

The Effect of Empagliflozin on Janus Kinase 2/ Signal Transducer and Activator of Transcription 3 Pathway in Patients with Type 2 Cardiorenal Syndrome

ABSTRACT

Background: Empagliflozin (EMPA) demonstrates cardioprotective effects on the patients with heart failure, but its effects in cardiorenal syndrome (CRS) remain unspecified. The purpose of the exploratory study was to investigate the effect of EMPA on patients with type 2 CRS and type 2 diabetes mellitus (DM).

Methods: This study was a randomized trial of patients with type 2 CRS and DM done between December 2020 and January 2022. Patients were randomly allocated to the control group and the EMPA group using EMPA as an add-on treatment. Serum interleukin 6 (IL-6), janus kinase 2 (JAK-2), and signal transducer and activator of transcription 3 (STAT-3) concentrations were measured in 102 patients with CRS and healthy individuals without any disease using enzyme-linked immunosorbent assay before and after treatment. The evaluation of renal function was measured by immunoturbidimetry, and cardiac function was estimated by doppler echocardiography. Rates of adverse events and major adverse cardiac events (MACE) were documented.

Results: The results showed that EMPA decreased the level of IL-6 but increased the level of JAK-2 and STAT-3 in patients. Additionally, the results suggest EMPA significantly reduced the incidence of MACE compared to the control group, while the rate of adverse events did not significantly differed.

Conclusions: Our study suggested that the cardiorenal benefits conferred by EMPA might be driven by anti-inflammatory effects, cooperated with the activation of JAK2/STAT3 signaling pathways, leading to modest short-term improvements in patients with type 2 CRS. The overall safety and low complication make EMPA a significant choice for clinical application.

Keywords: Empagliflozin, Janus kinase 2/signal transducer and activator of transcription 3 signaling pathway, cardiorenal syndrome, major adverse cardiac events

INTRODUCTION

Cardiorenal syndrome (CRS) has been known as a spectrum of disorders of the heart and kidneys, whereby acute or chronic dysfunction of one organ may induce acute or chronic dysfunction in the other. Different syndromes have been categorized into 5 subtypes and type 2 occurred when chronic abnormalities in cardiac function result in progressive chronic kidney injury or dysfunction, leading to high mortality.¹ Heart failure is a major public health problem affecting more than 23 million patients worldwide, and nearly 25% of the patients have type 2 CRS which is associated with reduced survival.^{2,3}

Consequently, there is a profound and urgent need for new drug therapies that prevent, treat, or slow the progression of CRS type 2. In recent years, there is evidence that empagliflozin (EMPA), a sodium-dependent glucose transporter 2 inhibitor, significantly reduced the incidence of cardiovascular and renal events in patients with or without type 2 diabetes mellitus (T2DM).⁴⁻⁶ The update of the Canadian Cardiovascular Society guidelines for the management of heart failure recommended that regardless of diabetes status, these

ORIGINAL INVESTIGATION

Pei Zhou 

Xiangyu Tang 

Yunxia Deng 

Rong Wu 

Yuan Yi 

Hao Deng 

Qiongqiao Cao 

Department of Cardiology, The Loudi Central Hospital, University of South China, Hunan, China

Corresponding author:

Xiangyu Tang
✉ 373609826@qq.com

Received: September 4, 2023

Accepted: February 1, 2024

Available Online Date: March 5, 2024

Cite this article as: Zhou P, Tang X, Deng Y, et al. The effect of empagliflozin on janus kinase 2/signal transducer and activator of transcription 3 pathway in patients with type 2 cardiorenal syndrome. *Anatol J Cardiol.* 2024;28(5):229-235.



Copyright@Author(s) - Available online at anatoljcardiol.com.
Content of this journal is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.

DOI:10.14744/AnatolJCardiol.2024.2736

agents should be considered as standard or foundational therapy in patients with heart failure with reduced ejection fraction (HFrEF).⁷ Empagliflozin targets the kidney to inhibit the reabsorption of filtered glucose in the renal proximal tubule. The potential mechanisms responsible for the improved heart and renal outcomes are likely to be multifactorial, and direct renovascular and hemodynamic effects are postulated to play a central role, including the reductions in intraglomerular pressure, inflammation reaction, and albuminuria in urine.⁸⁻¹⁰ However, whether the janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) pathway participates in CRS is unknown, and the effects of EMPA on patients with CRS remain unclear. Thus, the purpose of this exploratory work was to observe the effect of EMPA on the patients with CRS type 2 and type 2 DM by detecting interleukin 6 (IL-6), JAK-2, STAT-3 in serum, to provide evidence for the clinical application of EMPA.

METHODS

Participants

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. All subjects have given their written informed consent and this study protocol was reviewed and approved by the Ethical and Experimental Committee of the Loudi Central Hospital, approval number [2021-ethics (research)-032].

