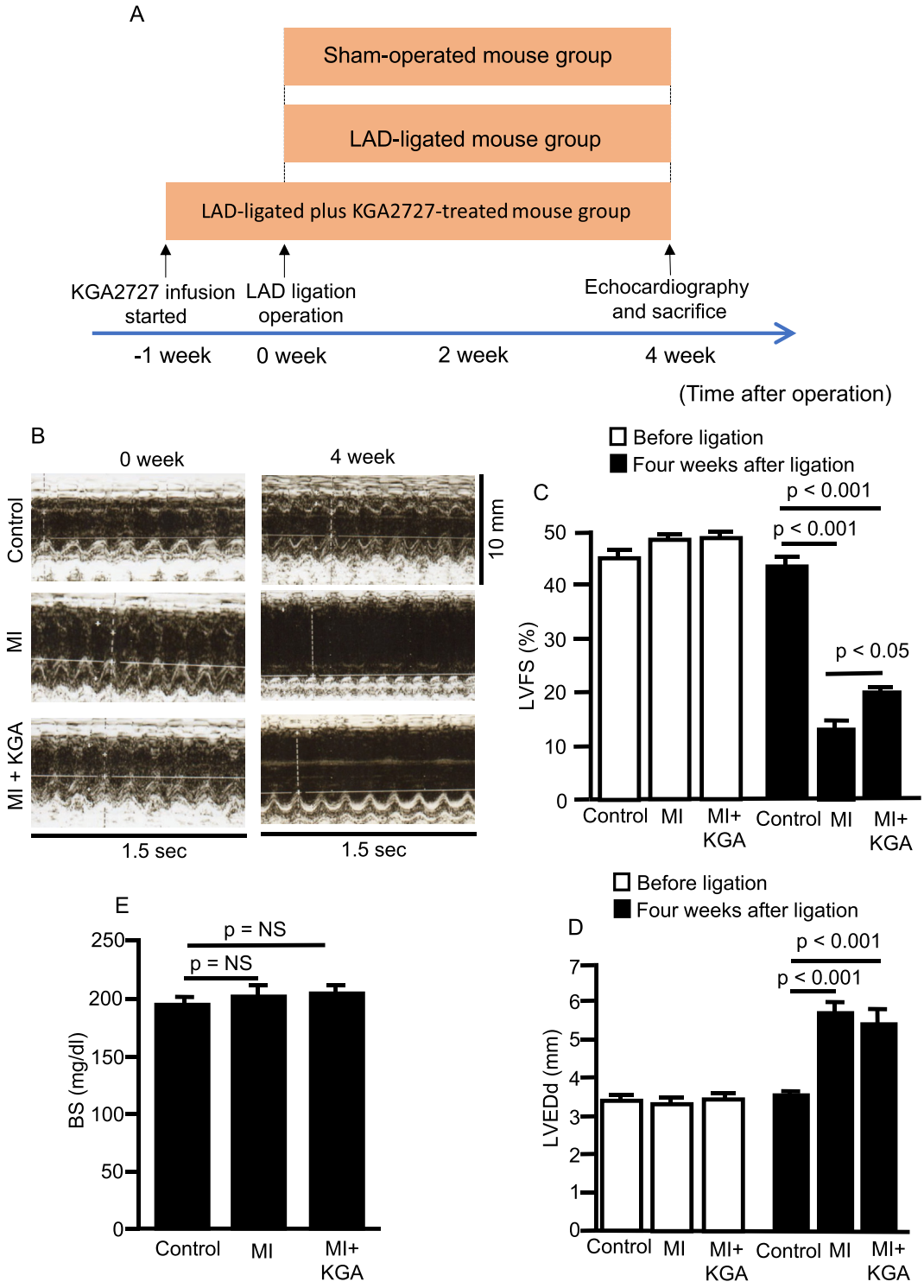

Fig. 1. Panel A, The schema of experimental protocols for each of the three groups (sham-operated, LAD-ligated, and LAD-ligated plus KGA-2727-treated mouse groups). Left anterior descending coronary artery (LAD) ligation was performed in two of the three groups. The infusion of KGA-2727, a selective SGLT1 blocker, was started from 7 days before LAD ligation and then continued through the experimental periods in the LAD-ligated plus KGA-2727-treated mouse group. Panels B–E, Effects of KGA-2727 on myocardial infarction (MI)-induced left ventricular contractile dysfunction and blood sugar level. Representative M-mode echocardiograms (panel B) of the three different groups before and 4weeks after LAD ligation. Left ventricular fractional shortening (LVFS) (panel C), left ventricular end-diastolic dimension (LVEDd) (panel D), and the blood sugar level (BS, panel E) measured from the three different groups of mice. Data are the mean ± SE obtained from 6 to 8 mice for each group. Control, sham operated mice; MI, LAD-ligated mice; MI + KGA, LAD-ligated mice treated with KGA-2727.

