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Empagliflozin demonstrates neuroprotective and cardioprotective effects by reducing ischemia/reperfusion damage in rat models of ischemic stroke and myocardial infarction

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Sodium-glucose co-transporter 2 (SGLT2) inhibitors have demonstrated potential neuroprotective and cardioprotective effects in preliminary studies. This study evaluates the efficacy of empagliflozin (EMPA) in reducing ischemia/reperfusion damage in both the brain and heart using rat models. Ischemic stroke and myocardial infarction (MI) were induced in male Sprague-Dawley rats, which were randomized into three groups: (1) Control (no EMPA), (2) Acute treatment (EMPA, 10 mg/kg IV, administered 10 min before ischemia and 1 min before reperfusion), and (3) Chronic treatment (EMPA, 20 mg/kg in food for 7 days before ischemia). Stroke was induced by middle cerebral artery occlusion (MCAO) for one hour, followed by 3 h of reperfusion, and MI was induced by left coronary artery occlusion for 30 min, followed by 3 h of reperfusion. Brain and heart tissues were analyzed for anatomic size of myocardial infarction and stroke. In the brain, cerebral infarction was significantly smaller in both EMPA treatment groups compared to controls (acute: 3.7 ± 1.2%, chronic: 6.9 ± 2.1% vs. control: 14.5 ± 2.5%, $p < 0.05$). Edema was also reduced in the EMPA groups (acute: 5.5 ± 0.9%, chronic: 5.9 ± 0.8% vs. control: 9.6 ± 1.2%, $p < 0.05$). In the heart, MI size was significantly reduced in both EMPA groups (acute: 46.9 ± 2.0%, chronic: 48.8 ± 5.8% vs. control: 70.0 ± 2.6%, $p < 0.05$), and no-reflow size was smaller in the EMPA groups (acute: 36.3 ± 3.3%, chronic: 33.9 ± 4.3% vs. control: 53.4 ± 3.3%, $p < 0.05$). EMPA treatment, both acute and chronic, significantly reduces cerebral infarct volume and edema, as well as myocardial infarct size and no-reflow in rat models of ischemic stroke and myocardial ischemia/reperfusion, indicating substantial neuroprotective and cardioprotective effects.

Keywords Neuroprotection, Cardioprotective effects, Empagliflozin, Myocardial infarction, Ischemic stroke, Sodium-glucose co-transporter 2 inhibitors

Despite significant advancements in thrombolysis, thrombectomy, and stenting, acute ischemic stroke and myocardial infarction continue to be leading causes of death and long-term disability in adults worldwide. While current treatments focus on restoring blood flow in occluded arteries, there remains a critical need for novel therapies to mitigate cell death caused by ischemia/reperfusion injury in both the heart and brain. Sodium-glucose co-transporter 2 (SGLT2) inhibitors, originally developed to promote glucose excretion in type 2 diabetes mellitus, have demonstrated promising benefits in cardiac conditions, particularly in heart failure. Both clinical trials and experimental studies suggest that SGLT2 inhibitors have cardioprotective effects[1,2]. For

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instance, Seefeldt et al.[1] investigated the effects of empagliflozin (EMPA), an SGLT2 inhibitor, on myocardial ischemia/reperfusion injury in rats. The study involved 30 min of occlusion of the left coronary artery followed by 2 h of reperfusion. Rats were treated with either chronic oral EMPA (30 mg/kg) for 7 days prior to coronary occlusion or with acute doses administered 1.5 h before occlusion and at the onset of reperfusion. The results showed that chronic pretreatment with EMPA significantly reduced myocardial infarct size in non-diabetic rats, whereas acute administration had no such effect.

In contrast, the effects of SGLT2 inhibitors on cerebral ischemic stroke remain unclear and controversial[3,4]. A meta-analysis concluded that SGLT2 inhibitors have a neutral effect on stroke risk and found no evidence supporting improved post-stroke outcomes[5]. Vercalsteren et al.[6] studied the effects of empagliflozin (10 mg/kg/day) in a stroke model using both type 2 diabetic and non-diabetic C57BL/6J mice. Their findings indicated that chronic empagliflozin treatment enhanced recovery in diabetic mice but had no impact on infarct size or recovery in non-diabetic mice. Moreover, the potential effects of pre-stroke empagliflozin treatment on cerebral infarct volume in rat models have been insufficiently explored, despite the widespread use of this drug among diabetic and heart failure patients, who are at increased risk of stroke.

These findings highlight the need for further investigation into the optimal timing of EMPA administration and its protective effects in relation to diabetes status. Our study seeks to address these gaps by evaluating the neuroprotective and cardioprotective effects of EMPA, administered either as a 7-day pre-treatment prior to ischemia or acutely during cerebral or myocardial infarction. We utilized standard models, including intraluminal filament middle cerebral artery occlusion (MCAO) for stroke and left coronary artery occlusion/reperfusion for myocardial infarction, using non-diabetic rats.

Methods

All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee at the Huntington Medical Research Institutes (HMRI), which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. The experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85 – 23, National Academy Press, Washington DC, revised 2011). This study adheres to the ARRIVE guidelines. Male Sprague-Dawley rats (approximately 300 g body weight) were purchased from Charles River Laboratories (Hollister, CA) and housed in the HMRI Animal Facility until the time of experimentation. The rats were kept in a controlled environment with a temperature of 22 °C, a 12-hour light/12-hour dark cycle, and had unrestricted access to food and water.

The present study comprised two separate sets of experiments (ischemic stroke and myocardial infarction) with similar therapeutic strategies. In both studies, male Sprague-Dawley rats were randomized into three groups: (1) Control group: rats received regular diet alone (without EMPA, LabDiet 5001 Laboratory Rodent Diet) and subjected to MCAO and reperfusion or proximal left coronary artery occlusion and reperfusion without therapy; (2) Acute treatment group: EMPA (10 mg/kg, IV) given at 10 min prior to middle cerebral artery or left coronary artery occlusion and one minute prior to reperfusion; (3) Chronic treatment group (7 days group): EMPA given by food (20 mg/kg) daily starting 7 days prior to artery occlusion and reperfusion. A dose of 20 mg/kg/day of EMPA has been selected based on its use in previously published studies to evaluate its therapeutic effects in rats[7–9]. In this study, we selected a dose of 0.04% EMPA in the diet, based on the typical daily food intake of 5–6 g per 100 g body weight for SD rats[10], with rats weighing approximately 300 g. A seven-day duration was chosen for chronic treatment based on Seefeldt's study[1].

EMPA (Cat. No.: HY-15409) was purchased from MedChemExpress USA (Monmouth Junction, NJ 08852). For acute intravenous injection in group 2, EMPA was dissolved into dimethyl sulfoxide (DMSO, Cat. No.: D2650, Sigma-Aldrich) in 10 mg/100µl and stored at − 20 ℃. The solution was diluted with Ringer's solution of 0.9 mL before use. For administration by diet in group 3, 0.04% EMPA was mixed with 99.96% Laboratory Rodent Diet (5001), which was prepared by Newco Distributors Inc. (Rancho Cucamonga, CA 91730).

Investigation of neuroprotective effects using MCAO /reperfusion model

Rats were anesthetized with intraperitoneal ketamine (90 mg/kg) and xylazine (10 mg/kg). A cannula was inserted into the trachea and connected to the respirator. Rats were ventilated with room air at 60 strokes/min and 10 ml/kg tidal volume. Body temperature was maintained within 37 ± 0.5 °C range with a heating pad during the procedures. Confirmation of anesthesia was done by lack of response to toe and tail pinch.

Focal cerebral ischemia was induced by a standard intraluminal filament transient occlusion of the right MCAO using the method of Zea Longa et al.[11]. In brief, the right common carotid artery was exposed through a midline incision, and both the external and the internal carotid arteries (ECA and ICA) were isolated and carefully separated from the adjacent vagus nerve. The ECA was dissected further distally and ligated, and a silicon-coated nylon filament (Doccol Corporation, Sharon, MA) was introduced into the ECA lumen through a puncture and was then gently advanced from the ECA to the ICA lumen, and then into the Circle of Willis until mild resistance was felt (around 22 mm length) to effectively block the origin of the middle cerebral artery. Adequate cerebral ischemia was confirmed by continuous laser doppler flowmetry (LDF) probe (moorVMS-LDF, Moor Instruments, Axminster, Devon, UK) and electroencephalography (EEG) monitoring (Fig. 1). After 60 min of MCAO, the suture was withdrawn to restore blood flow for 3 h of reperfusion. Animal body temperature was continuously monitored with a rectal probe and maintained at around 37 °C with a heating blanket during the surgical procedure.

