Curcumin Attenuates Ferroptosis-Induced Myocardial Injury in Diabetic Cardiomyopathy through the Nrf2 Pathway

Zhang Wei,¹ Qian Shaohuan,¹ Kang Pinfang,¹ and Shi Chao²

¹Department of Cardiovascular Medicine of the First Affiliated Hospital of Bengbu Medical College, Bengbu City, Anhui, China 233000
²Department of Cardiac Surgery of the First Affiliated Hospital of Bengbu Medical College, Bengbu City, Anhui, China 233000

Correspondence should be addressed to Shi Chao; wuweishichao@126.com

Received 25 May 2022; Accepted 1 July 2022; Published 15 July 2022

1. Introduction

Diabetes is a worldwide chronic metabolic condition. Serious complications affecting the nerves and blood vessels can occur as the course of diabetes progresses. Specifically, cardiovascular disease is one of the most common clinical complications of diabetes [1]. Elevated blood sugar levels can lead to lipid metabolism problems among vascular endothelial cells and cardiomyocytes and the accumulation of local inflammatory factors. This leads to coronary stenosis, myocardial fibrosis, and myocardial systolic dysfunction. Subsequently, severe heart failure can develop, lowering the long-term quality of life of these patients [2].

Ferroptosis is a necrotic inflammatory reaction caused by the accumulation of intracellular peroxides brought on by a variety of inflammatory factors and lipid metabolism disorders [3]. According to previous research, pancreatic beta cells express low levels of antioxidant enzymes making them prone to excess reactive oxygen species (ROS) accumulation which can trigger a variety of inflammatory responses. When in vitro human pancreatic beta cells are treated with the ferroptosis inducer erastin, glucose-stimulated insulin secretion capacity is significantly reduced [4]. Furthermore, during diabetic cardiomyopathy, cardiomyocyte cells obtain almost all of their energy from fatty acid oxidation resulting in excessive fatty acid oxidation, accumulation of peroxides and inflammatory factors, and irreversible cardiomyocyte damage [5, 6].

Curcumin is a polyphenolic compound extracted from the rhizomes of the turmeric plant and has a wide range of biological activities, including free radical scavenging and antioxidant, anti-inflammatory, and anticancer properties.
Figure 1: Continued.
According to Wang et al., curcumin can reduce aflatoxin B1-induced liver damage by regulating pyroptosis and the nuclear factor erythroid-2-related factor 2 (Nrf2) signaling pathway [9]. Nrf2 is one of the most important regulators of antioxidative stress in the body. By regulating the expression of glutathione synthesis and nicotinamide adenine dinucleotide phosphate generation, the Nrf2/ARE signaling pathway can neutralize the accumulated superoxide and inhibit oxidative stress [10, 11]. Therefore, this study is aimed at investigating whether curcumin can regulate Nrf2 expression and alleviate ferroptosis-induced myocardial injury in diabetic animal and cellular models.

### 2. Materials and Methods

#### 2.1. Construction of Diabetic Rabbit Model

Two-month-old male New Zealand rabbits purchased from the Medical Experimental Animal Center of Bengbu Medical College were used as experimental subjects. Streptozotocin was dissolved in sterile saline and intraperitoneally injected into the rabbits at a dose of 80 mg/kg. The rabbits were allowed to eat freely after receiving the injection. The fasting blood glucose levels of the rabbits were monitored regularly. The diabetic rabbit model was considered successfully established when the fasting blood glucose level was measured as 11 mmol/L twice or 14 mmol/L once. Following successful modelling, grouping was performed as follows: blank control group (Con-Group), diabetic rabbit group (DM-Group), diabetic rabbit + every other day curcumin administration group (Qod-Group), and diabetic rabbit + daily administration group (Qd-Group). Six rabbits were randomly assigned to each experimental group and raised in the same environment for 3 months. A curcumin solution was prepared using 300 mg/L of polybrene. The cardio- myocytes were cultured in 96-well plates. When they reached ~70% confluence, different concentrations of curcumin were added and the cardiomyocytes were cocultured for 24 h. CCK-8 reagent was added, and relative cell activity was measured using a microplate reader to determine the optimal curcumin concentration. Three duplicate wells were set up for each group.

#### 2.2. Rabbit Myocardial Slices

Rat H9C2 cardiomyocytes were used as experimental cell models (long-term culture cryopreserved in the laboratory). Cells were either treated with a normal glucose concentration medium of 5.5 mmol/L (Nor-Group) or a high-glucose concentration medium of 30 mmol/L (H-Group).

#### 2.3. Grouping of Cardiomyocytes

Rat H9C2 cardiomyocytes were cultured in 96-well plates. When they reached ~70% confluence, different concentrations of curcumin were added and the cardiomyocytes were cocultured for 24 h. CCK-8 reagent was added, and relative cell activity was measured using a microplate reader to determine the optimal curcumin concentration. Three duplicate wells were set up for each group.

