Dapagliflozin Protection against Myocardial Ischemia by Modulating Sodium-glucose Transporter 2 Inhibitor, Silent Information Regulator 1, and Fatty Acid Synthase Expressions

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Abstract

BACKGROUND: The emerging role of sodium-glucose transporter 2 (SGLT2) inhibitors drugs as potential therapeutic agents in myocardial ischemic (MI) injury treatment has raised the concern for possible mechanisms of action. AIM: The current experimental study aimed to investigate the possible protective effects of dapagliflozin (DAPA) a SGLT2, on isoproterenol (ISO)-induced MI in rats. MATERIALS AND METHODS: Thirty Wistar rats were divided randomly and equally into three groups. Group 1 (control group): Received 1.0 mL of normal saline through an orogastric tube for 14 days. Group 2 (ISO group): Received 1.0 mL of normal saline orally through an orogastric tube for 14 days. In the last 2 days (days 13 and 14), ISO (100 mg/kg) was freshly dissolved in normal saline and injected subcutaneously once daily. Group 3 (ISO + DAPA-treated group): Received DAPA 1.0 mg/kg/day orally for 14 days. In the last 2 days (days 13 and 14), ISO (100 mg/kg) was introduced like that described in Group 2. RESULTS: DAPA protects MI development by reversal of blood pressure changes, electrocardiographic alterations, stabilization of cardiac enzymes, inflammation restoration, oxidative stress, and lipid profile. SGLT2 was overexpressed in the ISO-induced MI, which declined in the ISO + DAPA group. Moreover, DAPA induced silent information regulator 1 (SIRT1)/fatty acid synthase (FASN) overexpression in ISO-induced MI. DAPA could have a potential protective role against acute MI. CONCLUSION: DAPA protects against acute MI by modulating SIRT1 and FASN expression in cardiac muscles, suppressing oxidative stress, and downregulating inflammatory mediators.

Introduction

Acute myocardial ischemia (MI) is a critical cardiovascular disorder estimated as the global cause of morbidity and mortality [1]. MI results from coronary artery obstruction because of atherosclerotic clots or artery spasms [2]. Obstruction of the coronary arteries results in MI due to oxygen and nutrient deprivation [3]. Furthermore, the release of reactive oxygen species (ROS) depletes the antioxidant enzymes inducing lipid peroxidation and protein and nucleic acid degeneration, which worsens the cardiac inflammatory reaction and cardiomyocyte apoptosis [4]. The activation of the nuclear factor-kappa beta (NF-κB) pathway has been recognized as a key factor in cardiac remodeling failure, inflammation, and fibrosis through the initiation of innate immunity and the inflammatory process [5]. In addition, the lack of regenerative capacity of the heart cells interferes with the regenerative process with the development of myocardial fibrosis, the major pathogenesis of ischemic cardiomyopathy, and systolic and diastolic dysfunctions [6].

The emerging role of sodium-glucose transporter 2 (SGLT2) inhibitors as potential therapeutic agents in MI treatment has raised concerns for possible mechanisms of action [7]. Moreover, SGLT2 is a transmembrane protein that is pivotal in renal sodium and glucose reabsorption [8]. In addition, SGLT2 inhibitors are used as monotherapy or combination therapy with other antidiabetic drugs to control hyperglycemia [9]. Along with the hypoglycemic effect of the SGLT2 inhibitors, several studies have correlated SGLT2 inhibitors to have antihypertensive effects through systolic and diastolic blood pressure reduction without altering the heart rate or fainting episodes [10]. The SGLT2 expression in the heart tissue is a matter of controversy. Thus, SGLT2 inhibitors could exert their function indirectly through autophagy downregulation [11].