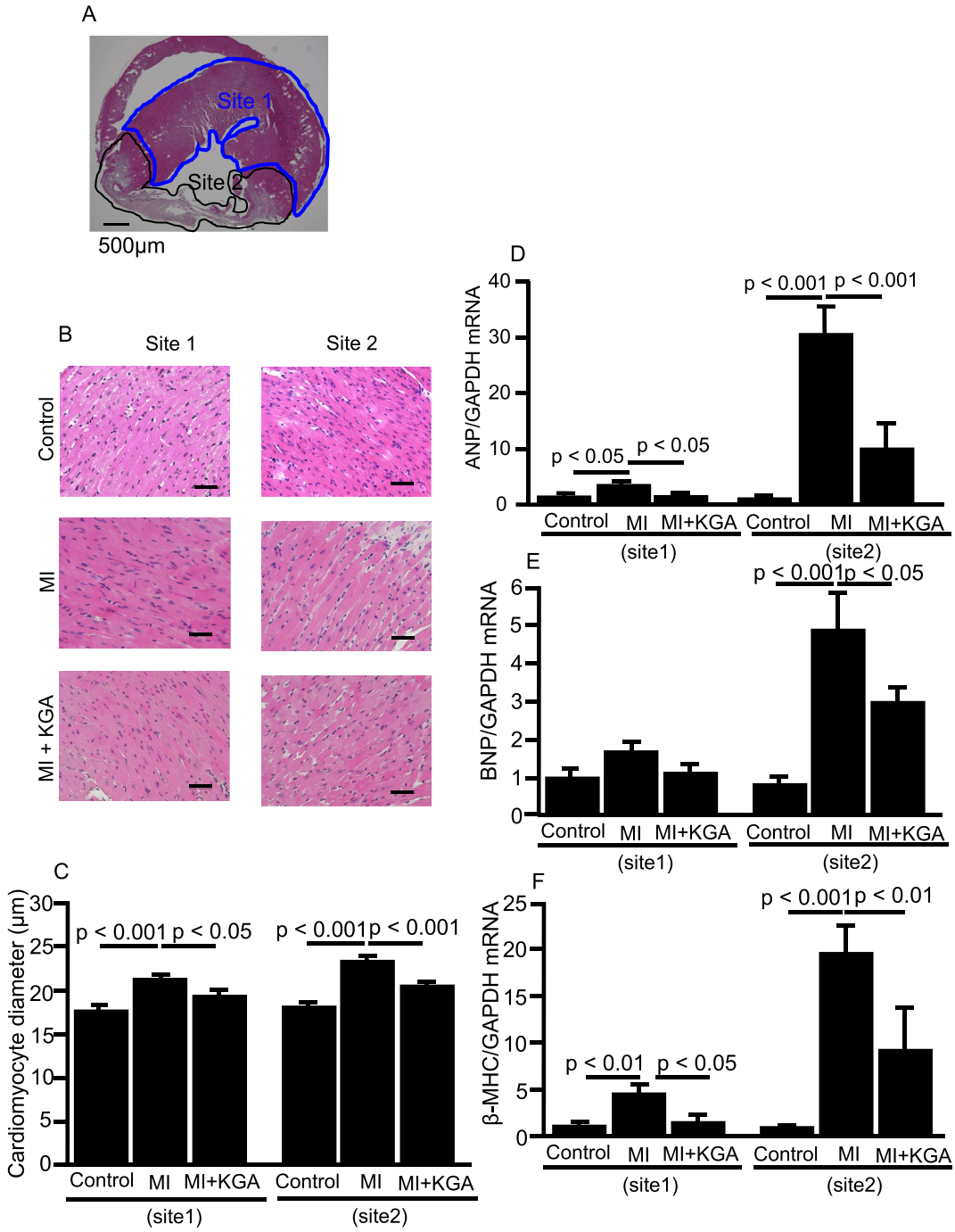

Fig. 2. Effects of KGA-2727 on myocardial infarction (MI)-induced left ventricular hypertrophy, and gene expression of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and β-myosin heavy chain (β-MHC). Representative example of left ventricular histology stained with hematoxylin/eosin in the three different groups (panel B). Original magnification: 40 × Scale bar = 50 μm. Comparison of the cross-sectional diameter of left ventricular cardiomyocytes (cardiomyocyte diameter) (panel C) from sites 1 and 2 of the left ventricle shown in panel A in the three different groups of mice. Quantitative analyses of left ventricular ANP (panel D), BNP (panel E), and β-MHC (panel F) mRNA expression levels measured at sites 1 and 2 of the left ventricle in the three different groups of mice. Data for each mRNA expression level were normalized to those of GAPDH. Data are the mean ± SE obtained from 6 mice in each group.