#### 2.4. CCK-8 Measured Cell Viability

The H9C2 cardiomyocytes were cultured in 96-well plates. When they reached ~70% confluence, different concentrations of curcumin were added and the cardiomyocytes were cocultured for 24 h. CCK-8 reagent was added, and relative cell activity was measured using a microplate reader to determine the optimal curcumin concentration. Three duplicate wells were set up for each group.

#### 2.5. Nrf2 Virus Construction and Transfection

The Shanghai Jikai Company in China provided Nrf2 viral vectors. When the cardiomyocytes had reached ~70% confluence, an appropriate amount of viral vector was mixed into 3 mL of fresh medium containing 6 μg/mL of polybrene. The cardiomyocytes were cultured in the medium, and transfection efficiency was assessed using a fluorescence microscope. For multiple transfections, the best virus transfection
Figure 2: Continued.

(a) Relative cell viability

Relative cell viability

Carboxin concentration

(b) Localization
Figure 2: Continued.
multiplicity of infection value was used. Transfection efficiency was verified using western blotting (WB).

2.6. Extraction of Nuclear Proteins. After cell digestion and centrifugation, an appropriate amount of cytoplasmic protein extraction reagent was added, thoroughly mixed, and placed in an ice bath for 10 min before centrifuging at 12000 g for 15 min to remove the supernatant. Then, the required amount of nuclear protein extraction reagent was added, thoroughly mixed, placed in an ice bath for 10 min, and centrifuged at 12000 g for 15 min. The supernatant was collected and used to measure nuclear protein.

2.7. Main Experimental Reagents. Curcumin (Sigma, America); Nrf2 virus vectors (Gikai, Shanghai, China); Heme Oxygenase 1 Rabbit Polyclonal Antibody, Cox1 Rabbit Polyclonal Antibody, Nrf2 Rabbit Polyclonal Antibody, Gpx4 Rabbit Polyclonal Antibody, One-Step TUNEL Assay Kit, Reactive Oxygen Species Assay Kit (Beyotime Biotechnology, Shanghai, China); Acsl4 (Abcam, United Kingdom); Dylight 594-Goat Anti-Rabbit IgG (Abbkine Scientific, Wuhan, China); DMEM high-sugar medium (Hyclone, American); and Nuclear Protein Extraction Kit (Solarbio Life Science, Beijing, China) were used.

2.8. Statistical Analysis. SPSS 25.0 software was used to process all statistical data. All experiments were conducted at least three times, and data are expressed as mean ± SD. t-tests were used for data analysis between two groups. Images were analyzed using ImageJ software. Statistical significance was set at a P value of <0.05.

3. Results

3.1. Myocardial Tissue Sections and Western Blotting. According to WB results, diabetes increased the expression of cyclooxygenase 1 (Cox1) and fatty acid coenzyme A ligase (Acsl4) proteins in the ferroptosis pathway in myocardial tissue (Figure 1(a)). Immunofluorescence results revealed that Gpx4 protein levels were significantly reduced in rabbit model diabetic myocardial tissue (Figure 1(b)). In contrast, curcumin reduced the expression of ferroptosis-related proteins in myocardial tissue (Figures 1(a) and 1(b)). Hematoxylin and eosin staining revealed that curcumin greatly improved disordered myocardial cell arrangement brought on by diabetes, alleviated myocardial cell edema, and decreased lipid accumulation between tissues. Masson’s staining and tissue collagen fluorescence data confirmed that diabetes increased myocardial fibrosis (Figures 1(c) and 1(d)). However, curcumin decreased the expression of collagen in myocardial tissue, reducing the degree of myocardial fibrosis (Figures 1(c) and 1(d)).

3.2. Curcumin Reduces Cell Damage Caused by High Glucose Levels. In the CCK-8 assay, the optimal curcumin concentration was found to be 10 μmol/L (Figure 2(a)). The cells in each group were cocultured for 24 h. Immunofluorescence colocalization was used to examine Nrf2 expression in the nucleus which revealed that curcumin promoted Nrf2 nuclear translocation. The Pearson correlation coefficient was 0.59 indicating a strong association between the two fluorescence groups (Figure 2(b)). WB reinforced that curcumin promoted nucleus translocation of Nrf2 (Figure 2(e)). Intranuclear transfer of Nrf2 increased the expression of antioxidant factor Heme Oxygenase-1 (HO-1), reducing cardiomyocyte
Figure 3: Continued.
accumulation of intracellular oxides, alleviating Gpx4 deple-
tion, and inhibiting the progression of glucose-induced fer-
roptosis (Figures 2(c)–2(e)).