Silent information regulator 1 (SIRT1) is a histone deacetylase of nicotinamide adenine dinucleotide (NAD+) found inside the nucleus and has a crucial role in cell proliferation, differentiation, and autophagy [12]. SIRT1 promotes the activity...
of antioxidant enzymes and interacts directly with NF-κB, inhibiting pro-inflammatory signaling in heart tissue [5]. In addition, SIRT1 activation maintains the proper functioning of the mitochondria and peroxisomes and reprograms the metabolic pathways mainly in gluconeogenesis and fatty acid oxidation. The two common downstream effectors of SIRT1 stimulation are the peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1α) and fibroblast growth factor 21 (FGF21). Fatty acid synthase enzyme (FASN) is a member of lipogenic pathways that are downregulated by FGF21 activation [13]. In addition, FASN is a housekeeping protein that can be used for energy storage, membrane assembly, and repair and secretion in the form of lipoprotein triglycerides during fasting [14]. However, prolonged FASN activation could result in inflammation, lipid peroxidation, and fibrosis [15]. However, the role of FASN activation in cardiac tissue is a matter of controversy [16].

The emerging role of SGLT2is drugs as potential therapeutic agents in MI injury treatment despite the absence of known heart receptors has raised the concern for possible mechanisms of action. Therefore, the current experimental study aimed to evaluate the protective effects of dapagliflozin (DAPA) a SGLT2 inhibitor in a rat model of MI induced by isoproterenol (ISO). In addition, the immunohistochemical expression of SIRT1 and FASN was assessed as a possible mechanism of DAPA-induced protective function.

Materials and Methods

Animals

This study followed the institutional experimental animal care and the ethical committee [IRB No: 00269/2021]. The Animal Research: Reporting of Observational Studies in Experimental Animals (ARRIVE) guidelines were rigorously adhered during the execution of all operations described in this publication [17]. Rats were kept dry, insulated with paper towels, and warmed with a heated towel (maintained at 37°C) throughout the trial to avoid heat loss.

The sample size was calculated at a 95% confidence interval and study power of 80% (0.8). Moreover, the sample size was 30 male Wistar albino rats with the same age (weight, 170–200 g). Rats were kept randomly inside animal cages at 23°C for a 12 h light/dark cycle with easy access to normal laboratory food and water.

Drugs

ISO hydrochloride powder (Sigma-Aldrich, St. Louis, MO, USA), DAPA (FORXIGA©, AstraZeneca Pharmaceuticals LP, Bangkok, Thailand), and phosphate buffer saline (PBS) (Biodiagnostic CO, Dokki, Giza, Egypt) were used.

Study design and MI induction

Rats were randomized into three experimental groups (n = 10/group) as follows:

Group 1 (control group): Received 1.0 mL of normal saline through an orogastric tube for 14 days.

Group 2 (ISO group): Received 1.0 mL of normal saline orally through an orogastric tube for 14 days. In the last 2 days (days 13 and 14), ISO (100 mg/kg) was freshly dissolved in normal saline and injected subcutaneously once daily [18].

Group 3 (ISO + DAPA-treated group): Received DAPA 1.0 mg/kg/day orally for 14 days. In the last 2 days (days 13 and 14), ISO (100 mg/kg) was introduced like that described in Group 2. On day 15, the following measurements were recorded:

The systolic blood pressure (SBP) of all groups was measured by tail-cuff plethysmography (Harvard Apparatus Ltd., Edenbridge, England) in conscious rats. To obtain consistent results, the animals must be handled, warmed, and restrained. Before measurement, the rats were trained to restrain themselves for 30 min/day for 2 days.

After 5 min of acclimation, we performed five consecutive measurements with a 1 min interval between returns. To obtain a consistent reading, the average of the five readings was taken [19].

Electrocardiography (ECG) monitoring was performed following the protocol of Balea et al. [20]. The animals were administered xylazine (2.6 mg/kg, i.p.) and ketamine (26 mg/kg, i.p.) as general anesthesia. The animals were then placed supine on a board for 15 min. Each rat’s paw pads were fitted with electrodes, and an ECG was recorded in lead II using the BIOPAC MP36 system (Goleta, CA, USA). Moreover, ECG was analyzed using BIOPAC Student Lab 3.7.7 (Goleta, CA, USA). RR intervals (in millisecond), PR segments (in millisecond), QRS duration (in millisecond), QT intervals (in millisecond), and ST-segment alterations (in millivolts) were calculated. The RR interval was used to compute the heart rate (HR; in beats per minute) following the formula: HR = 60,000/RR [21].