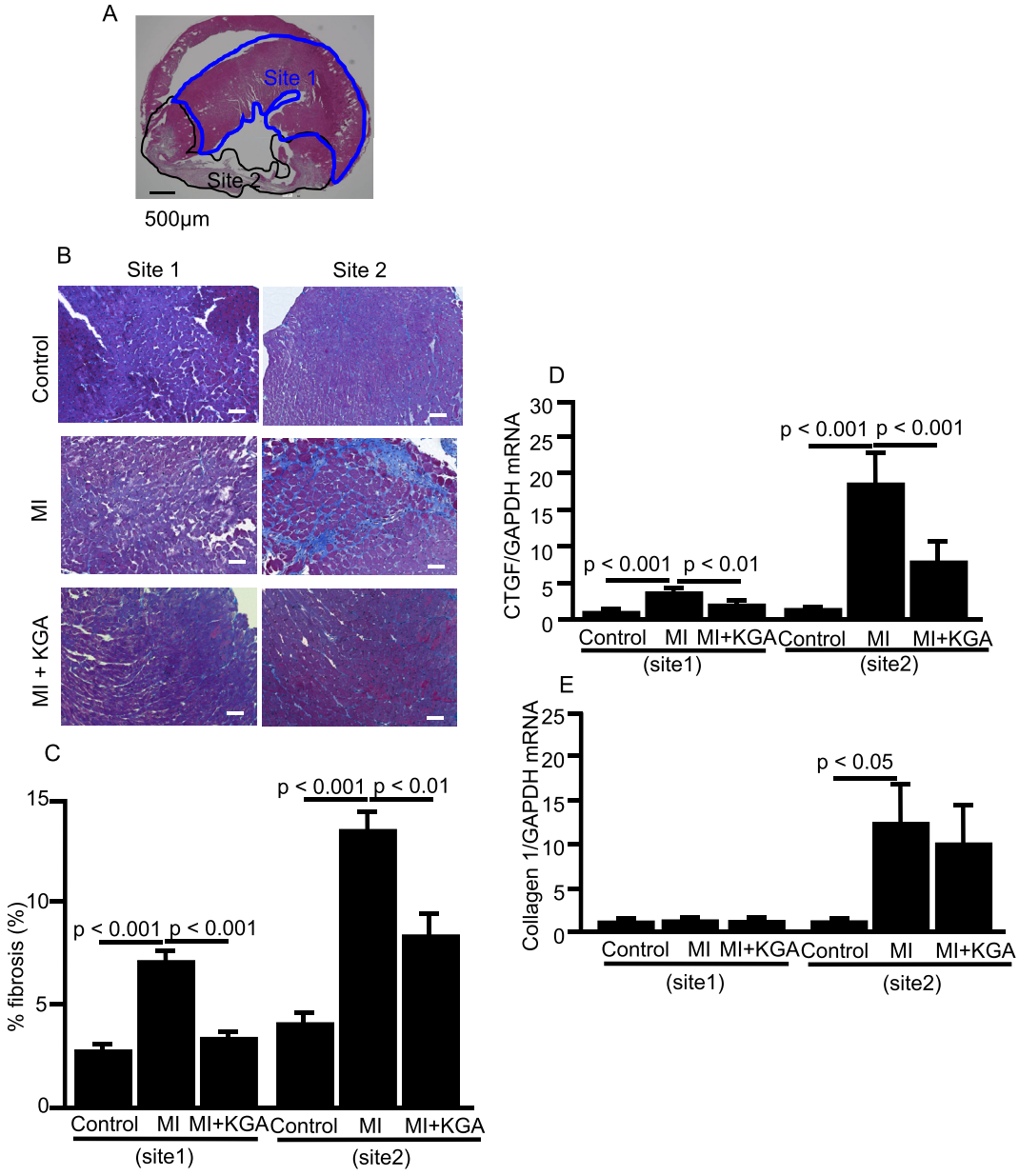

Fig. 3. Effects of KGA-2727 on myocardial infarction-induced left ventricular fibrosis, and gene expression of ventricular connective tissue growth factor (CTGF) and collagen type 1 (collagen 1). Representative example of the left ventricular histology (panel B) stained with Masson's trichrome in the three different groups of mice. Original magnification: 40 × Scale bar = 50 μm. Comparison of the fibrosis fraction (panel C) from sites 1 and 2 of the left ventricle shown in panel A in the three different groups of mice. Quantitative analyses of CTGF (panel D) and collagen 1 (panel E) mRNA expression levels at sites 1 and 2 of the left ventricle in the three different groups of mice. Data are the mean ± SE obtained from 6 mice in each group. N.S., not significant.

2.5. Data analysis

All data are shown as the mean ± SE. An analysis of variance with Bonferroni's test was used for the statistical analysis of multiple comparisons of data. $P < 0.05$ was considered significant.

3. Results

3.1. KGA2727 partially but significantly improved MI-induced left ventricular contractile dysfunction

Four weeks after LAD ligation, representative examples of M-mode echocardiograms demonstrated that LVEDd was significantly greater in LAD-ligated MI hearts regardless of KG-A2727 pretreatment than in the control. Interestingly, KGA-2727 improved left ventricular posterior wall motion in a LAD-ligated MI mouse. Fig. 1C, D shows LVFS and LVEDd in the three different groups of mice. Before LAD ligation, LVFS did not differ among the three different experimental groups regardless of KGA-2727 pretreatment (Fig. 1C). Four weeks after LAD ligation, LVFS and LVEDd were significantly reduced and dilated in LAD-ligated MI mouse hearts compared with control hearts, respectively. KGA-2727 pretreatment significantly improved the decreased LVFS but not the dilated LVEDd in LAD-ligated MI hearts. Four weeks after LAD ligation, KGA-2727 had no effect on blood sugar levels (Fig. 1E). Moreover, the heart rare was similar among the three different experimental groups (Control = 477 ± 14 beats/min, MI = 505 ± 26 beats/min, MI + KGA = 480 ± 10 beats/min, ANOVA, $p > 0.05$).