The trial was designed as a randomized trial of EMPA 10 mg or not for at least 12 weeks and ran between December 2020 and January 2022. The blood samples (4 mL) were collected from CRS patients and healthy subjects who were diagnosed or received routine examinations at the Loudi Central Hospital. Patients with CRS were eligible if they were aged between 40 and 80, had type 2 DM, prevalent cardiovascular disease typically of atherosclerotic origin (coronary artery disease, hypertension), and an estimated glomerular filtration rate of 45-60 mL/min/1.73 m². A total of 102 eligible participants were randomly assigned to the control group (n=52) and experimental group (n=50) treated with EMPA (10 mg once daily) added to their standard care, including HFrEF (n=20), heart failure with mid-range ejection fraction (HfmrEF) (n=36), and heart failure with

preserved ejection fraction (HFpEF) (n=36). All patients met the 2019 American Heart Association (AHA) diagnostic criteria for CRS type 2¹¹ and were treated with conventional heart failure and antihyperglycemic therapy. Another group of normal person (n=20) was selected as the healthy group. Fasting blood samples and echocardiographic measurements were performed before and 12 weeks after treatment. Enzyme-Linked Immunosorbent Assay (ELISA) was performed using the supernatants to measure the levels of IL-6, JAK-2, and STAT-3. The evaluation of renal function was measured by immunoturbidimetry, and cardiac function was estimated by Doppler echocardiography. The rates of adverse reactions and major adverse cardiac events (MACE) during the treatment were recorded.

Blood Testing

Blood samples were obtained via peripheral vein after 12 weeks of treatment and at admission. Plasma after receiving treatment of EMPA was separated by centrifugation and stored at -80°C. IL-6, JAK-2, and STAT-3 were measured by an ELISA kit (RENJIEBIO, RJ11850, RJ13240, RJ14355, Shanghai, China) according to the manufacturer's instructions. Serum creatinine (Ser), blood urea nitrogen (BUN), and uric acid (UA) were measured by the same biochemical detector.

Echocardiography

After 12 weeks of treatment and at admission, Doppler echocardiography was performed in all patients using an echo machine (EPIQ7, PHILIPS, Netherlands) equipped with a 10 MHz phased array linear transducer for serial assessment of cardiac structure and function, as previously described.

According to standard procedures, to survey left atrium diameter (LAD), left ventricular end diastolic (LVED), and left ventricular ejection fraction (LVEF), all measurements performed with an off-line analysis system by 2 practiced sonographers who were blinded to prior results, were based on the average of 3 to 6 consecutive cardiac cycles.

Adverse and Major Adverse Cardiac Events

Although well tolerated, there are known adverse effects with EMPA that require clinical monitoring, such as incidence of urinary and genital tract infections, diabetic ketoacidosis, volume depletion particularly in the setting of concomitant diuretic use, hypoglycemia, kidney failure, and anaphylactic response. Major adverse cardiac events events include nonfatal myocardial infarction, serious cardiac arrhythmias, stroke, heart failure hospitalization, and cardiovascular mortality.

Statistical Analysis

Before data was collected, a statistical power analysis was performed for estimating the required sample size. The analysis revealed that at least 114 participants and 20 healthy controls would be required to detect significant differences in serum IL-6 levels between patients with EMPA or not, with a power of 90% and an alpha error of 10%. The power was 86% in our experiment. Data was analyzed with SPSS version 25.0 (IBM Corp, Armonk, NY, USA). Continuous data were

HIGHLIGHTS

- Effects of empagliflozin (EMPA) on cardiorenal syndrome (CRS) remain unclear. The purpose of the exploratory study was to investigate its effect on patients with type 2 CRS and type 2 diabetes mellitus.
- Empagliflozin decreased the level of interleukin 6 (IL-6) while increasing the level of janus kinase 2 (JAK-2) and signal transducer and activator of transcription 3 (STAT-3) in patients.
- The cardiorenal benefits conferred by EMPA might be driven by anti-inflammatory effects, cooperated with the activation of JAK2/STAT3 signaling pathways.

Table 1. Baseline Clinical Characteristics of Patients with Empagliflozin and Controls