Blood sampling and tissue homogenate

All rats were sacrificed by head decapitation, and the venous blood samples of each rat were collected using heparinized capillary tubes from the retro-orbital plexus. The collected samples were centrifuged at 500xg for 20 min at 4°C to obtain serum and kept at ~80°C. The heart of each rat was
then dissected out, washed with saline, soaked, and weighed immediately. A small part of heart tissue was stored in the formaldehyde (40%) for histopathology. Part of the cardiac tissue was used to prepare tissue homogenate. The homogenate was prepared with 0.1 ± 0.05 g of myocardial tissue ground in 200 μL of PBS. The final tissue homogenate amount in each sample was adjusted to 10% by adding 700 μL of PBS. The homogenate was then centrifuged at 800×g for 10 min then stored at −80°C for biochemical analysis.

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Creatine kinase-MB (CK-MB) and cardiac troponin I (cTnI) were measured by the enzyme-linked immunoassay (ELISA) technique (Chongqing Biospes Co., Ltd., Chongqing, China; CK-MB BEK1248, cTnI BEK1253), following the manufacturer’s procedure.

Part of the cardiac tissue was used for the preparation of tissue homogenate. The homogenate was prepared with 0.1 ± 0.05 g of myocardial tissue ground in 200 μL of PBS. The homogenate was then centrifuged at 800×g for 10 min then stored at −80°C for biochemical analysis.

Determination of serum cardiac biomarkers

Creatine kinase-MB (CK-MB) and cardiac troponin I (cTnI) were measured by the enzyme-linked immunoassay (ELISA) technique (Chongqing Biospes Co., Ltd., Chongqing, China; CK-MB BEK1248, cTnI BEK1253), following the manufacturer’s procedure.

Determination of oxidative stress markers and antioxidant levels

Malondialdehyde (MDA), superoxide dismutase (SOD), and reduced glutathione (GSH) enzymes were measured in tissue homogenates by colorimetric assay using available commercial kits (Bio Diagnostic Co., Dokki, Giza, Egypt; MDA MD 25 29, SOD SD 25 21, GSH GR 25 11). The results were expressed in units in milligram protein.

Determination of cardiac inflammatory markers

Tumor necrosis factor-α (TNF-α) was measured using rat ELISA kits (ERT2010-1, Assaypro LLC, Saint Charles, MO, USA), and interleukin-6 (IL-6) was measured using rat ELISA kits (ab100772, Abcam, Cambridge, UK). The results were expressed as picograms per milliliter.

Pathological assessment

Heart tissue was collected from sacrificed rats. Representative sections were processed and embedded in paraffin blocks after fixation in neutral buffered formalin for 24 h.

Sections of 4–5 μm thickness were cut, deparaffinized, dehydrated, and stained with H&E for the assessment of the histopathological changes.

A rabbit polyclonal SGLT2 antibody (A20271) diluted at 1:100 was obtained from ABclonal, Woburn, Massachusetts, USA. SIRT1 antibody diluted at 1:50 (YPA2140) was obtained from Chongqing Biospes Co., Ltd., Chongqing, China, and FASN antibody (sc-55580) diluted at 1:200 was obtained from Santa Cruz Biotechnology Inc., Dallas, Texas, USA. After tissue deparaffinization and rehydration, antigen retrieval using a high pH EDTA solution (Dako, Ref K8000, Glostrup, Denmark) was performed, followed by cooling at room temperature. The primary antibodies were incubated overnight at 4°C. Secondary antibody using UltraVision Detection System: Anti-Polyvalent HRP/DAB, ready-to-use, NeoMarker was applied, and staining was visualized using a DAB chromogen substrate and Mayer’s hematoxylin as a counterstain. Positive and negative controls were used in each run.

Statistical analysis

The collected data of the BP, ECG, CK-MB, cTnI, MDA, SOD, GSH, TNF-α, and IL-6 levels were recorded and tabulated. Moreover, histopathological and immunohistochemical data were recorded. All findings were analyzed using one-way analysis of variance (ANOVA) and the Bonferroni post hoc test and expressed as mean ± standard error of mean.
(SEM), while categorical data were analyzed using Monte Carlo test. GraphPad Prism 9 was used to statistically analyze the data (GraphPad Software, San Diego, CA, USA). Statistical significance was defined as a value of \( p < 0.05 \).