3.2. KGA-2727 inhibits MI-induced cardiomyocyte hypertrophy and increases in hypertrophic gene expression

The cardiomyocyte diameter increased at both sites (sites 1 and 2) of a LAD-ligated compared with those of a sham-operated mouse left ventricle (Fig. 2B). KGA-2727 improved the increased cardiomyocyte diameter at both sites of a LAD-ligated mouse left ventricle (Fig. 2B). Quantitative analysis demonstrated that the cardiomyocyte diameter was significantly increased at both sites of LAD-ligated compared with those of sham-operated mouse left ventricles, and KGA-2727 decreased the increased cardiomyocyte diameter at both sites of LAD-ligated left ventricles (Fig. 2C). Moreover, mRNA expression levels of ANP and β-MHC were significantly increased at both sites of LAD-ligated compared with those of sham-operated mouse left ventricles. KGA-2727 reduced both of the increased mRNA expression levels in LAD-ligated left ventricles (Fig. 2D, F). BNP gene expression was increased and KGA-2727 reduced this increase at only site 2 of LAD-ligated left ventricles.

3.3. KGA-2727 inhibits MI-induced myocardial fibrosis and increases in fibrotic gene expression

Extensive interstitial fibrosis at both sites of the left ventricle was observed in a LAD-ligated mouse compared with a sham-operated mouse (Fig. 3B). Quantitative analysis demonstrated that the degree of myocardial fibrosis at both sites of the left ventricle was significantly greater in LAD-ligated than in sham-operated mice, and KGA-2727 decreased the degree of myocardial fibrosis at both sites of LAD-ligated left ventricles (Fig. 3C). Moreover, expression of the CTGF gene was significantly upregulated at both sites of LAD-ligated compared with that of sham-operated WT mouse ventricles. KGA-2727 reduced the increased gene expression of CTGF at both sites of LAD-ligated ventricles (Fig. 3D). In contrast, collagen 1 gene expression was significantly upregulated at only site 2 of LAD-ligated compared with sham-operated WT mouse ventricles. KGA-2727 failed to decrease the increased gene expression of collagen 1 at site 2 of LAD-ligated ventricles (Fig. 3E).

3.4. SGLT1 protein expression increased in LAD-ligated left ventricles and glucose transporter (GLUT) gene expressions

Representative examples of immunohistochemistry on staining with anti-SGLT1 antibody demonstrated that SGLT1 protein expression was increased at site 2 of a LAD-ligated compared with that of a sham-operated mouse left ventricle (Fig. 4A, middle right). KGA-2727 did not change the SGLT1 protein expression level at site 2 of a LAD-ligated mouse left ventricle (Fig. 4A, lower right). In contrast, KGA-2727 tended to increase the SGLT1 protein expression level at site 1 of a LAD-ligated compared with that of a sham-operated mouse left ventricle (Fig. 4A, lower left). Quantitative analysis demonstrated that the degree of SGLT1 protein expression at site 2 of the left ventricle was significantly greater in LAD-ligated than in sham-operated mice regardless of KGA-2727 pretreatment (Fig. 4B). In contrast, the degree of SGLT1 expression at site 1 of the left ventricle varied by each mouse and did not differ among the three different groups of mice. There were no differences in gene expression levels of GLUT1, 4 and 8 at both sites of the left ventricle among three different groups of mice (Fig. 5).

3.5. Mechanism for KGA-2727-induced inhibition of MI-induced ventricular remodeling

The mRNA expression of IL-18 but not IL-1β was significantly increased at site 2 of LAD-ligated compared with those of sham-operated mouse left ventricles (Fig. 4C, D). Interestingly, the gene expression level of IL-18 was similar between LAD-ligated left ventricles treated with KGA-2727 and sham-operated left ventricles (Fig. 4D). Moreover, the mRNA expressions of MMP-2 and MMP-3 were significantly upregulated at site 2 of LAD-ligated compared with those of sham-operated mouse ventricles (Fig. 4E, F). KGA-2727 inhibited increases in gene expression of MMP-3 but not MMP-2 in LAD-ligated mouse ventricles (Fig. 4E, F).