3.3. Nrf2 Overexpression Reduced Glucose-Induced Cell
Injury. Cardiomyocyte Nrf2 expression was assessed using
WB after lentivirus transfection (Figure 3(a)). Nrf2 nuclear
overexpression dramatically increased the expression of the
downstream antioxidant factor HO-1 (Figures 3(c) and
3(d)). HO-1 and Gpx4 protein expression was dramatically
reduced in the knockdown group; however, Cox1 protein
expression in ferroptosis was significantly elevated which
led to decreased cell activity in this group (Figures 3(b)–
3(d)). Therefore, overexpression of Nrf2 has the effect of
inhibiting myocardial injury caused by high glucose.

3.4. Curcumin and Nrf2 Protect against Ferroptosis-Inducing
Agents. The ferroptosis inducer erastin was used to treat
H9C2 cardiomyocytes, and the dose of erastin was set at
1 mmol/L based on relevant literature [12]. After 24h of
cocultivation, erastin was added to Nrf2-overexpressing car-
diomyocytes and curcumin-treated cardiomyocytes. The
results showed that when cardiomyocytes were treated with
erastin, ROS levels significantly increased while cell viability
significantly decreased (Figure 4(a)). Cardiomyocyte curcu-
min treatment and Nrf2 overexpression increased the
expression of Gpx4 in the cytoplasm and Nrf2 in the nucleus
and reduced ROS accumulation and ferroptosis-induced cell
damage (Figure 4(b)).

4. Discussion

Elevated blood sugar causes cardiomyocyte insulin resis-
tance, cellular lipid peroxidation, activation of inflammatory
factors, cellular microenvironments of oxidative stress and
inflammatory factors, RASS, and sympathetic nerve stimula-
tion activation. All these result in myocardial fiber changes
and decreased myocardial function [13–15]. Myocardial
slices and collagen staining showed that diabetes caused car-
diomyocyte hypertrophy, disordered cell arrangements, and
increased myocardial fibrosis. Furthermore, diabetes
decreased myocardium Gpx4 and nuclear Nrf2 expression,
increased the expression of downstream-related ferroptosis
factors, and increased superoxide accumulation, demon-
strating that ferroptosis is closely related to diabetic cardiomypathy.

Ferroptosis is a process in which cells are stimulated to
cause excessive intracellular accumulation of iron ions and
lipid peroxides resulting in oxidative stress and cell death
[16]. Park et al. discovered that reducing Gpx4 expression
in myocardial infarction mouse models resulted in a worsen-
ing of myocardial cell ferroptosis and impaired myocardial
cell activity [17]. This experiment demonstrated that at the
animal and cellular levels, glucose-induced continuous car-
diomyocyte peroxide accumulation triggers ferroptosis and
causes cell damage. Curcumin was discovered to increase
the expression of liver insulin-degrading enzymes and main-
tain the integrity of islets as an oxygen free radical scavenger
[18, 19]. According to Ali’s research, curcumin can counter-
act D-galactose-induced oxidative stress in rat brain and
heart by lowering Bax and CASP-3 expression. This is a common misunderstanding [20]. This study showed that curcumin promotes the transfer of Nrf2 into the nucleus, increases the expression of antioxidant factor HO-1, reduces cardiomyocyte ROS accumulation, and reduces the degree of ferroptosis caused by high glucose levels.

By regulating the expression of glutathione peroxidase, glutathione s-transferase, and glutathione reductase, Nrf2 maintains reduced levels of intracellular glutathione and cellular redox homeostasis [21, 22]. When cells are disrupted by ROS, Nrf2 translocates to the nucleus and binds to the antioxidant response element/electrophilic response element (ARE/EpRE) of target genes via heterodimerization alongside somite Maf protein which induces the expression of downstream protective genes [23, 24]. Furthermore, Li discovered that curcumin reduces mercury-induced stem cell damage by promoting Nrf2 nucleus translocation [25]. This experiment found that curcumin promotes the transfer of

![Figure 4: Curcumin prevents cardiac damage caused by erastin. Era-G: erastin-treated cardiomyocytes; Era+Nrf2-G: Nrf2 overexpressing +erastin-treated cardiomyocytes; Era+Cur-G: curcumin+erastin-treated cardiomyocytes. (a) Terminal deoxynucleotidyl transferase dUTP nick-end labelling (TUNEL) and reactive oxygen species (ROS) assays revealed that ferroptosis inducers caused massive myocardial cell death. The apoptosis rate and intracellular ROS were significantly reduced vs. the Era-G cells (×200). (b) Western blot detected the expression levels of nuclear Nrf2 and ferroptosis-related factors (*P < 0.05).](image-url)
Nrf2 into the nucleus, prompts cells to express a large number of antioxidant factors including HO-1, reduces the excessive downregulation of Gpx4, and inhibits the progression of cell ferroptosis. Curcumin can also reduce erastin-induced ferroptosis, accumulation of oxidative products, and degree of cell damage. Although further studies are required, the results of this study may provide a new therapeutic direction for the treatment of diabetic cardiomyopathy.