After 3 hours of MCA reperfusion, rats were euthanized at the end of the experiment under deep anesthesia by intraperitoneal injection of ketamine/xylazine followed by intravenous injection of KCL (149 mg/ml, 0.5 ml/250 gm body weight). Ether was not used for euthanasia in this study. The brains were harvested and sectioned into 2 mm coronal slices with a rodent brain matrix. The brain slices were stained for 15 min at 37 °C with 1% triphenyltetrazolium chloride (TTC). The infarction areas were determined by the absence of TTC

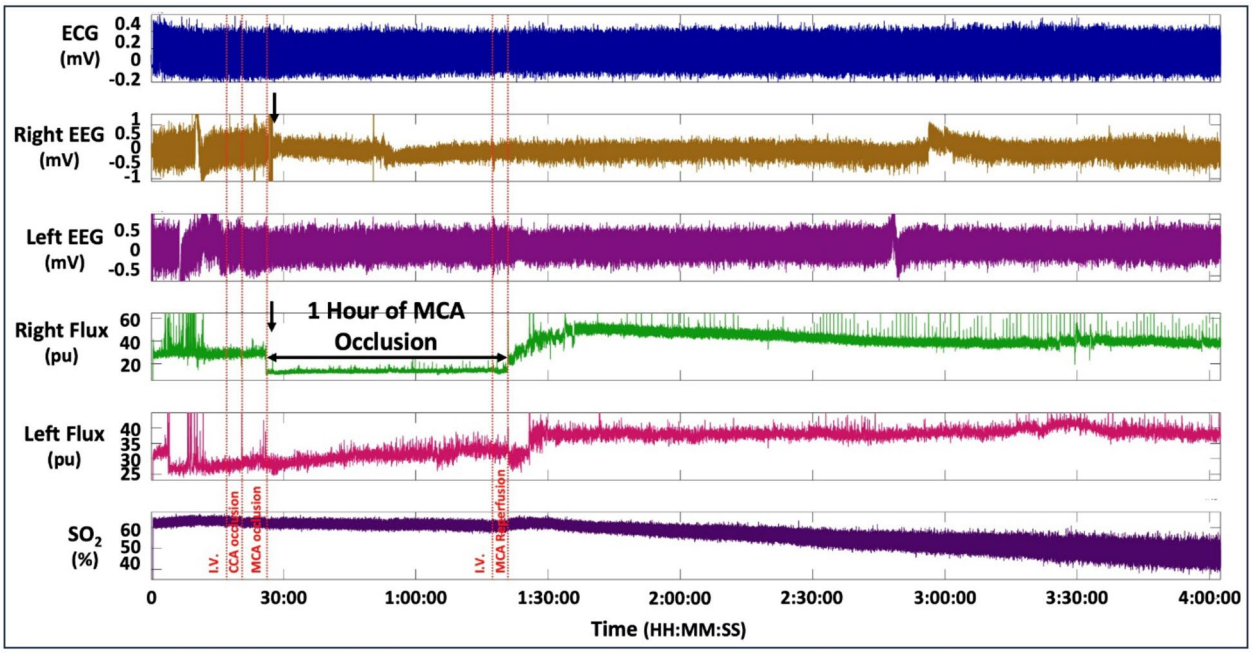

Fig. 1. Representative continuous monitoring of electrocardiogram (ECG), electroencephalography (EEG), cerebral blood flow, and cerebral tissue oxygen using PowerLab LabChart 8.0. Channel 1(top channel): ECG; Channel 2: EEG of right cerebral hemisphere: Electrical activity in EEG leads may not restore after reperfusion due to neuronal damage, mitochondrial dysfunction, excitotoxicity, and blood-brain barrier disruption, with studies[34–36] showing persistent disturbances in brain activity post-reperfusion. 3: EEG of left cerebral hemisphere; Channel 4: Cerebral blood flow of right cerebral hemisphere; Channel 5: Cerebral blood flow of left cerebral hemisphere; Channel 6 (bottom channel): Cerebral tissue oxygen of right cerebral hemisphere. Channel numbering is from the top (Channel 1) to the bottom (channel 6). The amplitude of EEG (channel 2) and blood flow (channel 4) in the right cerebral hemisphere significantly decreased after right cerebral middle artery occlusion (black arrows).

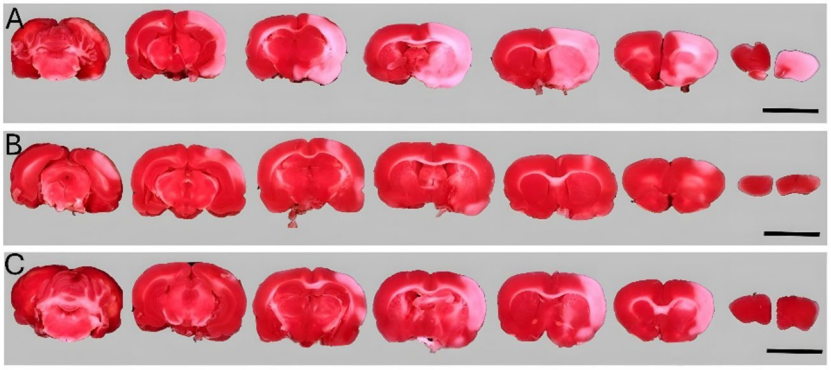

Fig. 2. The rat brain was serially sectioned into seven coronal slices (2 mm thickness) and stained with 1% TTC to observe infarct areas. Viable brain tissue was stained brick red, while infarct areas stained white. Panel A: a sample from the control group; Panel B: a sample from the acute intravenous EMPA treatment group; Panel C: a sample from the 7 days chronic oral EMPA treatment group. EMPA treatment significantly reduced the infarct area in MCAO reperfusion rats. (scale bar = 10 mm).

staining (white color), while the viable areas were stained brick red color (Fig. 2). Hemispheric and infarct volumes were measured using ImageJ software (NIH, Bethesda, MD, USA). Infarct volume was corrected for swelling using the recommended Reglodi's method[12]. In brief, the average of the of the entire contralateral and ipsilateral hemisphere area, and infarct area were obtained from anterior and posterior views. The volume of the contralateral hemisphere (control area that did not contain the infarct) and ipsilateral hemisphere (that contained the infarct), and cerebral absolute unadjusted infarct volume (mm^3) were obtained using the planimetered area multiplied by the slice thickness (2 mm) and summed across all slices to give the total volume. The total absolute

edema-adjusted infarct volume was calculated according to the formula as described by Reglodi[13]: *Absolute edema-adjusted cerebral infarct volume = absolute unadjusted infarct volume × (contralateral hemisphere volume / ipsilateral hemisphere volume)*. The ratio of the infarct volume to the whole brain volume was obtained by dividing the infarct volume by the 2 × contralateral hemisphere volume. Cerebral edema was calculated according to the formula: *(ipsilateral hemisphere volume - contralateral hemisphere volume)/ contralateral hemisphere volume × 100*.

Investigation of cardioprotective effects using left coronary artery occlusion/reperfusion model

Myocardial infarction was induced using previously described methods[14,15]. In brief, the rats were intubated and ventilated with room air after being anesthetized with intraperitoneal ketamine (90 mg/kg) and xylazine (10 mg/kg). The rats were placed on the heated pad to maintain body temperature of 37 ± 0.5 ℃. After shaving and cleaning the neck and chest area, a cutdown was made to expose the right jugular vein and common carotid artery. A PE50 tubing catheter was inserted into the right jugular vein for fluid delivery. A Millar Mikro-Tip® pressure catheter was inserted into the common carotid artery to record arterial pressure and heart rate, then advanced into left ventricular cavity to measure left ventricular pressure and ± dp/dt using a PowerLab Data Acquisition System (ADInstruments Inc. Colorado Springs, CO 80907). A left thoracotomy was conducted at the 4th intercostal space. The pericardium was opened to expose the heart. A 4 – 0 silk suture was passed around the left coronary artery and the ends of the suture were passed by a short polyethylene tube to form a snare for later coronary artery occlusion and reperfusion. The snare was clamped against the heart surface for occlusion and the snare was released for reperfusion. Changes in the color of the left ventricular wall during coronary artery occlusion were examined to confirm the ischemia. The left coronary artery was occluded for 30 min followed by 3 h of reperfusion.