4. Discussion

We demonstrated that pretreatment with KGA-2727 protected against cardiac remodeling and HF induced by LAD ligation in mice, suggesting that SGLT1 inhibitors are effective for the treatment of ischemic cardiomyopathy. It is known that SGLT2 inhibitors have indirect and direct cardioprotective effects.[14] SGLT2 inhibitors can modify risk factors of major cardiovascular events through indirect effects such as decreases in blood glucose and blood pressure in patients with diabetes mellitus and hypertension, respectively.[15] Moreover, they also directly attenuate cardiac inflammation and oxidative stress,[16,17] which improves cardiac structural and functional remodeling. It is known that SGLT1 exists in small intestine, heart, kidney, skeletal muscle, and brain. We demonstrated that blood sugar levels did not differ among three different groups of mice, suggesting that KGA-2727 does not have indirect cardioprotective effects through SGLT1 existed in small intestine and kidney. It is known that cardiac functions are both directly and indirectly controlled by autonomic nerve activation. Whether KGA-2727 acts autonomic nerves through SGLT1 existed in brain is uncertain. Our result demonstrated that the heart rate did not differ among three different groups of mice, suggesting that KGA-2727 does not act autonomic nerve. Nevertheless, whether KGA-2727 specifically inhibited SGLT1 in the heart is still unclear. Present study demonstrated that MI increased SGLT1 protein expression in the left ventricle and KGA-2727 inhibited cardiac remodeling, suggesting that KGA-2727 effectively inhibit SGLT1 in the heart and causes cardioprotective effects. Moreover, while a previous study demonstrated that insulin induced positive inotropic effects through SGLT1 in human ventricular myocardium,[18] KGA-2727 treatment did not change the left ventricular contractile function before LAD ligation, suggesting that KGA-2727 does not induce negative inotropic effects through SGLT1 inhibition. Li et al[9] demonstrated that SGLT1 knock-down protected hearts from ischemia-reperfusion injury in mice. In contrast, Phlorizin, a nonselective SGLT blocker, has been demonstrated to impair left ventricular contractile recovery after reperfusion has started and increase the infarct size of the heart during ischemia-reperfusion in mouse hearts.[7] Differences in the effects between phlorizin and knock-down of SGLT1 on ischemia/reperfusion-induced cardiac injury might depend on the effect of phlorizin on glucose transporters different from SGLT1. In fact, phlorizin decreased uptake of the glucose analog 2-deoxy-D-glucose (2-DG)[7,8] which is considered to be a poor substrate for SGLT1, suggesting that phlorizin-induced increases in I/R injury may be secondary to the inhibition of GLUTs rather than SGLT1. Therefore, KGA-2727, which is a selective SGLT1 inhibitor, might be useful for the treatment of ischemic cardiomyopathy.

SGLT1 gene and/or protein expression was found to be elevated in hypertrophic, ischemic, and diabetic cardiomyopathy in humans.[3,4] We demonstrated that myocardial infarction increased SGLT1 protein expression in the left ventricles. It is well known that myocardial ischemia activates AMP-activated kinase (AMPK). Moreover, the activation of AMPK upregulated SGLT1 gene expression in cardiomyocytes and translocated it to the plasma