Results

**Blood pressure**

The SBP of the ISO group was significantly decreased compared with those of the control group (87 ± 0.68 vs. 147.4 ± 0.91 mmHg; \( p < 0.05 \)). Moreover, the ISO + DAPA-treated group showed a significant increase in the SBP (104.5 ± 0.92 vs. 87 ± 0.68 mmHg) compared with the ISO group (\( p < 0.05 \), Figure 1).

![Figure 1: Dapagliflozin elevates ISO induced reduction of systolic blood pressures. Data were expressed as mean ± SEM (n = 10 for each group). BP, blood pressure; SPB, systolic blood pressure; ISO, isoproterenol; DAPA, dapagliflozin. *\( P < 0.05 \) vs. control; #\( P < 0.05 \) vs. ISO.](image)

**Electrocardiography**

ECG tracing revealed normal heart activity in both the control and DAPA-treated groups. Significant ECG alterations with shortened RR interval and hence tachycardia, ST-segment elevation, QRS, and QT interval prolongation were observed in the ISO group compared with the control group (\( p < 0.001 \)). However, such ECG abnormalities improved in the ISO + DAPA-treated group (\( p < 0.001 \)) as demonstrated by the normalization of the RR interval, HR, ST-segment, and QT intervals when compared with the ISO group (Table 1).

**Biochemical results**

Regarding cardiac enzymes, the serum CK-MB (400.2 ± 39.72 vs. 153.9 ± 2.64 U/L) and cTnI (501.4 ± 2.37 vs. 57.7 ± 0.6 pg/mL) of the ISO group were significantly increased compared with those of the control group (\( p < 0.05 \) for both). However, the ISO + DAPA-treated group showed a significant decrease in the CK-MB (307 ± 3.51 vs. 400.2 ± 39.72 U/L) and cTnI (307 ± 3.51 vs. 501.4 ± 2.37 pg/mL) compared with the ISO group (\( p < 0.05 \) for both; Figure 2a and b).

Regarding serum lipid profile, both cholesterol (233.12 ± 1.10 vs. 145.5 ± 0.41 mg/dL) and TG (179.13 ± 1.71 vs. 120.56 ± 0.96 mg/dL) were significantly increased in the ISO group compared with the control group (\( p < 0.05 \) for both). Moreover, the ISO + DAPA-treated group showed a significant decrease in the cholesterol (191.39 ± 1.09 vs. 233.12 ± 1.10 mg/dL) and TG levels (119.12 ± 1.16 vs. 179.13 ± 1.71 mg/dL) compared with the ISO group (\( p < 0.05 \) for both; Figure 2c and d).

![Figure 2: The electrocardiographic effect of dapagliflozin in isoproterenol-induced myocardial ischemic.](image)

**Table 1: The electrocardiographic effect of dapagliflozin in isoproterenol-induced myocardial ischemic.**

| Parameters | Group   | ISO | ISO + DAPA |
|-----------|---------|-----|------------|
| HR (b/min) | Control | 382 ± 1.15 | 392 ± 0.58* |
|           |         | 87 ± 0.68 | 104.5 ± 0.92* |
| RR (ms)   | Control | 154.67 ± 0.88 | 154.00 ± 0.58* |
|           |         | 104.67 ± 0.88* | 32.00 ± 0.58* |
| QRS (ms)  | Control | 54.00 ± 0.58* | 46.00 ± 1.53* |
| QT (ms)   | Control | 0.02 ± 0.00 | 0.04 ± 0.00* |
| ST (mV)   | Control | 0.02 ± 0.00 | 0.04 ± 0.00* |

*Significantly different from control group, #significantly different from ISO group. HR, RR, QRS, QT, and ST intervals. Data were expressed as mean ± SEM and analyzed using one-way ANOVA followed by Bonferroni’s post hoc test at \( p < 0.05 \). n = 10.

ANOVA: Analysis of variance, DAPA: Dapagliflozin, ISO: Isoproterenol, SEM: Standard error of mean.
2.75 ± 0.07 nM/mg tissue protein) compared with the ISO group (p < 0.05; Figure 3b and c).