Group (n)	Healthy Group (20)	Control (52)	EMPA (50)	P
Age, years	69.40 ± 4.74	71.21 ± 8.47	68.18 ± 8.41	.160
Men, %	11 (55.00)	30 (57.69)	32 (64.00)	.723
BMI, kg/m ²	23.63 ± 0.89	23.38 ± 2.17	23.18 ± 1.80	.637
Smoker, %	5 (25.00)	27 (51.92)	27 (54.00)	.073
Alcohol abuse, %	2 (10.00)	10 (19.23)	6 (12.00)	.478
Hypertension, %	-	26 (50.00)	22 (44.00)	.546
Hyperlipidemia, %	-	14 (26.92)	20 (40.00)	.163
Chronic bronchitis, %	-	15 (28.85)	8 (16.00)	.123
NT-proBNP, ng/L	402.5 ± 73.16 ^a	5771.70 (1962.50, 13141.97) ^b	5983.00 (1980.00, 12999.47) ^b	.000
cTnI, mg/L	0.01 (0.01-0.01) ^a	0.01 (0.01,0.01) ^b	0.01 (0.01,0.03) ^b	.000
D-dimer, mg/L	0.5 (0.20-3.29)	366.50 (1.32, 1392.50)	416.00 (1.09, 1400.00)	.857
FBG, mmol/L	5.04 ± 0.58 ^a	5.99 (5.14, 7.63) ^b	6.82 (5.31, 8.39) ^b	.000
HbA1c, %	5.10 ± 0.60 ^a	7.25 (6.50, 8.50) ^b	7.35 (6.88, 8.75) ^b	.000
ALT, U/L	20.00 (15.00-25.50)	18.50 (14.75,25.00)	18.50 (15.00,27.5)	.844
AST, U/L	19.55 ± 6.85	15.00 (12.00, 24.00)	15.50 (12.00, 32.00)	.522
TC, mmom/L	3.49 ± 0.70 ^a	4.33 ± 1.37 ^b	4.34 ± 1.33 ^b	.026
TG, mmom/L	1.27 ± 0.39	1.23 (0.84,1.84)	1.52 (1.01, 2.12)	.144
HDL-C, mmom/L	1.19 ± 0.39	0.91 (0.74,1.14)	0.90 (0.74,1.16)	.070
LDL-C, mmom/L	2.06 ± 0.72	2.22 (1.56, 2.90)	2.26 (1.61, 3.18)	.374

^{a,b,c}The same letters show that there is no statistically significant difference between them, and different letters show that there is a statistically significant difference. ALT, alanine aminotransferase; aminotransferase; AST, aspartate transaminase; cTnI, cardiac troponin; EMPA, empagliflozin; FBG, fasting blood-glucose; HbA1c, hemoglobin a1c; HDL-C, high density lipoprotein; LDL-C, low density lipoprotein; NT-proBNP, n-terminal b-type natriuretic peptide; TC, total cholesterol; TG, triglyceride.

presented as means ± standard deviations, while categorical variables or median and interquartile range for non-normally distributed variables. We compared the experimental parameters and the rate of adverse events and MACE between groups were performed using Mann-Whitney *U*-tests. The main effects of treatment of EMPA were determined using 2-way analysis of variance and Kruskal-Wallis tests. If the main or interaction effects were found to be significant in the Kruskal-Wallis tests, then multiple comparison testing between the means of different groups was performed using the Least Significant Difference (LSD) or Bonferroni post hoc test. Chi-square test or the Fisher's exact test was used for categorical data. Differences were considered statistically significant at *P* < .05.

RESULTS

Baseline Clinical Characteristics and Medications of Patients with Empagliflozin and Controls

The health group had a low level of NT-proBNP, D-dimer, FBG, and HbA1c compared with patients with CRS. While basic information (e.g., age, sex, etc.), baseline biomarkers (e.g., BMI, NT-proBNP, cTnI, blood lipids), and past medical history (e.g., hypertensive disease, hyperlipidemia, chronic bronchitis) between these groups were not significantly different (Table 1). All patients continued with their background medications for glycemic control (e.g., metformin, sulfonylureas, thiazolidinedione, glucagon-like peptide-1 agonists, and insulin), heart failure medications, anti-hypertensive therapy, lipid-lowering therapy, and no significant difference was found between the 2 groups (Table 2).

Effect of Empagliflozin on IL-6, JAK-2 and STAT-3 of Cardiorenal Syndrome Patients Compared with Healthy people

Patients with CRS have a higher level of IL-6, and low expression of JAK-2 and STAT-3 was statistically significant compared to the control group (*P* < .05). These phenomena remind us of a possible connection between IL-6 and JAK2/STAT3 signaling, indicating that CRS activates inflammatory reactions by inhibiting JAK2/STAT3 signaling. However, the level of IL-6 decreased after the treatment of EMPA, while JAK-2 and STAT-3 were increased, suggesting that EMPA exerts anti-inflammatory effects in patients with type 2 CRS (Table 3).

Table 2. Baseline Medications of the Patients

	Healthy Group (20)	Control (52)	EMPA (50)	χ ² value	P
Diuretic, %	-	49 (94.23)	46 (92.00)	0.198	.656
Cardiotonic, %	-	16 (30.77)	19 (38.00)	0.591	.533
Nitrate, %	-	24 (46.15)	23 (46.00)	0.000	1.000
Antiplatelet drug, %	-	33 (63.46)	26 (52.00)	1.373	.316
ACEI/ARB, %	-	20 (38.46)	24 (48.00)	0.945	.424
CCB, %	-	26 (50.00)	22 (44.00)	0.368	.559
Lipid-lowering drug, %	-	29 (58.00)	30 (60.00)	0.187	.693

ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blockers; CCB, calcium-channel blockers; EMPA, empagliflozin.