Transthoracic echocardiography was performed before coronary artery occlusion, and at 3 min before reperfusion and 3 h after reperfusion using Philips iE33 E Cart Echocardiography System equipped with 15 – 7 MHz Linear Transducer. The left ventricular end-diastolic internal diameter (LVIDd) and left ventricular end-systolic internal diameter (LVIDs) at the level of papillary muscles were recorded and measured using Two-dimensional guided M-mode echocardiography. Left ventricular fractional shortening (LVFS) was calculated based on the formula *LVFS = (LVIDd – LVIDs)/LVIDd × 100%* in all rats.

At 3 h after coronary artery reperfusion, 4% Thioflavin S solution (0.3 ml) was injected into the jugular vein to assess the distribution of the no-reflow zone to delineate the microvascular obstruction, which appeared dark or non-fluorescent when heart slices are visualized under UV light, while areas receiving perfusion demonstrate a yellow green fluoresces of the Thioflavin S dye. Then the left coronary artery was re-occluded at the original site of occlusion, and Unisperse blue dye (Ciba-Geigy, Hawthorne, NY, USA) was injected into the jugular vein to show the ischemic zone, which appears pink when viewing heart slices under standard white light, while perfused areas appeared blue. Finally, the rats were euthanized with intravenous injections of 1 ml of KCL (149 mg/ml) while the rats were under deep anesthesia to stop the heart in a relatively diastolic or relaxed state. The heart was excised and gently washed in clear saline. The harvested hearts were transected into 4 transverse slices from apex to base and were photographed under white light to delineate the ischemic risk zone and the nonischemic regions (Fig. 5A, B and C). The heart slices were then photographed under ultraviolet light (254 nm wavelength) to delineate the areas of perfusion by Thioflavin S (yellow green fluorescent areas) versus the no-reflow zones (non-fluorescent) (Fig. 5G, H, and I). In order to measure the necrotic zone, the hearts were incubated in TTC for 15 min at 37 °C. TTC staining showed viable cells as brick red and dead or necrotic cells appeared white to yellow (Fig. 5D, E and F). The photographs were used for planimetry and corrected for the weight of each slice to determine the percentage of each heart slice that was at risk, infarcted, or contained no-reflow using software Image J (NIH, Bethesda, MD, USA).

Statistical analysis

The results are expressed as means ± SEM. Statistical analysis was performed using SigmaPlot 12.0. The differences among the 3 groups were evaluated by one-way ANOVA followed by all pairwise multiple comparison procedures (Holm-Sidak method). The results were considered statistically significant when $p < 0.05$.

Results

Results of ischemic stroke study

A total of 47 animals were randomized into 3 groups, and no rats were excluded from this study. Histochemical examination demonstrated that the total absolute edema-adjusted infarct volume was significantly smaller in the acute intravenous EMPA treatment group (60.2 ± 20.1 mm^3, $n = 16$) and chronic oral therapy EMPA group (110.1 ± 33.5 mm^3, $n = 15$) compared with the control group (232.4 ± 40.5 mm^3, $n = 16$; $p = 0.005$). The size of the cerebral infarction, expressed as a percentage (%) of the infarct volume to the whole brain volume, was significantly smaller in both the acute EMPA treatment group (3.7 ± 1.2%, $n = 16$) and chronic EMPA group (6.9 ± 2.1%, $n = 15$) compared with the control group (14.5 ± 2.5%, $n = 16$; $p = 0.005$). The edema of the ipsilateral hemisphere, expressed as a percentage of the edema volume to the contralateral hemisphere volume (%), was significantly reduced in both the acute EMPA group (5.5 ± 0.9%, $n = 16$) and chronic EMPA group (5.9 ± 0.8%, $n = 15$) compared to the control group (9.6 ± 1.2%, $n = 16$; $p = 0.009$) (Fig. 3, and Table 1).

LDF values and the changes in laser doppler signal were continuously recorded before ischemia, during the 1 h of MCA occlusion and 3 h of reperfusion on MCAO ipsilateral (infarcted) hemispheres (the 4th channel in Fig. 1). Baseline LDF values were comparable among control (71.9 ± 7.7 PU (perfusion unit)), acute (91.1 ± 12.8 PU), and 7 days group (71.2 ± 12 PU; $p = 0.392$). The cerebral blood flow dropped similarly during the ischemic

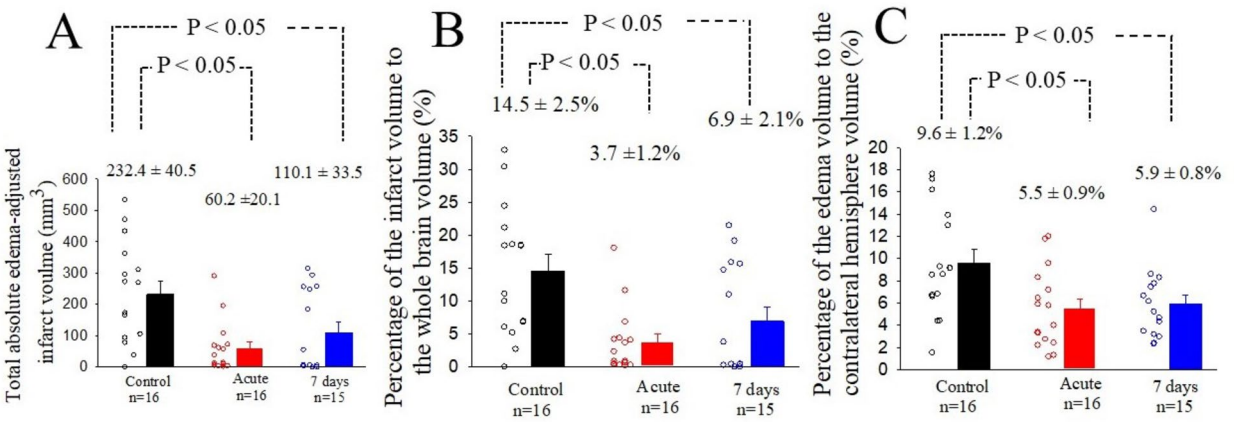

Fig. 3. Both acute and chronic EMPA treatment significantly reduced cerebral infarction and edema in a rat ischemic stroke model. A: Absolute edema-adjusted cerebral infarct volume. B: The ratio of the infarct volume to the whole brain volume. C: Cerebral edema.

	Control	Acute	7 Days	
	n = 16	*n* = 16	*n* = 15	*p* Value
Infarct volume (mm^3)	232.4 ± 40.5	60.2 ± 20.1	110.1 ± 33.5	0.005*
Infarct size (%)	14.5 ± 2.5	3.7 ± 1.2	6.9 ± 2.1	0.005#
Edema size (%)	9.6 ± 1.2	5.5 ± 0.9	5.9 ± 0.8	0.009&

Table 1. Cerebral absolute edema-adjusted infarct volume (mm^3), infarct size and edema size (%). *Control vs. acute $p < 0.05$; control vs. 7days $p < 0.05$. #Control vs. acute $p < 0.05$; control vs. 7days $p < 0.05$. &Control vs. acute $p < 0.05$; control vs. 7days $p < 0.05$. Infarct size was expressed as the ratio of the infarct volume to the whole brain volume (%). Edema size = (ipsilateral hemisphere volume - contralateral hemisphere volume)/ contralateral hemisphere volume × 100).

phase among the 3 groups (Fig. 4A and B). However, administration of 7 days of EMPA in diet had a trend to normalize the cerebral blood flow at 3 h after reperfusion back to the pre-ischemic levels (Fig. 4C).

Results of myocardial infarction study

A total of 48 animals were randomized into 3 groups and operated upon, of which 4 rats in the control group, 4 rats in the acute group, and 1 rat in the 7 days group died during the surgical procedure and were excluded from analysis. Therefore, there were 13 successful rats in each of the 3 groups. As shown in Table 2, there was no significant difference in ischemic risk size, which was expressed as a percentage of LV mass, among the 3 groups. However, as presented in Fig. 5 and Fig. 6; Table 2, both acute (intravenous injection) and chronic (7 days diet with EMPA) administration of EMPA significantly reduced infarction size (expressed as a percentage of LV ischemic risk zone mass) and no-reflow size (expressed as a percentage of LV ischemic risk zone mass) in comparison with the control group.