The inflammatory mediators' level was measured, and a significant TNF-α (140.11 ± 0.72 vs.
43.03 ± 3.87 pg/mL) and IL-6 (145.65 ± 0.65 vs. 12.88 ± 0.26 pg/mL) increase in the ISO group compared with the control group (p < 0.05 for both) were found. Moreover, the ISO + DAPA-treated group showed a significant decrease in TNF-α (75.74 ± 1.38 vs. 140.11 ± 0.72 U/mg tissue protein) and IL-6 (39.32 ± 0.62 vs. 145.65 ± 0.65 pg/mL) compared with the ISO group (p < 0.05 for both; Figure 3d and e).

**Histopathological findings**

Histological myocardium examination of both the control and DAPA-treated groups revealed a normal myofibrillar structure with clear transverse striations, centrally located nuclei, acidophilic sarcoplasm, and inflammatory infiltrate absence (Figure 4a and b). The ISO group showed marked disturbed and fragmented myocardial fibers with vacuolation, edema, peripheral pyknotic nuclei, and interstitial edema compared with the control group (p < 0.001). In addition, significant moderate congestion and inflammation in between myocardial cells were observed (p < 0.001; Figure 4d and e). Moreover, the ISO + DAPA group revealed a significant improvement of the histopathological changes with mild myocardial damage and edema (p = 0.003 and p = 0.012) and complete inflammatory infiltrate clearing (p < 0.001), Figures 4g and h. Table 2 illustrated the histopathological changes in the studied groups.

**MTC stain**

No collagen fibers were noted between cardiac myocytes in both the control and DAPA-treated groups. Collagen deposition was highlighted by MTC in between myocardial fibers and the wall of blood vessels in the ISO group. However, a minimal degree of collagen deposition was observed in the ISO + DAPA-treated group (Figure 4c, f, and i).
Table 2: Comparison between the histopathological changes in the studied groups

| Variables                              | Control (%) | ISO group (%) | ISO + DAPA (%) | p     |
|----------------------------------------|-------------|---------------|----------------|-------|
| Degenerated muscle                     |             |               |                |       |
| Absent                                 | 10 (100)    | 0             | 0              | p1 < 0.001 |
| Mild                                   | 0           | 10 (100)      | 2 (20)         | p2 < 0.001 |
| Moderate                               | 0           | 2 (20)        | 8 (80)         | p3 = 0.003 |
| Marked                                 | 0           | 8 (80)        |                |       |
| Intestinal hemorrhage and congestion   |             |               |                |       |
| Absent                                 | 10 (100)    | 0             | 0              | p1 < 0.001 |
| Mild                                   | 0           | 0             | 6 (60)         | p2 < 0.001 |
| Moderate                               | 0           | 9 (90)        | 4 (40)         | p3 = 0.012 |
| Marked                                 | 0           | 1 (10)        |                |       |
| Inflammation                           |             |               |                |       |
| Absent                                 | 10 (100)    | 0             | 0              | p1 < 0.001 |
| Mild                                   | 0           | 0             | 9 (90)         | p2 < 0.001 |
| Moderate                               | 0           | 9 (90)        | 1 (10)         | p3 < 0.001 |
| Marked                                 | 0           | 1 (10)        |                |       |

Data were expressed as frequency (%) and analyzed using Monte Carlo test at p < 0.05. p1 control regarding ISO. p2 control regarding ISO + DAPA. p3 ISO regarding ISO + DAPA. DAPA: Dapagliflozin, ISO: Isoproterenol.

Immunohistochemical results

A significant SGLT2 overexpression was observed in the ISO group compared with the control group (p < 0.001). The expression was significantly decreased in the ISO + DAPA-treated group compared with the ISO group (p < 0.001; Figure 5a-c).

The SIRT1 and FASN expressions as modulators of fatty acid oxidation and autophagy were also assessed. SIRT1 nuclear expression declined at a nonsignificant level (p = 0.427) in the ISO group compared with the control group. However, SIRT1 was overexpressed in the ISO + DAPA group compared with the control and ISO groups (p = 0.047 and p < 0.001, respectively; Figure 5d-f).