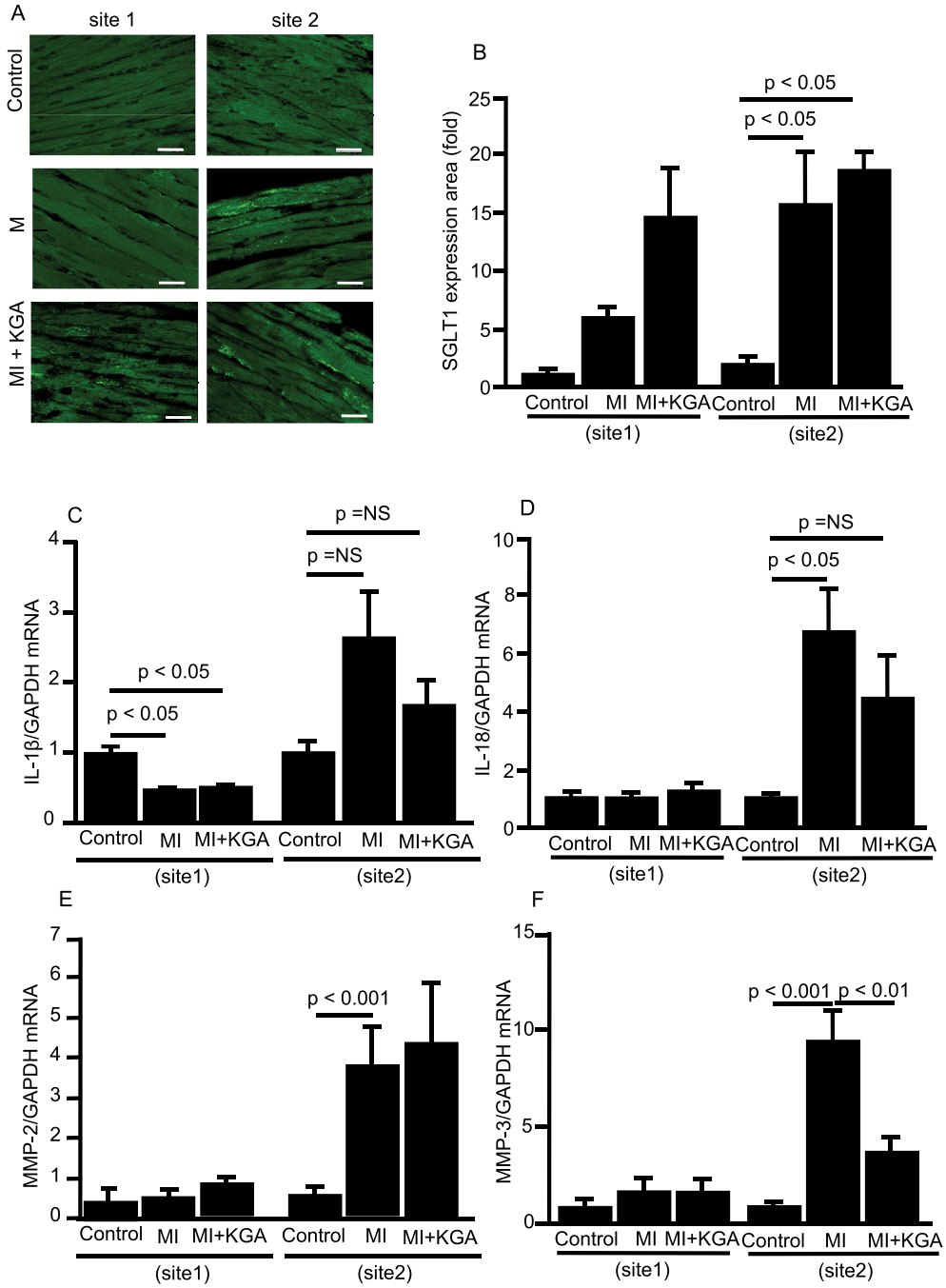

Fig. 4. Panels A–B, Protein expression of SGLT1 by immunohistochemistry. Examples (panel A) of left ventricular immunohistochemistry stained with ant-SGLT1 antibody (green) in each mouse of the three different groups. Original magnification: 100 × Scale bar = 20 μm. Relative expression levels are shown in bar graphs as the SGLT1 expression area/ventricular myocyte area ratio calculated at sites 1 and 2 of the left ventricle in the three different groups of mice (panel B). Data are the mean ± SE obtained from 5 mice in each group. Panels C–F, Quantitative analyses of IL-1β (panel C), IL-18 (panel D), MMP-2 (panel E), and MMP-3 (panel F) mRNA expression levels at sites 1 and 2 of the left ventricle in the three different groups of mice. Data are the mean ± SE obtained from 6 mice for each group. NS, not significant.

membrane.[19] Therefore, LAD ligation might activate AMPK, leading to upregulation of SGLT1 in the left ventricles.

Cardiac overexpression of SGLT1 increases the cardiomyocyte size, collagen 1 gene expression, and interstitial fibrosis, and inhibition of SGLT1 expression reversed all of these remodeling phenomena in mouse hearts.[5] We demonstrated that LAD-ligated MI increased SGLT1 protein expression, and ANP, BNP, β-MHC, CTGF, and collagen 1 gene expression, and induced cardiomyocyte hypertrophy and interstitial fibrosis. Pretreatment with KGA-2727 inhibited cardiomyocyte hypertrophy and interstitial fibrosis in LAD-ligated MI mouse ventricles with increased SGLT1 protein expression. Moreover, although KGA-2727 did not inhibit increases in collagen 1 gene expression, it inhibited increases in ANP, BNP, β-MHC, CTGF gene expressions in LAD-ligated mouse left ventricles. These results suggest that LAD-ligated MI induces cardiomyocyte hypertrophy and interstitial fibrosis, in part, through increases in SGLT1 protein expression, and that KGA-2727 inhibits these forms of cardiac remodeling by blocking SGLT1.