Hemodynamic data from the carotid artery and left ventricle were obtained using a Millar catheter at 3 distinct time points: At the onset of the experiment under basal conditions, immediately prior to coronary artery reperfusion following 30 min of myocardial ischemia, after 3 h of reperfusion. The recorded and analyzed hemodynamic parameters included systolic and diastolic blood pressure, mean blood pressure, heart rate, LV end-systolic pressure (Pes), LV end-diastolic pressure (Ped), LV maximum increase in systolic pressure (dp/dt max), LV minimum decrease in diastolic pressure (dp/dt min), and the tau-Weiss time constant (tau-Weiss). These data were presented in Table 3. At baseline prior to coronary artery occlusion, hemodynamic parameters reflected identical conditions across 3 analyzed groups; there were no significant differences as anticipated. After 30 min of ischemia just before coronary artery reperfusion, Pes was significantly lower in the 7 days treatment group compared to the acute treatment group (Fig. 7). At 3 h after reperfusion, all of the hemodynamic values were comparable among the 3 groups.

Assessment of cardiac function with echocardiography, including LVIDd, LVIDs, and LVFS were accomplished in all rats and presented in Table 4; Fig. 8. There was no significant differences among the 3 groups at the 3 checked timepoints.

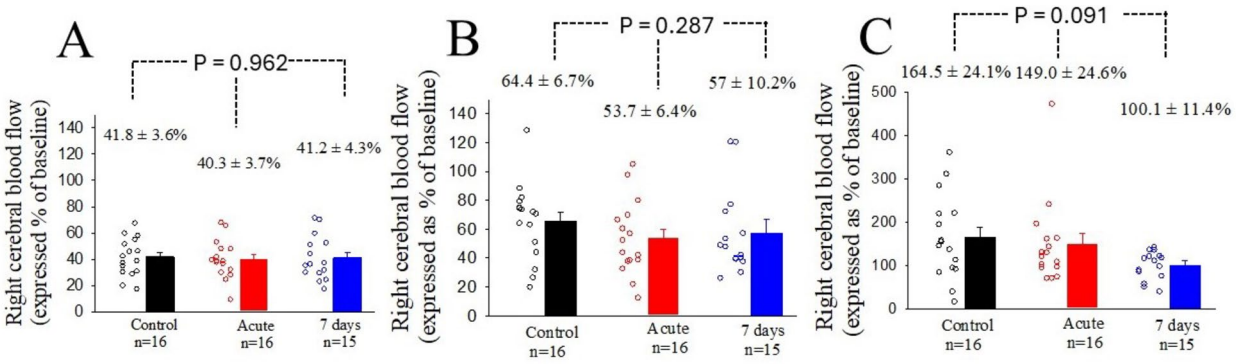

Fig. 4. Cerebral blood flow was evaluated by laser Doppler flowmetry signal (LDF) in the right cerebral hemisphere. The cerebral blood flow data was expressed as the percentage of the baseline LDF signal values prior to MCAO. Panel A and B: The cerebral blood flow measured by the LDF signal decreased at the time immediately after filament insertion into the MCA and were similar among the 3 groups (panel A) and at the time just prior to the withdraw of the filament from the MCA (panel B). Panel C: At 3 h after MCA reperfusion, the cerebral blood flow was 164.5 ± 24.1% of the baseline blood flow value in the control group; but was only 100.1 ± 11.4% of the baseline blood flow value in the 7 days group. Chronic EMPA treatment has a trend to normalize cerebral blood flow back to the pre-ischemic levels at 3 h after reperfusion, suggesting the treatment has a less reactive hyperemia after MCA reperfusion and potentially prevents the ischemic-induced cerebral blood vessel injury.

	Control	Acute	7 days	
	n = 13	*n* = 13	*n* = 13	*p* value
AR/LV	47.9 ± 2.1%	44.1 ± 2.7%	42.1 ± 2.1%	0.215
AN/AR	70.0 ± 2.6%	46.9 ± 2.0%	48.8 ± 5.8%	0.005*
A-NR/AR	53.4 ± 3.3%	36.3 ± 3.3%	33.9 ± 4.3%	0.012#

Table 2. Myocardial ischemia risk zone, infarct size, and no-reflow size ($n = 13$ in each group). AR = ischemic risk area; AN = necrosis area; A-NR = no-reflow area; LV = left ventricular area. *Control vs. acute $p = 0.009$; control vs. 7 days $p = 0.012$. #Control vs. acute $p = 0.019$; control vs. 7 days $p = 0.031$.

Discussion

The key findings of our present study are that both acute and chronic SGLT2 inhibitor EMPA treatment significantly reduced cerebral infarction and edema in a nondiabetic rat ischemic stroke model; and significantly reduced myocardial infarct size and no-reflow in a nondiabetic rat myocardial ischemia/reperfusion model.

Recent studies have demonstrated the expression of SGLT2 transporter in many areas of the brain, including the cerebellum and hippocampus[16], suggesting the critical role of SGLT2 in maintaining neuronal health. EMPA has higher selectivity for SGLT2 (2500-fold of affinity for SGLT2 compared to SGLT1) compared to dapagliflozin (1200-fold) and canagliflozin (250-fold). Several studies confirmed that SGLT2 inhibitors can cross the blood-brain barrier due to their partially lipid soluble property, which suggests the potential therapeutic neuroprotective role of SGLT2 inhibitors in brain injury[3]. Although different types of SGLT2 inhibitors have been studied in acute cerebral ischemia, only a few animal studies are available to demonstrate the neuroprotective of EMPA in ischemic induced damage in brain. Bdel-Latif et al.[17]induced global cerebral ischemia/reperfusion injury through occlusion of the bilateral common carotid arteries for 30 min followed by one-hour reperfusion in male Wistar rats. The rats received EMPA treatment twice (at 1 and 24 h after reperfusion) in two different doses (1 and 10 mg/kg, p.o., respectively). EMPA reduced neuronal death, infarct size and ameliorated cognitive impairment in a dose-dependent manner compared with the vehicle-treated group. In Amin's study[18], rats with streptozotocin-induced hyperglycemia were subjected to transient cerebral ischemia/reperfusion by bilateral common carotid artery occlusion for 30 min, followed by 24 h of reperfusion. EMPA was administered at 1 and 24 h after reperfusion. The treatment significantly reduced cerebral infarct volume, potentially through its glycemic control and associated antioxidant, anti-inflammatory, and anti-apoptotic effects. Our results uniquely provide evidence that EMPA treatment significantly reduces cerebral infarct volume and edema in a standard intraluminal filament middle cerebral occlusion stroke model in non-diabetic rats.

Cerebral autoregulation is impaired during cerebral ischemia/reperfusion injury process[19]. The brain is dependent on a constant supply of oxygen and energy substrates delivered through cerebral blood flow, which is relatively independent of changes in mean arterial pressure within a certain range, termed "cerebrovascular autoregulation". Cerebrovascular autoregulation can become dysfunctional and fail to maintain constant cerebral blood flow. In general, occlusion of the middle cerebral artery produces a severe reduction in cerebral blood flow within the ischemic territory, and reperfusion of the occluded artery will induce a transient increase in cerebral

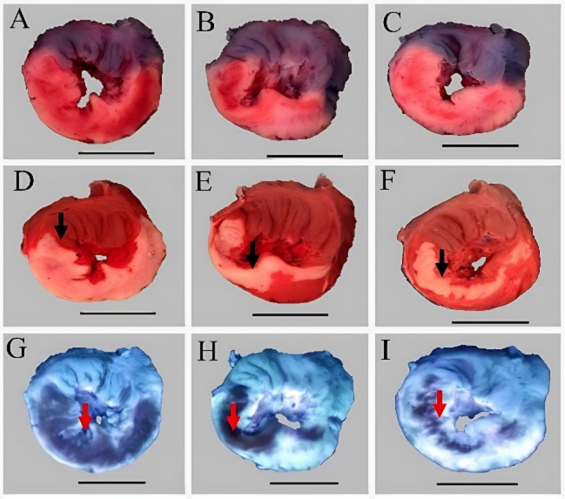

Fig. 5. Representative transverse left ventricular slices showing the ischemic risk zone (panel A, B and C), infarct zone (panel D, E and F), and zone of no-reflow (panel G, H and I) of the heart. Heart slices in panels A, B, and C show the risk area. Blue represents the region that received blue dye during occlusion and is therefore the nonischemic area. The pink area did not receive blue dye and represents the ischemic risk area. Panels D, E and F show infarct area. Heart slice after incubation in TTC to visualize viable tissue (brick red) versus necrosis or infarct tissue (white area). The infarct areas appear as a homogeneous white area (black arrows). Panels G, H and I show the no-reflow area (non-fluorescent). The heart slice shown under UV light to distinguish areas of no reflow area (non-fluorescent) from the areas receiving flow during reperfusion (fluorescent). Non fluorescent perfusion defect that did not receive thioflavin S is visualized as dark blue zone (red arrows). Panel A, D and G are from a heart received saline (non-treated control); Panel B, E and H are from a heart that received acute I.V. EMPA; Panel C, F and I are from a heart that received 7 days of oral EMPA in the diet (scale bar = 5 mm). The area at risk is similar in all 3 groups, but infarct size and no reflow are smaller in both acute and chronic EMPA treatment groups.