Table 3. Effect of Empagliflozin on Interleukin 6, Janus Kinase 2, Signal Transducer and Activator of Transcription 3

	Healthy Group (20)	Control (52)	EMPA (50)	P
IL-6 (pg/mL)	1.37 (1.08, 2.90) ^a	6.12 (4.54, 14.59) ^b	5.94 (3.37, 11.78) ^b	.000
Before JAK-2 (ng/mL)	6.55 ± 1.26	5.77 ± 1.33	5.81 ± 1.53	.095
STAT-3 (pg/mL)	574.94 ± 85.47	135.24	118.56	.074
IL-6 (pg/mL)	2.12 (1.20, 2.50) ^a	4.49 (2.87, 6.71) ^b	2.81 (2.01, 4.00) ^c	.000
After JAK-2 (ng/mL)	6.65 ± 0.89 ^a	6.19 ± 1.36 ^b	6.89 ± 1.02 ^a	.011
STAT-3 (pg/mL)	573.61 ± 81.52	539.32 ± 80.45	573.75 ± 72.03	.056

^{a,b,c}The same letters show that there is no statistically significant difference between them, and different letters show that there is a statistically significant difference. EMPA, empagliflozin; IL-6, interleukin 6; JAK-2, janus kinase-2; STAT-3, signal transducer and activator of transcription-3.

Effect of Empagliflozin on Renal Function of Cardiorenal Syndrome Patients Compared to Healthy People

To determine whether EMPA was associated with changes in renal function, we performed a detailed analysis of 3 critical parameters (Ser, BUN, and UA). Patients with CRS have a higher level of Ser and BUN compared to healthy people, but UA has no significant difference between these groups. The detailed analysis revealed that renal injury was alleviated in the EMPA and control group. The level of BUN was decreased, but Ser revealed no significant decrease in the control group after treatment. Interestingly, the level of BUN and Ser were both decreased in the EMPA group, indicating EMPA exerts a nephroprotective effect to some extent (Figure 1).

Effect of Empagliflozin on Echocardiography of Cardiorenal Syndrome Patients Compared to Healthy People

Next, we examined the echocardiography. Patients with CRS have a higher level of LAD, LVED, and low level of LVEF compared to healthy people. However, the difference in LAD, LVED, and LVEF was not statistically significant when comparing the EMPA and control group, showing improved cardiac function. Therefore, this experiment did not show the cardioprotective effect of empagliflozin on patients with CRS, which may be related to the short duration of experimental research (Figure 2). Thus, EMPA therapy expected benefit for cardioprotective need further research.

Effects of Empagliflozin on the Rate of Adverse Events and Major Adverse Cardiovascular Events

Finally, we examined the rate of adverse reactions and found that the incidence of adverse reactions in the 2 groups was 13.46% and 24.00% respectively, which were not significantly different among the experimental groups (Table 4). However, the analysis in this trial showed that changes in the rates of MACE respectively mediated 40.38% and 20.00% of the effect of EMPA on the risk of cardiovascular death, indicating that continuous treatment with EMPA improves the short-term prognosis safely (Table 4).

DISCUSSION

The pathogenesis of CRS is complex, including chronic activation of the renin-angiotensin-aldosterone system and the sympathetic nervous system, contributing to cellular hypertrophy, inflammation, apoptosis, fibrosis, and oxidative stress in both the heart and the kidney. Augmented levels of reactive oxygen species can also stimulate the production of pro-inflammatory mediators such as IL-6 and TGF-β.¹² Recent studies revealed an increased risk of developing macrovascular and microvascular complications in diabetic injury, which are associated with insulin resistance, further oxidative damage, advanced glycation end products (AGE),