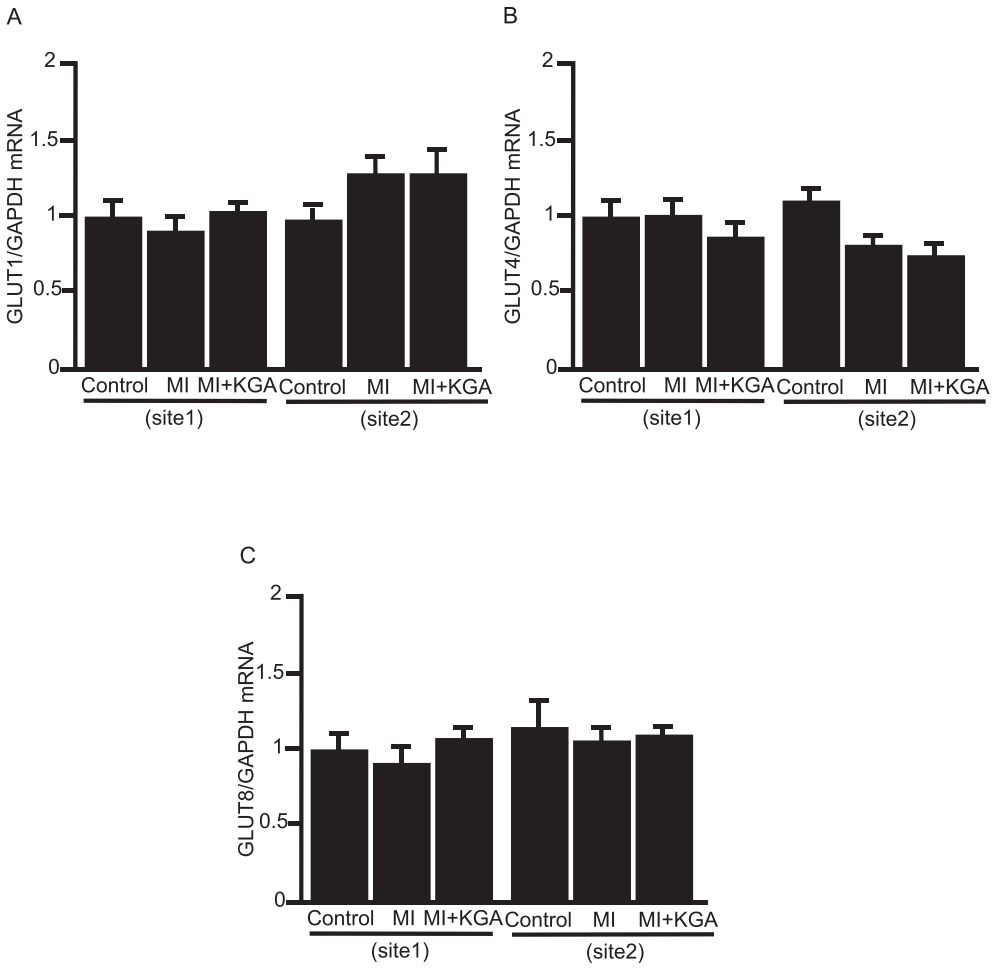

Fig. 5. Panels A–C, Quantitative analyses of glucose transporter 1 (GLUT1) (panel A), GLUT4 (panel B), and GLUT8 (panel C) mRNA expression levels at sites 1 and 2 of the left ventricle in the three different groups of mice. Data are the mean ± SE obtained from 6 mice for each group.

Chronic pressure overload increased SGLT1 and IL-18 gene expressions, leading to the development of cardiac hypertrophic cardiomyopathy in mice.[6] Myocardial infarction increased left ventricular IL-18 expression, which reduces myocardial contractility in mice.[20] Our results demonstrated that the IL-18 gene expression level increased in the LAD-ligated mouse ventricles. Moreover KGA-2727 failed to decrease IL-18 gene expression in the LAD-ligated mouse ventricles, suggesting that KGA-2727 does not contribute to the improvement of the decreased myocardial contractility through the inhibition of IL-18 gene expression in MI hearts. MMP-2 plays an important role in chronic pressure overload-induced left ventricular hypertrophy and contractile dysfunction.[21] Moreover, upregulation of SGLT1 by high glucose treatment was associated with increases in MMP-2 protein expression in human cardiac fibroblasts.[22] KGA-2727 failed to inhibit increases in collagen 1 or MMP-2 gene expression in LAD-ligated mouse left ventricles. However, hypoxia reduces MMP-2 activity in the absence of decreases in MMP-2 protein expression.[23] Therefore, MMP-2 activity may be reduced in LAD-ligated hypoxic mouse left ventricles even if gene expression is upregulated. The improved ventricular contractile function was associated with decreases in MMP-3 activity in LAD-ligated mice.[24] Moreover, increases in circulating MMP-3 levels are associated with left ventricular dysfunction, adverse left ventricular remodeling, and the prognosis in patients after acute myocardial infarction.[25] KGA-2727 pretreatment decreased MMP-3 gene expression, which may contribute to the effects of KGA-2727 to protect against left ventricular contractile dysfunction.

Ramratnam et al[5] have demonstrated that cardiac overexpression of SGLT1 increases cardiomyocyte size, collagen 1 gene expression, and interstitial fibrosis in mouse hearts even under non-diabetic condition. We demonstrated that myocardial infarction increased SGLT1 protein expression, and ANP, BNP, β-MHC, and CTGF gene expression, leading to cardiac hypertrophy and interstitial fibrosis in mouse ventricles. However, the precise mechanisms for SGLT1-induced cardiac hypertrophy and fibrosis is still uncertain. Recent study demonstrated that enhanced Na^+-glucose co-transport via SGLT1 elevated intracellular Na^+ concentration in hearts,[26] and elevated intracellular Na^+ concentration followed by intracellular Ca^{2+} overload might be linked to a higher propensity for cardiac hypertrophy and fibrosis. In fact, it is known that intracellular calcium can induce cardiac hypertrophy[27] and the proliferation of cardiac fibroblasts.[28] Therefore, increased SGLT1 expression in the left ventricle might elevate intracellular Na^+ concentration followed by intracellular Ca^{2+} overload, leading to cardiac hypertrophy and fibrosis. Thus, KGA-2727 may inhibit intracellular Ca^{2+} overload, leading to improvement of cardiac hypertrophy and fibrosis.