Similarly, FASN expression followed a similar SIRT1 expression manner in all studied groups. FASN expression was declined at a nonsignificant level (p = 0.452) in the ISO group compared with the control group. However, FASN was overexpressed in the ISO + DAPA group compared with the control and ISO groups (p = 0.013 and p < 0.001, respectively; Figure 5g-i). Table 3 illustrated the detailed markers expression.
Table 3: Comparison between sodium-glucose transporter 2, silent information regulator 1, and fatty acid synthase immunohistochemical expression in the studied groups

| Studied markers | Control | ISO | ISO+DAPA | p  |
|-----------------|---------|-----|---------|----|
| SGLT2           | 14.50 ± 3.45 | 80.00 ± 12.53 | 27.50 ± 5.01 | p1 = 0.001 |
|                 | p2 = 1.000 | p3 = 0.001 | p3 = 0.001 |   |
| SIRT1           | 77.50 ± 16.16 | 62.50 ± 8.92 | 131.50 ± 19.03 | p1 = 0.427 |
|                 | p2 = 0.047 | p3 = 0.001 | p3 = 0.001 |   |
| FASN            | 84.50 ± 14.38 | 71.50 ± 8.88 | 99.00 ± 26.29 | p1 = 0.452 |
|                 | p2 = 0.013 | p3 = 0.001 |   |   |

Data were expressed as mean ± SEM and analyzed using one-way ANOVA followed by Bonferroni’s post-hoc test at p < 0.05. n = 10. p1 control regarding ISO. p2 control regarding ISO+DAPA. p3 ISO regarding ISO + DAPA. SGLT2: Sodium-glucose transporter 2, SIRT1: Silent information regulator 1, FASN: Fatty acid synthase. ANOVA: Analysis of variance. DAPA: Dapagliflozin. ISO: Isoproterenol. SEM: Standard error of mean.

Discussion

DAPA, a selective SGLT2 inhibitor, is reported to be a possible adjuvant therapy in HF treatment [26]. The previous studies reported the SGLT2 inhibitors’ therapeutic effect in MI despite the absence of known heart receptors [27]. Therefore, identifying the possible mechanism by which DAPA protects against MI progress is an ongoing focus.

We adopted the ISO-induced MI model due to the comparable pathophysiological and morphological alterations of this non-invasive model to those of actual heart disorders [28]. ISO-induced MI as evidenced by a significant decrease in BP, ECG changes, and elevation of cardiac enzymes in addition to diffuse histopathological changes in the cardiac tissues. Balea et al. found a significant ISO role in lowering the BP in acute administration, and prolonged administration increases the BP [20]. The ECG alterations in the ISO group, reduced RR interval, increased HR, and prolonged QRS complex could result from the direct agonist effect of ISO with β1 and β2 adrenoreceptor, slowing of ventricular conduction, and subendocardial ischemia and necrosis [20], [29]. In addition, ST depression could result from the loss of action potential in the myocardial cell membrane induced by ROS overproduction and oxidative stress [20]. Furthermore, ISO induced cardiac damage with the release of cytosolic destructive enzymes [30].

We observed different mechanisms by which ISO-induced acute MI picture by altering different mechanisms. ISO altered ROS production and the release of the inflammatory mediators. The ROS alterations in the cardiac tissue homogenates, increased MDA, and decreased SOD and GSH could participate in cardiac damage through the depletion of the antioxidant enzymes and mitochondrial dysfunction [31], [32]. Moreover, the activation of the inflammatory cytokines (e.g., TNF-α and IL-6) induced myocardial inflammation and subsequent fibrosis [33]. ISO induced MI by altering the lipid profile with the elevation of cholesterol and TG levels, the key risk factors in the development of coronary artery diseases [34].

DAPA pre-treated rats showed a significant reversal of ISO-induced MI changes. SBP improvement, lack of ECG alterations, restoration of muscle integrity with normalization of cardiac enzymes level, reduction of ROS levels and inflammatory mediators, and dramatic improvement in the histopathological changes were significantly observed. The previous studies reported a possible modulatory role of DAPA in enhancing cardiac hemodynamic and cell integrity, reducing free radical levels, and inhibiting lipid peroxidation [35], [36], [37], [38], [39]. The cardioprotective activity of DAPA could be directly induced through the stabilization of the cardiac cells by modulating sodium influx and Ca2+ homeostasis [40]. In addition, DAPA could inhibit the production of different pro-inflammatory cytokines [41]. DAPA could indirectly induced cardioprotection effect by lowering serum cholesterol and TG levels, the common risk factors for vascular endothelitis and cardiac fibrosis [42].