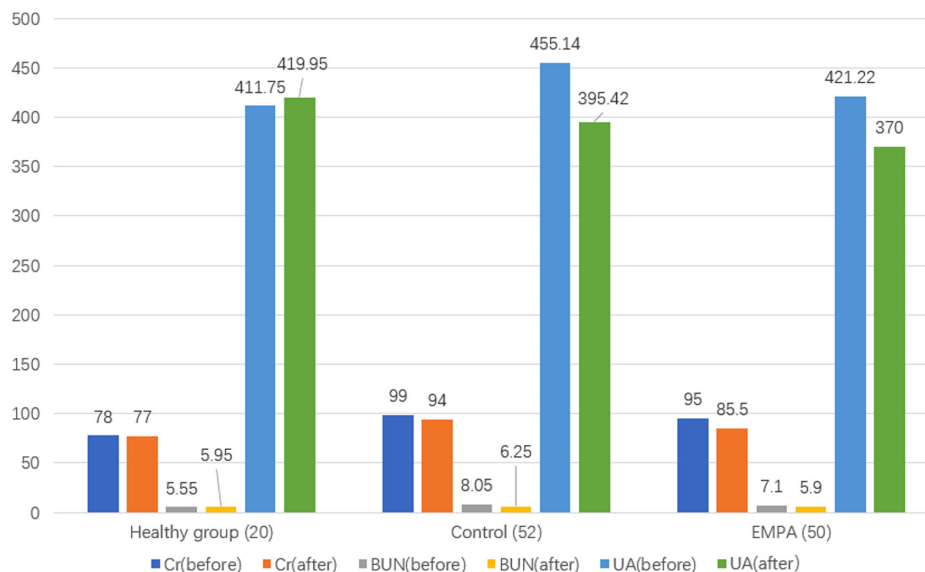


Figure 1. Effect of EMPA on renal function. Compared to the healthy group, Cr, and BUN are meaningful in both the control group and the EMPA group (P < .05). BUN, blood urea nitrogen; Cr, serum creatinine; EMPA, empagliflozin; UA, uric acid.

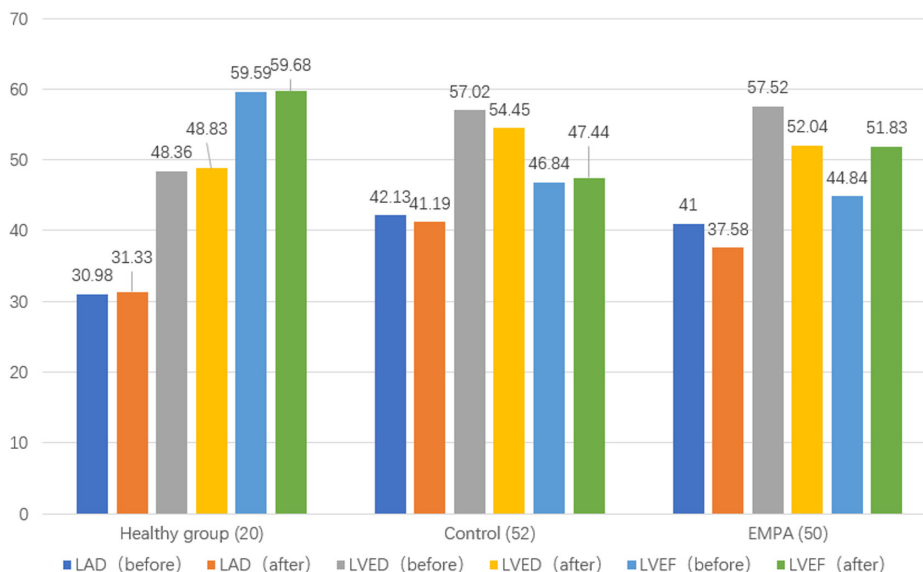


Figure 2. Effect of EMPA on echocardiography. Compared to the healthy group, LAD, LVED, and LVEF are significantly different in both the control group and the EMPA group ($P < .05$). Compared to the control group, LAD, LVED, and LVEF are significantly different in the EMPA group ($P < .05$). EMPA, empagliflozin; LAD, left atrium diameter; LVED, left ventricular end diastolic; LVEF, left ventricular ejection fraction.

leading to high morbidity and mortality in CRS.¹³ Despite this, the role of renal mitochondrial oxidative stress in CRS type 2 pathology remains to be investigated.

While the data on the role of renal mitochondria in the setting of CRS type 2 is limited, there is considerable evidence that augmented levels of tumor necrosis factor alpha (TNF- α) and soluble tumor necrosis factor receptor type-1 within heart failure were significantly associated with congestion and renal impairment, and were independent predictors of a composite outcome of death and heart failure hospitalisation.¹⁴ In this study, in patients hospitalized for CRS the high level of IL-6 was associated with inflammation and the anti-inflammatory actions of EMPA were confirmed. Moreover, EMPA reduced inflammatory cytokines (IL-1 β , IL-18), and substances related to oxidative stress (H₂O₂, 3-nitrotyrosine, lipid peroxide, etc.).^{15,16}

The JAK/STAT pathway, an important high-profile pathway of cytokine signaling, plays pivotal roles in proliferation,

differentiation, apoptosis, and various inflammatory responses, participating in the occurrence and development of various diseases. In IL-6 signaling, the ligand binds IL-6R and generates hetero-dimeric IL-6/IL-6R complexes that associate with glycoprotein130 (gp130), resulting in the activation of gp130 and inducing the phosphorylation of JAKs, which in turn triggers STAT-3 homodimerization, nuclear translocation, DNA binding, and the activation of STAT-3 transcription targets genes.^{17,18} Dysregulation of JAK2/STAT3 signaling pathways is associated with the pathogenesis of heart failure, but the expression of it is still controversial. Zhang et al¹⁹ found that when rats with transverse aortic constriction were accepted a stepped therapy with captopril, the expression of capase 3, bcl2-associated X protein, phosphorylated JAK-2, phosphorylated STAT-3 decreased, but the expression of B cell lymphoma-2 increased. Thus, they demonstrated that captopril could attenuate myocardial hypertrophy and cardiac apoptosis of mice via suppression of JAK2/STAT3 and Wnt3a/ β -catenin signaling pathways. In addition, it is reported that heart failure activates the JAK2/STAT3 system and enhances myocardial fibrosis and remodeling.^{20,21} However, there is no study on the expression of JAK2/STAT3 in patients with CRS.