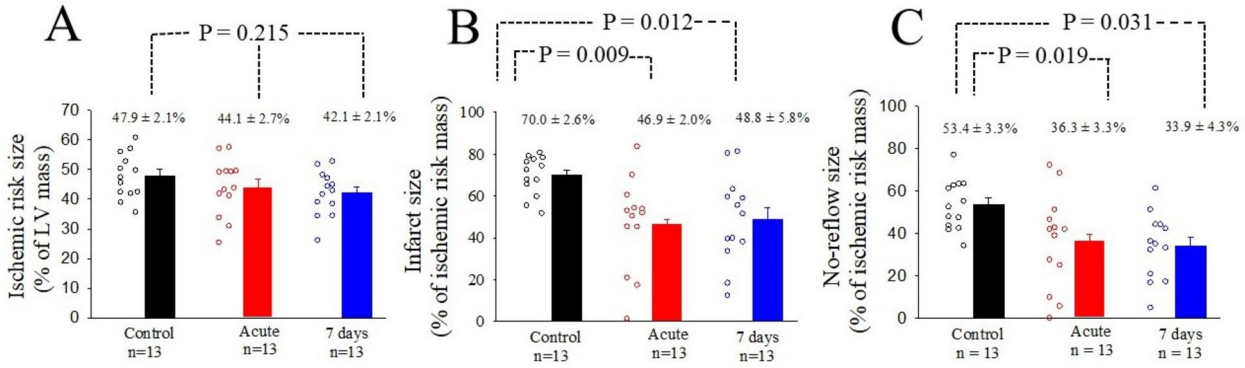

Fig. 6. A: Ischemic risk size, which was expressed as a percentage of LV mass, was comparable among the 3 groups. B: The infarct size, which was expressed as percentage of left ventricle ischemia risk mass that developed necrosis, was significantly reduced in both acute EMPA treated group and chronic EMPA group compared to the control group. C: The no-reflow size, which was expressed as percentage of left ventricle ischemia risk mass, was significantly smaller in the acute EMPA treated group and chronic EMPA group compared to the control group.

blood flow (post-ischemic hyperperfusion or hyperemia) followed by a more sustained reduction in cerebral blood flow (hypoperfusion). In our present study, both acute and chronic EMPA treatment did not affect the cerebral blood flow drop during the ischemic phase compared to the control group (Fig. 4A and B). However, administration of 7 days' EMPA in food does have a trend to normalize cerebral blood flow back to the pre-ischemic levels at 3 h after reperfusion (Fig. 4C). The role of this phenomenon in the prevention of ischemic induced cerebral damage and edema remains to be clarified.

SGLT2 transporter is not expressed in the heart under normal conditions, but it is transiently expressed in ischemic myocardial tissue, which allows SGLT inhibitors to directly affect cardiomyocyte metabolism and attenuate infarct size[20]. In Cai's study[21], the cardioprotective effects of EMPA were evaluated using both

	Control	Acute	7 days	*p* value
Prior to coronary occlusion	*n* = 13	*n* = 13	*n* = 13	
Systolic Pressure (mmHg)	92 ± 2	95 ± 4	91 ± 3	0.582
Diastolic Pressure (mmHg)	68 ± 2	71 ± 3	67 ± 2	0.53
Mean Pressure (mmHg)	78 ± 2	82 ± 3	76 ± 3	0.419
Heart Rate (BPM)	254 ± 8	271 ± 8	271 ± 10	0.282
Pes (mmHg)	88 ± 2	92 ± 3	87 ± 2	0.476
Ped (mmHg)	8.9 ± 0.8	8.2 ± 1.1	9.8 ± 0.6	0.449
dP/dt max (mmHg/s)	5532 ± 222	6137 ± 268	5491 ± 394	0.182
dP/dt min (mmHg/s)	4819 ± 327	5219 ± 406	4627 ± 211	0.43
Tau (ms)	14.0 ± 1.0	12.5 ± 0.7	14.3 ± 1.0	0.325
Before coronary reperfusion				
Systolic Pressure (mmHg)	84 ± 3	84 ± 2	77 ± 1	0.138
Diastolic Pressure (mmHg)	62 ± 3	61 ± 3	55 ± 1	0.128
Mean Pressure (mmHg)	71 ± 3	70 ± 3	63 ± 1	0.099
Heart Rate (BPM)	243 ± 13	246 ± 11	245 ± 13	0.983
Pes (mmHg)	81 ± 2	85 ± 2	77 ± 1	0.01*
Ped (mmHg)	13.6 ± 1.3	12.1 ± 1.6	12.2 ± 1.2	0.681
dP/dt max (mmHg/s)	4714 ± 169	4950 ± 370	4198 ± 146	0.093
dP/dt min (mmHg/s)	3568 ± 182	3736 ± 220	3138 ± 186	0.1
Tau (ms)	18.2 ± 1.7	15.2 ± 0.9	16.8 ± 1.2	0.276
3 h after reperfusion				
Systolic Pressure (mmHg)	78 ± 3	78 ± 2	75 ± 2	0.618
Diastolic Pressure (mmHg)	55 ± 3	52 ± 2	52 ± 2	0.518
Mean Pressure (mmHg)	64 ± 3	62 ± 2	61 ± 2	0.644
Heart Rate (BPM)	268 ± 7	267 ± 7	274 ± 9	0.773
Pes (mmHg)	78 ± 3	79 ± 2	78 ± 2	0.932
Ped (mmHg)	9.8 ± 1.3	8.0 ± 0.9	8.3 ± 1.2	0.551
dP/dt max (mmHg/s)	3664 ± 225	3826 ± 222	3716 ± 131	0.84
dP/dt min (mmHg/s)	3224 ± 214	3405 ± 219	3186 ± 151	0.706
Tau (ms)	17.3 ± 1.6	15.0 ± 1.7	14.9 ± 1.2	0.33

Table 3. Hemodynamic parameters at baseline prior to coronary artery occlusion, at 1 min before coronary artery reperfusion, and at 3 h after coronary artery reperfusion. *Acute vs. 7 days $p = 0.008$; control vs. 7 days $p = 0.161$; acute vs. control $p = 0.158$.