KGA-2727 improved cardiomyocyte hypertrophy and interstitial fibrosis at site 1 in which there was no significant increase in SGLT1 expression. Cardiac remodeling such as cardiomyocyte hypertrophy and interstitial fibrosis is triggered by mechanical stretch and

several humoral factors. In fact, it has been demonstrated that increased mechanical stretch on myocardium induced by myocardial contractile dysfunction activates several factors, such as angiotensin II type 1 receptor,[29] different from SGLT1 in the heart, which induces cardiomyocyte hypertrophy and interstitial fibrosis. Recent studies demonstrated that high glucose[30] and protein kinase C activation[31] induced SGLT1 activation. In this study, serum glucose levels were similar among three different groups of mice, suggesting that serum glucose does not change SGLT1 activation level among the three different groups. In contrast, as AT_1 receptor activation can induce protein kinase C activation,[32] it is possible that myocardial stretch-induced AT_1 receptor activation induces SGLT1 activation, leading to cardiac hypertrophy and interstitial fibrosis. Therefore, KGA-2727 might improve cardiomyocyte hypertrophy with decreases in ANP and β-MHC gene expression and interstitial fibrosis with decreases in CTGF through the inhibition of SGLT1 activation at site 1 of the left ventricle where SGLT1 protein expression was not increased after LAD ligation.

Connelly et al[33] reported that dual inhibition of SGLT1 and 2 (the drug was T-1095) exacerbated cardiac contractile dysfunction following MI in rats. Although the differences between Connelly's and our results are unclear, there is a difference in the results regarding SGLT1 protein expression between the two studies. In fact, while LAD-ligated MI increased cardiac SGLT1 expression in our study, it did not increase the SGLT1 expression in Connelly's study, suggesting that the SGLT1-blocking action of T-1095 may not directly act to improve cardiac contractile dysfunction in rats with MI. However, Banerjee et al[19] demonstrated that AMPK activation increases the translocation of SGLT1 from the sarcoplasm to sarcolemma, which activates glucose uptake by SGLT1 even though its expression level is not changed. Moreover, as described above, high glucose and protein kinase C activation can induce SGLT1 activation. Although the effects of those factors on cardiac SGLT1 activation in Connelly's study remain uncertain it is possible that protein kinase C activation by the activated AT_1 receptors induces SGLT1 activation in MI hearts. Therefore, cardiac SGLT1 activity was likely to increase in the MI rats. Nevertheless, in contrast to our results SGLT1 and 2 blockade by T-1095 exacerbated cardiac contractile dysfunction. There are also several differences in study design between the two studies. For example, the species and age of animals and route of drug administration were different between the two studies. In fact, while KGA-2727 was subcutaneously administered in our study, T-1095 admixed in chow was orally administered in the previous study. In addition, the plasma T-1095 concentration was not measured in the study. Therefore, whether the plasma concentration is sufficient to block SGLT1 remains uncertain. KGA-2727 was continuously administered from 1 week before LAD ligation to 4 weeks after ligation. However, the timing of drug administration is unclear in Connelly's study, suggesting that the difference affects the results. In any case, our results suggest that KGA-2727 pretreatment can improve MI-induced left ventricular remodeling and ventricular contractile dysfunction. Thus, it requires scrupulous attention to use dual SGLT1/2 blockers for myocardial infarction therapy.

5. Study limitation

Our results demonstrated that there were no significant differences in GLUT1, 4, or 8 gene expressions among the three different groups of mice. However, Darvey et al[34] demonstrated that ischemia-induced translocation of GLUT4 to the sarcolemma can transport glucose into the cytosol, suggesting that sarcolemmal protein expression levels of GLUTs rather than mRNA levels of those transporters are more important to elucidate the effects of GLUTs on the myocardium. Therefore, further studies may be needed to more clearly elucidate whether GLUTs participate in the improvement of cardiac contractile dysfunction in LAD-ligated left ventricles.

Our present study demonstrated that KGA-2727 pretreatment improved MI-induced left ventricular remodeling and contractile dysfunction. These results suggest that KGA-2727 may become a new pharmacological therapy for ischemia-induced cardiomyopathy. However, only one dose of KGA-2727 was used to examine the effects of the drug on MI-induced left ventricular remodeling and contractile dysfunction. Therefore, whether the effects of KAG-2727 on MI hearts are based on dose-dependent effects is still unknown. Shibazaki et al[10] demonstrated that orally administered KGA-2727 dose-dependently decreases plasma glucose levels in Zucker diabetic fatty rats subjected to the oral glucose tolerance test, suggesting that it dose-dependently inhibits SGLT1 in the small intestine. Thus, KGA-2727 may dose-dependently inhibit cardiac SGLT1. In either case, further studies may be needed to more clearly elucidate whether SGLT1 blockade dose-dependently improves MI-induced myocardial remodeling and contractile dysfunction.

6. Conclusions

We found that pretreatment with KGA-2727 protects against MI-induced left ventricular functional and structural remodeling in mice. Moreover, KGA-2727 inhibited MI-induced increases in expressions of several cardiac genes, such as ANP, BNP, β-MHC, CTGF, IL-18, MMP-3. Therefore, KGA-2727 might be useful for the treatment of ischemia-induced cardiomyopathy.

Declaration of Competing Interest

All authors declare no potential conflict of interest.

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