A bunch of studies confirmed the expression of JAK2/STAT3 signaling was decreased in patients with heart failure. And our study is in agreement with those reported in an elegant study by Li et al²² showed that troxerutin, also known as vitamin P4, could inhibit the expression of oxidative stress, inflammation, and apoptosis of cardiomyocytes induced by H₂O₂ via activating the JAK2/STAT3 signaling. Similarly, researchers found that ligustrazine²³ and astragaloside IV²⁴ exert cardioprotective function by activating JAK2/STAT3 pathway. Additionally, it is demonstrated that remote ischemic preconditioning (RIPC) induced the cardioprotective

Table 4. Effects of Empagliflozin on the Rate of Adverse Events and Major Adverse Cardiovascular Events

	Healthy group (20)	Control (52)	EMPA (50)	χ^2 value	P
Adverse events, %	-	7 (13.46)	12 (24.00)	1.868	.208
MACE, %	-	21 (40.38) ^a	10 (20.00) ^b	5.007	.032

^{a,b}The same letters show that there is no statistically significant difference between them, and different letters show that there is a statistically significant difference. EMPA, empagliflozin; MACE, major adverse cardiovascular events. Adverse effects include urinary and genital tract infections, diabetic ketoacidosis, volume depletion particularly in the setting of concomitant diuretic use, hypoglycemia, kidney failure, and anaphylactic response. MACE events include nonfatal myocardial infarction, serious cardiac arrhythmias, stroke, heart failure hospitalization, and cardiovascular mortality.

effect by activating JAK/STAT pathway.²⁵ Our data shows that the anti-inflammatory actions of EMPA were mediated by JAK2/STAT3 activation. Why do different drugs play a therapeutic role through different ways? We found that this may be related to a variety of factors by consulting many papers. First, the expression of STAT-3 is related to the stage of the disease. Signal transducer and activator of transcription 3 increased during the process of cardiac hypertrophy, while the tyrosine-phosphorylation of JAK-2 and STAT-3 is reduced in patients with end-stage dilated cardiomyopathy, indicating impaired downstream activation of this critical pathway.^{26,27} In the trial conducted by Nikolaou et al,^{28,29} they confirmed that EMPA could improve myocardial function and reduce infarct size as well as improve redox regulation through STAT-3 activation. Second, it is reported that mice with cardiac myocyte restricted knockout of gp130 or STAT-3 develop heart failure with age, and cardiac STAT-3 levels are reported to be decreased in heart failure patients, thus the absence or inactivity of gp130 could turn cardiac hypertrophy into heart failure.³⁰ And there is a study²⁶ reported increased gp130 and diminished JAK-2, STAT-3 levels in patients with ischemic and dilated cardiomyopathy. Last but not least, the suppressors of cytokine signaling (SOCS) are recently discovered cytoplasmic proteins controlling negative feedback in key intracellular pathways, including JAK/STAT pathways. It is demonstrated that enhanced SOCS-3 expression is associated with compensated hypertrophy, while the overexpression of SOCS-1 myocardial signaling accelerates the transition to heart failure in a chronic pressure-overload cardiac rat model.^{31,32} Therefore, we speculate that EMPA can affect gp130 or SOCS directly or indirectly to produce a renoprotective effect by activating the JAK2/STAT3 pathway.

Study Limitations

The limitations of the present study include its small sample size, which was determined by budgetary constraints. Furthermore, to verify whether the effects of EMPA were associated with JAK2/STAT3 signaling, we are determined to detect gp130, SOCS-1, and SOCS-3 in further study as the effects of drugs on patients are long-lasting. Nevertheless, our study provides unique insights into the renal effects of EMPA with JAK2/STAT3 signaling and supports the application of EMPA as a therapeutic option in CRS patients with diabetes. Our experiment may be complementary to Zannad et al,³³ they confirmed the differential expression of a small select group of circulating proteins following SGLT2 inhibition after 52 weeks. Sodium-dependent glucose transporters-2 (SGLT2) inhibition is a new type of drug used to treat patients with CRS, and its exact physiological functions are not yet fully understood; therefore, its significance in JAK2/STAT3 signaling and prognosis in patients with CRS is limited. The relationship between EMPA and JAK2/STAT3 signaling warrants further investigation.

CONCLUSIONS

Empagliflozin therapy exerts anti-inflammatory effects and favorably affects to cardiac and renal function, associated with substantial improvements in short-term outcomes.

Consequently, it's demonstrated that EMPA might be of benefit in patients with CRS type 2 via activating JAK2/STAT3 signaling, worth application in medical treatment.