in vivo and in vitro models. In the in vivo model, mice were randomly assigned to either a sham operation group or a myocardial ischemia (45 min)/reperfusion (2 h) (ischemia/reperfusion) injury group. Ten-week-old mice were treated with EMPA (10 mg/kg/day) for seven days prior to myocardial ischemia/reperfusion injury. In the in vitro model, cardiac microvascular endothelial cells were isolated from mice after myocardial ischemia/reperfusion injury. The ischemia/reperfusion injury impaired endothelial barrier function and disrupted the integrity of endothelial cells, while EMPA preserved endothelial homeostasis and maintained cardiac microvascular structure and function. This study was the first to explore the therapeutic potential of EMPA for acute myocardial microvascular impairment. It revealed that EMPA mitigates reperfusion-induced cardiac microvascular damage by enhancing mitophagy, normalizing mitochondrial fission and fusion, reducing endothelial oxidative stress, and inhibiting mitochondrial apoptotic signaling, ultimately improving endothelial function and microvascular structure. Lu et al.[22] demonstrated that EMPA significantly reduces myocardial infarct size caused by ischemia/reperfusion injury in a left anterior descending artery (LAD) ligation model in mice. Using isolated cardiomyocytes and an ex vivo heart perfusion system, EMPA was shown to improve myocardial contractility under physiological and pathological conditions in the isolated cardiomyocytes from adult C57BL/6J mice. Biochemical analysis revealed that EMPA activates the cardiac AMPK signaling pathway and reduces mitochondrial superoxide production during hypoxia and reoxygenation. These findings suggest that EMPA's cardioprotective effects are mediated by AMPK activation and are independent of its hypoglycemic effects. Seefeldt et al.[1] reported that pretreatment with oral EMPA (30 mg/kg) for 7 days significantly reduced myocardial infarct size in non-diabetic rats that were subjected to 30 min of left coronary artery occlusion followed by 2 h of reperfusion, while oral administration at 1.5 h before coronary occlusion and at the onset of reperfusion did not. In our present study, intravenous injection of EMPA (10 mg/kg, IV) 10 min prior to left coronary artery occlusion combined with EMPA (10 mg/kg, IV) one minute prior to reperfusion significantly reduced both infarct size and no-reflow size in the 30 min coronary occlusion followed by 3 h reperfusion non-diabetic rats. Therefore, intravenous therapy rather than oral therapy may be needed for the acute treatment of myocardial infarction. Consistent with our results, Lahnwong et al.[23] reported that acute intravenous

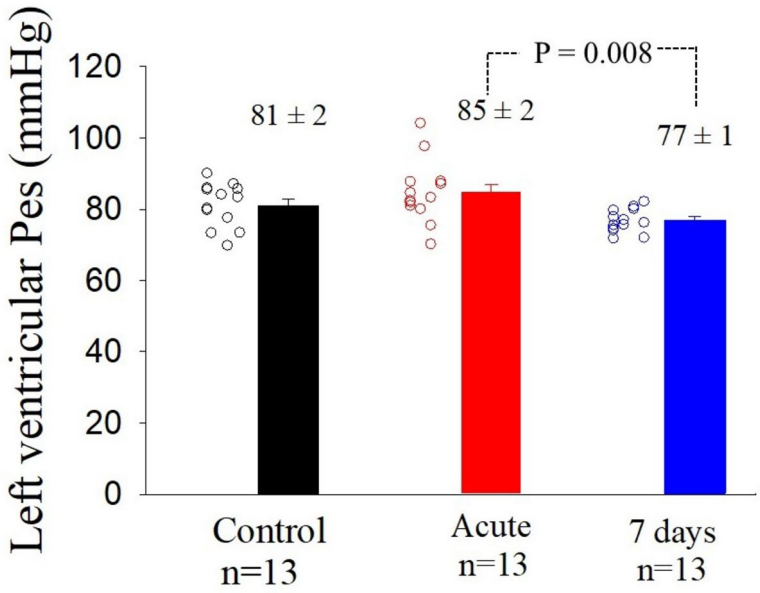

Fig. 7. There was a significant reduction in Pes in the 7-day treatment group compared to the acute treatment group at the time point just before coronary artery reperfusion following 30 min of ischemia.

	Control	acute	7 days	*p* value
Prior to coronary occlusion	*n* = 13	*n* = 13	*n* = 13	
Diastolic LV ID (mm)	6.58 ± 0.31	6.73 ± 0.08	6.97 ± 0.17	0.544
Systolic LV ID (mm)	3.36 ± 0.34	3.11 ± 0.16	3.34 ± 0.21	0.606
LVFS (%)	50.46 ± 3.04	53.99 ± 1.95	52.35 ± 2.46	0.616
At 1 min before reperfusion				
Diastolic LV ID (mm)	7.03 ± 0.23	6.54 ± 0.26	6.69 ± 0.19	0.313
Systolic LLV ID (mm)	4.97 ± 0.19	4.48 ± 0.23	4.66 ± 0.24	0.301
LVFS (%)	29.04 ± 2.11	31.63 ± 1.93	30.65 ± 2.52	0.597
At 3 h after reperfusion				
Diastolic LV ID (mm)	6.27 ± 0.22	6.01 ± 0.16	6.67 ± 0.16	0.096
Systolic LV ID (mm)	4.50 ± 0.22	4.01 ± 0.25	4.61 ± 0.23	0.357
LVFS (%)	27.96 ± 2.81	32.01 ± 3.44	31.06 ± 2.75	0.59

Table 4. Cardiac function assessed by echocardiography. LV = left ventricle; ID = internal dimension; LVFS = left ventricular fractional shortening.

administration of dapagliflozin (SGLT2 inhibitor, 1 mg/kg) 15 min before cardiac ischemia and at 15 min into the cardiac ischemic period exerted cardioprotective effects by attenuating myocardial infarct size, improving LV function and reducing arrhythmias in male Wistar rats with 30 min of coronary occlusion followed by 2 h of reperfusion. Sayour et al.[24] also demonstrated that an intravenous bolus of canagliflozin (SGLT2 inhibitor, 3 μg/kg bodyweight) 5 min after the onset of ischemia protected against myocardial ischemic reperfusion injury in non-diabetic male rats subjected to coronary artery occlusion for 30 min, followed by 120 min reperfusion in vivo. The discrepancy of cardioprotective effects of administration of SGLT2 inhibitor shortly before or during myocardial ischemia suggest that different drug routes of administration may affect the cardioprotective effects of EMPA, and the underlying mechanisms need to be further investigated.

Apart from glucose lowering, SGLT2 inhibitors have been shown to have hemodynamic effects through inhibiting the sympathetic nervous system, lowering blood pressure, reducing arterial stiffness, and improving vascular function[25,26]. In our present study, we observed that pre-ischemia administration of EMPA did not affect hemodynamic parameters during the pre-ischemia phase (Table 3). The fact that we did observe the lack of significant improvement in hemodynamic and ultrasound data with therapy of EMPA may be attributed to the phenomenon of stunned myocardium, where the recovery of function in reperfused ischemic tissue can be delayed for several days[27]. In the future we will carry out studies that allow reperfusion for several days which may be required to see improvements in function. However, compared to the intravenous administration of EMPA, chronic 7 days of diet administration of EMPA significantly reduced left ventricular end-systolic pressure, and showed a non-significant trend toward decreasing of mean blood pressure and left ventricular dp/