Despite the previously reported DAPA mechanisms, ongoing studies are on focus to identify the mechanism by which SGLT2 inhibitor protects against acute MI. Kashiwagi et al. reported a definite SGLT1 expression as a cardioprotective mechanism in low-glucose heart disease, but SGLT2 expression has not yet been well-defined [43]. Furthermore, the previous studies reported the absence of SGLT2 expression in either human or adult rat heart, excluding a direct SGLT2 inhibition action [44]. The relatively low SGLT2 expression in other studies could result from a crosstalk between the antibody and other SGLT isoforms normally expressed in heart tissue [44].

In the present study, SGLT2 expression was low in the cardiac muscle of the healthy control group, but the expression was elevated in the ISO group. Moreover, the SGLT2 expression markedly declined to reach an almost normal level in the ISO + DAPA group. Lee HW D explained a transient SGLT2 elevation in the acute MI limited to the infarction area while the level diminished with the chronicity of the disease [45]. Furthermore, Lee HW D explained the negative SGLT2 expression in the study by Di Franco due to the inadequacy of the experimental time required for the up- or down-regulation of proteins [46].

We further studied the SIRT1/FASN and SGLT2 expressions in untreated and treated MI groups. SIRT1 was expressed at a low level in the cardiac muscle, which is consistent with its physiological function. SIRT1 protects against aging and apoptosis by controlling the expression of several proteins responsive to oxidative stress [47]. However, the expression was not significantly declined in MI contrary to the previous studies [48]. The previous studies were conducted in diabetic mouse models. Diabetes impaired mitochondrial and peroxisomal stability, promoted the formation of unfolded proteins, and suppressed autophagy [49]. In addition, the discrepancy could be
due to the acute disease onset in our study in contrast to the chronic heart disease model in the previous studies.

The overexpression of SIRT1 in the ISO + DAPA-treated group could be result from a crosstalk between SGLT2 inhibitor and SIRT1. SIRT1 mediated the SGLT2i function through the activation of hypoxia-induced factor-2 alpha (HIF-2α). HIF-2α is the initial regulator for erythropoietin synthesis, the most powerful predictor of lowering the risk of HF in clinical trials [49]. SIRT1 activation promoted autophagy to alleviate oxidative stress and prevent cardiac injury in type 2 DM [50]. Moreover, SGLT2 inhibitors promoted ketogenic nutrient deprivation that induced SIRT1 activation and cardioprotection against HF [38].

In the present study, FASN expression followed the expression of SIRT1 in cardiac tissues. SIRT1 induced FASN expression to maintain lipid and glucose metabolism through the regulation of different mechanisms [51], [52]. However, the beneficial FASN role in cardiac disease is a matter of controversy. FASN induction in stressed myocardium represents a compensatory response to protect cardiomyocytes from pathological calcium flux [53]. On the contrary, FASN expression could be associated with decreased cardiac output and impaired oxygen supply, leading to HF [54]. A study performed by Hansmann et al. found that peroxisome proliferator-activated receptor-gamma activation triggered the FASN upregulation and signs of HF within 2 months [55]. Abd Alla et al. concluded that initial FASN upregulation may be beneficial by supplying more energy substrate to the heart muscle whereas long-term exposure may induce cardiomyopathy and HF [56]. Therefore, further studies are required to validate the functional role of FASN activation in heart tissue on short- and long-term activation. This could necessitate the usage of combinational SGLT2 and FASN inhibitors in MI prevention and subsequent HF.

**Conclusion**

Pre-treatment with SGLT2i (DAPA) may reduce the effects of ISO-induced oxidative stress, elevation of inflammatory cytokines (TNF- and IL-6), and overactivity of myocardial sympathetic flow, which raises the oxygen demand of the cardiac muscles. Although SGLT2 expression is modest in healthy cardiac muscle, acute MI may enhance its expression. The regulation of SIRT1 and FASN expression in the cardiac muscle may be the mechanism by which DAPA exerts its potential cardioprotective effect against MI.

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