Ethics Committee Approval: This study was performed in line with the principles of the Declaration of Helsinki. All subjects have given their written informed consent and this study protocol was reviewed and approved by the Ethics Committee of the Loudi Central Hospital (date: June 29, 2021; number: 2021-ethics (research)-032).

Informed Consent: Informed consent was obtained from all individual participants included in the study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – P.Z., X.T.; Design – P.Z., X.T.; Supervision – P.Z., X.T.; Resource – P.Z., X.T.; Materials – Y.D., R.W., Y.Y., H.D., Q.C.; Data Collection and/or Processing – Y.D., R.W., Y.Y., H.D., Q.C.; Analysis and/or Interpretation – P.Z., X.T., Y.D., R.W., Y.Y.; Literature Search – P.Z., X.T., H.D., Q.C.; Writing – P.Z., X.T., Y.D., R.W., Y.Y.; Critical Review – P.Z., X.T., Y.D., R.W., Y.Y., H.D., Q.C.

Declaration of Interests: The authors have no conflicts of interest to declare.

Funding: This work was funded by the scientific research project of Loudi Central Hospital (Y2021-23).

REFERENCES

1. Ronco C, McCullough P, Anker SD, et al. Cardio-renal syndromes: report from the consensus conference of the acute dialysis quality initiative. *Eur Heart J*. 2010;31(6):703-711. [CrossRef]
2. Dunlay SM, Roger VL. Understanding the epidemic of heart failure: past, present, and future. *Curr Heart Fail Rep*. 2014;11(4):404-415. [CrossRef]
3. Löfman I, Szummer K, Hagerman I, Dahlström U, Lund LH, Jernberg T. Prevalence and prognostic impact of kidney disease on heart failure patients. *Open Heart*. 2016;3(1):1-9. [CrossRef]
4. Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, cardiovascular outcomes, and mortality in Type 2 diabetes. *N Engl J Med*. 2015;373(22):2117-2128. [CrossRef]
5. Zannad F, Ferreira JP, Pocock SJ, et al. SGLT2 inhibitors in patients with heart failure with reduced ejection fraction: a meta-analysis of the EMPEROR-Reduced and DAPA-HF trials. *Lancet*. 2020;396(10254):819-829. [CrossRef]
6. Wanner C, Lachin JM, Inzucchi SE, et al. Empagliflozin and clinical outcomes in patients with type 2 diabetes mellitus, established cardiovascular disease, and chronic kidney disease. *Circulation*. 2018;137(2):119-129. [CrossRef]
7. McDonald M, Virani S, Chan M, et al. CCS/CHFS heart failure guidelines update: defining a new pharmacologic standard of care for heart failure with reduced ejection fraction. *Can J Cardiol*. 2021;37(4):531-546. [CrossRef]
8. Wanner C. EMPA-REG OUTCOME: the nephrologist's point of view. *Am J Cardiol*. 2017;120(1S):S59-S67. [CrossRef]
9. Georgianos PI, Vaios V, Dounousi E, Salmas M, Eleftheriadis T, Liakopoulos V. Mechanisms for cardiorenal protection of SGLT-2 inhibitors. *Curr Pharm Des*. 2021;27(8):1043-1050. [CrossRef]
10. Nambu H, Takada S, Fukushima A, et al. Empagliflozin restores lowered exercise endurance capacity via the activation of skeletal muscle fatty acid oxidation in a murine model of heart failure. *Eur J Pharmacol*. 2020;866:172810. [CrossRef]
11. Rangaswami J, Bhalla V, Blair JEA, et al. Cardiorenal syndrome: classification, pathophysiology, diagnosis, and treatment