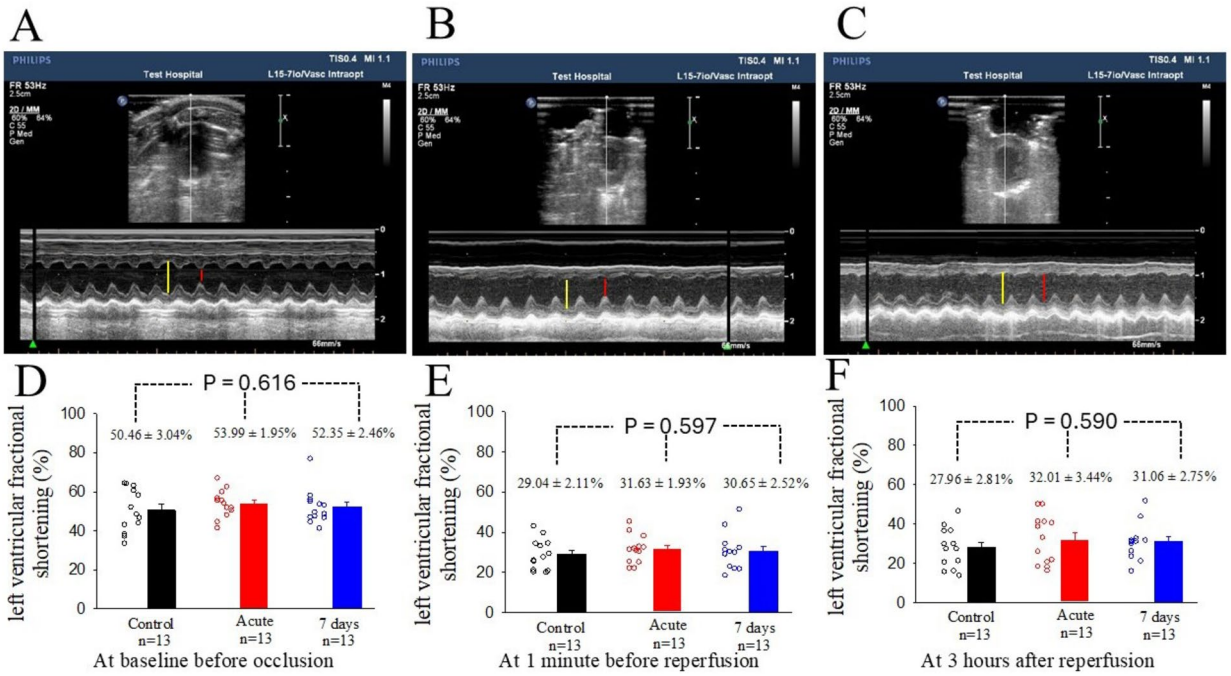

Fig. 8. Representative M-mode echocardiography images used to assess cardiac function in rats: (A) Baseline, prior to left coronary artery occlusion, showing normal left ventricular wall movement. (B) One minute before left coronary artery reperfusion, displaying dyskinesia of the ischemic anterior left ventricular wall. (C) Three hours after reperfusion, where the ischemic anterior left ventricular wall remains dyskinetic, indicative of stunned myocardium. Yellow lines denote the left ventricular diastolic internal diameter (LVIDd), while red lines indicate the left ventricular systolic internal diameter (LVIDs). Cardiac function is evaluated using left ventricular fractional shortening (LVFS), calculated based on LVIDd and LVIDs. There was no significant difference in LVFS among the three groups at the three assessed time points: baseline before left coronary artery occlusion (panel D), one minute prior to coronary reperfusion (panel E), and three hours after coronary reperfusion (panel F).

dt max (Table 3). The exact mechanisms for these different hemodynamic effects of EMPA between acute and chronic treatment are not fully understood and need to be further investigated.

Study limitations

Our study is the first to examine both acute IV and chronic oral treatment in two ischemia and reperfusion disease models: brain and heart. We used rats instead of mice, which were used in some other models, and employed non-hyperglycemic animals, as not all patients experiencing a stroke or heart attack are hyperglycemic. In the MI model, we measured both anatomic infarct size and no-reflow, while in the stroke model, we continuously monitored cerebral blood flow reductions using laser Doppler flowmetry and assessed cerebral function with EEG to ensure model reliability. However, our study has certain limitations that should be acknowledged: (1) Timing of EMPA Administration: In this study, EMPA was administered before the ischemic insult. It remains to be determined whether initiating therapy after occlusion, at reperfusion, or post-reperfusion could also reduce cerebral infarct size. (2) Short-Term Focus: We assessed the effects at 3 h post-artery reperfusion. Whether the reduction in acute cerebral infarct size translates into a sustained, long-term reduction remains unclear and requires further investigation. (3) Neurological and Cardiac Outcomes: It is not yet determined if the observed phenomenon is associated with improved long-term neurological and cardiac function, which will need further clarification. (4) Sex-Specific Outcomes: Our study was conducted on male rats, and whether similar effects would occur in female rats has not been investigated. The potential gender differences in the protective effects of EMPA require further investigation. While EMPA has been shown to reduce the risk of cardiovascular death and hospitalization for heart failure in both men and women[28], a meta-analysis of randomized controlled trials indicated that although both sexes benefit from EMPA, the reduction in primary composite outcomes was less pronounced in women[29]. (5) Preconditioning and postconditioning treatments were not used as positive controls in this study. Employing adaptive response methods for comparison could enhance the study design and outcomes and should be incorporated in future studies. However, previous studies from our laboratory have shown that ischemic preconditioning but not postconditioning reduces infract size in our rat model of myocardial infarction[30,31]. (6) Plasma concentrations of EMPA were not measured after 7 days, which should be considered in our future studies. (7) We note that a limitation of our study was the lack of investigation into underlying mechanisms; however, this was not the study's primary objective. Our goal was to assess the efficacy of an acute IV dose in the in vivo ischemia/reperfusion models in both the brain and heart, and to

determine whether this approach was as effective as chronic oral therapy. This is particularly relevant for patients who are not on SGLT-2 inhibitors for conditions like diabetes, heart failure, or chronic kidney disease but then experience an acute stroke.

Additionally, while the underlying mechanisms of EMPA were not explored in this study, our primary goal was to evaluate the cardioprotective and neuroprotective effects of this SGLT-2 inhibitor in two distinct models of ischemia/reperfusion injury. The mechanistic aspects of the drug's action are beyond the scope of this research, but will be a focus in future studies [for more details about underlying mechanisms, refer to references 3,25]. A recent review[32]provided a comprehensive summary of potential mechanisms underlying the benefits of SGLT2 inhibitors in ischemia/reperfusion phenomena by targeting multiple pathways, including metabolic optimization, ionic regulation, and inflammation reduction. By enhancing ketone body utilization, SGLT-2 inhibitors improved cardiac efficiency and reduced oxidative stress. SGLT2i also inhibit sodium-hydrogen exchangers, preventing calcium overload and ischemic injury, while also boosting mitochondrial function and reducing inflammation. With antiplatelet properties and arrhythmia prevention, SGLT2i offer a multifaceted cardiovascular benefits, though further research is needed to clarify their role during ischemia. Our study adds to this body of evidence by demonstrating the efficacy of EMPA in both acute and chronic settings, suggesting that its benefits may extend beyond short-term metabolic effects to longer-term structural and functional recovery in ischemia/reperfusion injury. Another recent review[33] focused on the clinical use of EMPA in post-myocardial infarction settings. Studies show that EMPA reduces N-terminal pro-brain natriuretic peptide (NT-proBNP) levels and improves structural and functional echocardiographic measures in patients with post-myocardial infarction. It also lowers body weight, blood pressure, and uric acid, while improving renal function and ejection fraction. EMPA is effective in treating heart failure related to recent MI and reduces functional decline, particularly in patients with type 2 diabetes and preserved estimated glomerular filtration rate (eGFR). Further trials are needed to confirm its long-term efficacy and benefits post-MI. Our preclinical data underscore the importance of timing and dosing in achieving optimal protective effects in ischemia/reperfusion models.

Conclusions

This study provides compelling evidence for the neuroprotective effects of EMPA in acute ischemic stroke and its cardioprotective effects in acute myocardial infarction in non-diabetic rats. Unlike previous studies that primarily focused on models involving metabolic dysfunction (e.g., hyperglycemia), our study demonstrates that EMPA significantly reduces infarct size and edema in non-diabetic rats. By replicating clinically relevant ischemia/reperfusion injury scenarios, our findings suggest that EMPA's protective effects extend beyond glycemic control and may involve additional mechanisms, such as anti-inflammatory, antioxidative, and cerebrovascular benefits.

Furthermore, our study reveals that intravenous administration of EMPA immediately before ischemia and prior to reperfusion effectively reduces both infarct size and no-reflow size. This contrasts with the findings of Seefeldt et al.[1], who reported that while oral EMPA pretreatment for seven days significantly reduced infarct size, acute oral administration at the onset of ischemia did not. Our results suggest that, for acute myocardial ischemia/reperfusion injury, intravenous administration may be a more effective strategy than oral delivery, likely due to pharmacokinetic factors influencing drug bioavailability and onset of action. Our findings underscore the potential importance of the administration route: while chronic oral EMPA therapy has shown protective effects in both preclinical and clinical settings, our results indicate that acute intravenous administration may be preferable for achieving immediate cardioprotective benefits during myocardial infarction.

Future studies should further investigate the mechanisms underlying these route-dependent differences in efficacy, particularly in relation to EMPA's effects on endothelial function, mitochondrial dynamics, and inflammatory signaling. Additionally, given that ischemia often occurs unexpectedly in clinical settings, where therapeutic intervention typically takes place at reperfusion or post-reperfusion, future research should also examine the effects of post-ischemic EMPA administration, long-term functional outcomes, and potential sex differences. A deeper understanding of the optimal timing and route of administration will be critical for translating these findings into clinical practice.

Data availability

The data presented in this study are available on request from the corresponding author Wangde Dai via email wangde.dai@hmri.org.

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References

1. Seefeldt, J. M. et al. Cardioprotective effects of empagliflozin after ischemia and reperfusion in rats. *Sci. Rep.* **11** (1), 9544 (2021).
2. Tavecchia, G. A., Gualini, E., Sacco, A. & Oliva, F. The role of sodium-glucose co-transporter 2 inhibitors in myocardial infarction: available evidence and future perspectives. *Eur. Heart J. Suppl.* **26** (Suppl 1), i84–i87 (2024).
3. Al Hamed, F. A. & Elewa, H. Potential therapeutic effects of sodium Glucose-linked cotransporter 2 inhibitors in stroke. *Clin. Ther.* **42** (11), e242–e249 (2020).
4. Takashima, M. et al. Low-dose sodium-glucose cotransporter 2 inhibitor ameliorates ischemic brain injury in mice through pericyte protection without glucose-lowering effects. *Commun. Biol.* **5** (1), 653 (2022).
5. Tsai, W. H. et al. Effects of SGLT2 inhibitors on stroke and its subtypes in patients with type 2 diabetes: a systematic review and meta-analysis. *Sci. Rep.* **11** (1), 15364 (2021).
6. Vercalsteren, E. et al. The SGLT2 inhibitor empagliflozin promotes post-stroke functional recovery in diabetic mice. *Cardiovasc. Diabetol.* **23** (1), 88 (2024).
7. Connelly, K. A. et al. Load-independent effects of empagliflozin contribute to improved cardiac function in experimental heart failure with reduced ejection fraction. *Cardiovasc. Diabetol.* **19** (1), 13 (2020).

8. Yaribeygi, H. et al. Sodium glucose Cotransporter-2 inhibitor empagliflozin increases antioxidative capacity and improves renal function in diabetic rats. *J. Clin. Med.* **12** (11), 3815 (2023).
9. Kim, S., Jo, C. H. & Kim, G. H. Effects of empagliflozin on nondiabetic salt-sensitive hypertension in uninephrectomized rats. *Hypertens. Res.* **42** (12), 1905–1915 (2019).
10. Sharp, P. & Villano, J. S. The Laboratory Rat. 2nd Edition. Published December 11, by CRC Press. ISBN 9781439829868. (2012).
11. Zea Longa, E. L., Weinstein, P. R., Carlson, S. & Cummins, R. Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke* **20**, 84–91 (1989).
12. Nouraee, C. et al. A brief review of Edema-Adjusted infarct volume measurement techniques for rodent focal cerebral ischemia models with practical recommendations. *J. Vasc Interv Neurol.* **10** (3), 38–45 (2019).
13. Reglodi, D. et al. Effects of pretreatment with PACAP on the infarct size and functional outcome in rat permanent focal cerebral ischemia. *Peptides* **23** (12), 2227–2234 (2002).
14. Dai, W. & Kloner, R. A. Effects of acetaminophen on myocardial infarct size in rats. *J. Cardiovasc. Pharmacol. Ther.* **8** (4), 277–284 (2003).
15. Dai, W. & Kloner, R. A. Experimental cell transplantation therapy in rat myocardial infarction model including nude rat Preparation. In: (ed Lee, R. J.) Stem Cells for Myocardial Regeneration, Methods in Molecular Biology™. Springer; (2010). Volume 660, Part 2, 99–109.
16. Piątkowska-Chmiel, I. et al. Molecular and neural roles of sodium-glucose cotransporter 2 inhibitors in alleviating neurocognitive impairment in diabetic mice. *Psychopharmacol. (Berl).* **240** (4), 983–1000 (2023).
17. Bdel-Latif, R. G., Rifaai, R. A. & Amin, E. F. Empagliflozin alleviates neuronal apoptosis induced by cerebral ischemia/reperfusion injury through HIF-1α/VEGF signaling pathway. *Arch. Pharm. Res.* **43**, 514–525 (2020).
18. Amin, E. F., Rifaai, R. A. & Abdel-Latif, R. G. Empagliflozin attenuates transient cerebral ischemia/reperfusion injury in hyperglycemic rats via repressing oxidative-inflammatory-apoptotic pathway. *Fundam Clin. Pharmacol.* **34** (5), 548–558 (2020).
19. Kunz, A. & Iadecola, C. Cerebral vascular dysregulation in the ischemic brain. *Handb. Clin. Neurol.* **92**, 283–305 (2009).
20. Lee, S. Y. et al. Sodium/glucose Co-Transporter 2 inhibitor, Empagliflozin, alleviated transient expression of SGLT2 after myocardial infarction. *Korean Circ. J.* **51** (3), 251–262 (2021).
21. Cai, C. et al. Empagliflozin attenuates cardiac microvascular ischemia/reperfusion through activating the AMPKα1/ULK1/FUNDC1/mitophagy pathway. *Redox Biol.* **52**, 102288 (2022).
22. Lu, Q. et al. Empagliflozin attenuates ischemia and reperfusion injury through LKB1/AMPK signaling pathway. *Mol. Cell. Endocrinol.* **501**, 110642 (2020).
23. Lahnwong, S. et al. Acute Dapagliflozin administration exerts cardioprotective effects in rats with cardiac ischemia/reperfusion injury. *Cardiovasc. Diabetol.* **19** (1), 91 (2020).
24. Sayour, A. A. et al. Acute Canagliflozin treatment protects against in vivo myocardial ischemia/reperfusion injury in non-diabetic male rats and enhances endothelium-dependent vasorelaxation. *J. Transl Med.* **17** (1), 127 (2019).
25. Lopaschuk, G. D. & Verma, S. Mechanisms of cardiovascular benefits of sodium glucose Co-Transporter 2 (SGLT2) inhibitors: A State-of-the-Art review. *JACC Basic. Transl Sci.* **5** (6), 632–644 (2020).
26. Durante, W., Behnammanesh, G. & Peyton, K. J. Effects of Sodium-Glucose Co-Transporter 2 inhibitors on vascular cell function and arterial remodeling. *Int. J. Mol. Sci.* **22** (16), 8786 (2021).
27. Kloner, R. A. Stunned and hibernating myocardium: where are we nearly 4 decades later?? *J. Am. Heart Assoc.* **9** (3), e015502 (2020).
28. Htoo, P. T. et al. Cardiorenal effectiveness of empagliflozin vs. glucagon-like peptide-1 receptor agonists: final-year results from the EMPRISE study. *Cardiovasc. Diabetol.* **23** (1), 57 (2024).
29. Rivera, F. B. et al. Sex differences in cardiovascular outcomes of SGLT-2 inhibitors in heart failure randomized controlled trials: A systematic review and meta-analysis. *Am. Heart J. Plus.* **26**, 100261 (2023).
30. Li, Y. & Kloner, R. A. The cardioprotective effects of ischemic 'preconditioning' are not mediated by adenosine receptors in rat hearts. *Circulation* **87** (5), 1642–1648 (1993).
31. Dow, J. & Kloner, R. A. Postconditioning does not reduce myocardial infarct size in an in vivo regional ischemia rodent model. *J. Cardiovasc. Pharmacol. Ther.* **12** (2), 153–163 (2007).
32. Quentin, V., Singh, M. & Nguyen, L. S. A review of potential mechanisms and uses of SGLT2 inhibitors in ischemia-reperfusion phenomena. *World J. Diabetes*. **13** (9), 683–695 (2022).
33. Ravendran, K. et al. The use of empagliflozin post myocardial infarction. *Cureus* **15** (6), e40602 (2023).
34. Sysoev, Y. I. et al. Changes in brain electrical activity after transient middle cerebral artery occlusion in rats. *Neurol. Int.* **14** (3), 547–560 (2022).
35. Liu, P. et al. Electrophysiological signatures in global cerebral ischemia: neuroprotection via chemogenetic Inhibition of CA1 pyramidal neurons in rats. *J. Am. Heart Assoc.* **13** (24), e036146 (2024).
36. Amini, N. et al. Improve baroreflex sensitivity and nucleus tractus solitarius electrical activity in renal Ischemia-Reperfusion injury. *Arq. Bras. Cardiol.* **117** (2), 290–297 (2021).

Author contributions

The authors made substantial contributions to the conception or design of the work (R.A.K.) or to the acquisition, analysis, or interpretation of data for the work (W.D., J.L., J.C., N.M.P., R.A., R.A.K); participated in critically revising the manuscript (W.D., J.L., J.C., N.M.P., R.A., R.A.K); approved the final version to be published (W.D., J.L., J.C., N.M.P., R.A., R.A.K); and agreed to be accountable for all aspects of the work (W.D., J.L., J.C., N.M.P., R.A., R.A.K).

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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