- strategies: A scientific statement from the American Heart Association. *Circulation*. 2019;139(16):e840-e878. [\[CrossRef\]](#)
12. Giam B, Kaye DM, Rajapakse NW. Role of renal oxidative stress in the pathogenesis of the cardiorenal syndrome. *Heart Lung Circ*. 2016;25(8):874-880. [\[CrossRef\]](#)
 13. Cortassa S, Caceres V, Tocchetti CG, et al. Metabolic remodeling of glucose, fatty acid and redox pathways in the heart of type 2 diabetic mice. *J Physiol*. 2020;598(7):1393-1415. [\[CrossRef\]](#)
 14. Pugliese NR, Fabiani I, Conte L, et al. Persistent congestion, renal dysfunction and inflammatory cytokines in acute heart failure: a prognosis study. *J Cardiovasc Med (Hagerstown)*. 2020;21(7):494-502. [\[CrossRef\]](#)
 15. Sukhanov S, Higashi Y, Yoshida T, et al. The SGLT2 inhibitor Empagliflozin attenuates interleukin-17A-induced human aortic smooth muscle cell proliferation and migration by targeting TRAF3IP2/ROS/NLRP3/Caspase-1-dependent IL-1 β and IL-18 secretion. *Cell Signal*. 2021;77:109825. [\[CrossRef\]](#)
 16. Koliijn D, Pabel S, Tian Y, et al. Empagliflozin improves endothelial and cardiomyocyte function in human heart failure with preserved ejection fraction via reduced pro-inflammatory-oxidative pathways and protein kinase G α oxidation. *Cardiovasc Res*. 2021;117(2):495-507. [\[CrossRef\]](#)
 17. Pilati C, Zucman-Rossi J. Mutations leading to constitutive active gp130/JAK1/STAT3 pathway. *Cytokine Growth Factor Rev*. 2015;26(5):499-506. [\[CrossRef\]](#)
 18. Lokau J, Garbers C. Activating mutations of the gp130/JAK/STAT pathway in human diseases. *Adv Protein Chem Struct Biol*. 2019;116:283-309. [\[CrossRef\]](#)
 19. Zhang Y, Zhang L, Fan X, et al. Captopril attenuates TAC-induced heart failure via inhibiting Wnt3a/ β -catenin and Jak2/Stat3 pathways. *Biomed Pharmacother*. 2019;113:108780. [\[CrossRef\]](#)
 20. Chen Y, Surinkaew S, Naud P, et al. JAK-STAT signalling and the atrial fibrillation promoting fibrotic substrate. *Cardiovasc Res*. 2017;113(3):310-320. [\[CrossRef\]](#)
 21. Shen Y, Zhang W, Lee L, et al. Down-regulated microRNA-195-5p and up-regulated CXCR4 attenuates the heart function injury of heart failure mice via inactivating JAK/STAT pathway. *Int Immunopharmacol*. 2020;82:106225. [\[CrossRef\]](#)
 22. Li H, Yang M, Lou D. Troxerutin regulates HIF-1 α by activating JAK2/STAT3 signaling to inhibit oxidative stress, inflammation, and apoptosis of cardiomyocytes induced by H₂O₂. *Drug Dev Res*. 2021;7:1-12. [\[CrossRef\]](#)
 23. Chen CB, Chen TB, Xu YB. Mechanism of ligustrazine on mitochondrial autophagy reducing myocardial ischemia reperfusion injury through JAK2/STAT3 signaling pathway. *Chin J Immunol*. 2021;37(07):819-823. [\[CrossRef\]](#)
 24. Sui YB, Wang Y, Liu L, Liu F, Zhang YQ. Astragaloside IV alleviates heart failure by promoting angiogenesis through the JAK-STAT3 pathway. *Pharm Biol*. 2019;57(1):48-54. [\[CrossRef\]](#)
 25. Billah M, Ridiandries A, Allahwala UK, et al. Remote ischemic preconditioning induces cardioprotective Autophagy and Signals through the IL-6-Dependent JAK-STAT Pathway. *Int J Mol Sci*. 2020;21(5):1-25. [\[CrossRef\]](#)
 26. Podewski EK, Hilfiker-Kleiner D, Hilfiker A, et al. Alterations in Janus kinase (JAK)-signal transducers and activators of transcription (STAT) signaling in patients with end-stage dilated cardiomyopathy. *Circulation*. 2003;107(6):798-802. [\[CrossRef\]](#)
 27. Zhang J, Wei X, Zhang W, Wang F, Li Q. MiR-326 targets MDK to regulate the progression of cardiac hypertrophy through blocking JAK/STAT and MAPK signaling pathways. *Eur J Pharmacol*. 2020;872:172941. [\[CrossRef\]](#)
 28. Nikolaou PE, Efentakis P, Abu Qourah F, et al. Chronic empagliflozin treatment reduces myocardial infarct size in nondiabetic mice through STAT-3-Mediated protection on microvascular endothelial cells and reduction of oxidative stress. *Antioxid Redox Signal*. 2021;34(7):551-571. [\[CrossRef\]](#)
 29. Andreadou I, Efentakis P, Balafas E, et al. Empagliflozin limits myocardial infarction in vivo and cell death in vitro: role of STAT3, mitochondria, and redox aspects. *Front Physiol*. 2017;8:1077. [\[CrossRef\]](#)
 30. Zouein FA, Kurdi M, Booz GW. LIF and the heart: just another brick in the wall? *Eur Cytokine Netw*. 2013;24(1):11-19. [\[CrossRef\]](#)
 31. Gao J, Guo Y, Chen Y, Zhou J, Liu Y, Su P. Adeno-associated virus 9-mediated RNA interference targeting SOCS3 alleviates diastolic heart failure in rats. *Gene*. 2019;697:11-18. [\[CrossRef\]](#)
 32. Cittadini A, Monti MG, Iaccarino G, et al. SOCS1 gene transfer accelerates the transition to heart failure through the inhibition of the gp130/JAK/STAT pathway. *Cardiovasc Res*. 2012;96(3):381-390. [\[CrossRef\]](#)
 33. Zannad F, Ferreira JP, Butler J, et al. Effect of empagliflozin on circulating proteomics in heart failure: mechanistic insights into the EMPEROR programme. *Eur Heart J*. 2022;43(48):4991-5002. [\[CrossRef\]](#)