5. Conclusions
Diabetic cardiomyopathy can cause heart vessel blockages, myocardial contractile dysfunction, and a significant reduction in quality of life. The pathogenesis of diabetic cardiomyopathy is not entirely known as well as what the most effective prevention and treatment methods are.

Curcumin has been shown to have antioxidant properties and to maintain cell activity. However, its effect on diabetic cardiomyopathy has not yet been established. This study found that feeding diabetic rabbits curcumin significantly improved myocardial structure, including myocardial fibrosis and cell arrangement disorder. The therapeutic effect of curcumin significantly improved as dosing frequency increased. Curcumin was also found to increase Nrf2 transfer into the nucleus, increase the expression of Gpx4 and HO-1, reduce glucose-induced myocardial cell damage, and reverse myocardial cell damage caused by erasit. This study confirmed the role of ferroptosis in the pathogenesis of diabetic cardiomyopathy and provided a new theoretical direction for diabetic cardiomyopathy prevention and treatment.

Data Availability
The data covered in this article are included in the article.

Ethical Approval
Ethical approval was given by the medical ethics committee of Bengbu Medical College with the following reference number: 2022:031.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

Authors’ Contributions
Zhang Wei is in charge of the main experiment’s operation process; Qian Shaohuan and Kang Pinfang are responsible for data collation and paper polishing; and Shi Chao provides experimental design and data integration.

Acknowledgments
The present study was supported by research grants from the Science and Technology Project of Anhui Province (1804h08020280).

References
[1] W. Crasto, V. Patel, M. J. Davies, and K. Khunti, “Prevention of microvascular complications of diabetes,” Endocrinology and Metabolism Clinics of North America, vol. 50, no. 3, pp. 431–455, 2021.
[2] G. Murtaza, H. U. H. Virk, M. Khalid et al., “Diabetic cardiomyopathy - a comprehensive updated review,” Progress in Cardiovascular Diseases, vol. 62, no. 4, pp. 315–326, 2019.
[3] Y. Sun, P. Chen, B. Zhai et al., “The emerging role of ferroptosis in inflammation,” Biomedicine & Pharmacotherapy, vol. 127, p. 110108, 2020.
[4] A. Bruni, A. R. Pepper, R. L. Pawlick et al., “Ferroptosis-inducing agents compromise in vitro human islet viability and function,” Cell Death & Disease, vol. 9, no. 6, p. 595, 2018.
[5] M. Tong, T. Saito, P. Zhai et al., “Mitophagy is essential for maintaining cardiac function during high fat diet-induced diabetic cardiomyopathy,” Circulation Research, vol. 124, no. 9, pp. 1360–1371, 2019.
[6] M. Nakamura and J. Sadoshima, “Cardiomyopathy in obesity, insulin resistance and diabetes,” The Journal of Physiology, vol. 598, no. 14, pp. 2977–2993, 2020.
[7] C. M. White, V. Pasupuleti, Y. M. Roman, Y. Li, and A. V. Hernandez, “Oral turmeric/curecumin effects on inflammatory markers in chronic inflammatory diseases: a systematic review and meta-analysis of randomized controlled trials,” Pharmacological Research, vol. 146, p. 104280, 2019.
[8] N. E. Rainey, A. Moustapha, and P. X. Petit, “Curcumin, a multifaceted hormetic agent, mediates an intricate crosstalk between mitochondrial turnover, autophagy, and apoptosis,” Oxidative Medicine and Cellular Longevity, vol. 2020, Article ID 3656419, 23 pages, 2020.
[9] Y. Wang, F. Liu, M. Liu et al., “Curcumin mitigates aflatoxin B1-induced liver injury via regulating the NLRP3 inflammasome and Nrf2 signaling pathway,” Food and Chemical Toxicology, vol. 161, p. 112823, 2022.
[10] J. Li, K. Lu, F. Sun et al., “Panaxylod attenuates ferroptosis against LPS-induced acute lung injury in mice by Keap1-Nrf2/HO-1 pathway,” Journal of Translational Medicine, vol. 19, no. 1, p. 96, 2021.
[11] J. Ren, D. Su, L. Li et al., “Anti-inflammatory effects of Aureusidin in LPS-stimulated RAW264.7 macrophages via suppressing NFκB and activating ROS- and MAPKs-dependent Nrf2/HO-1 signaling pathways,” Toxicology and Applied Pharmacology, vol. 387, article 114846, 2020.
[12] Y. T. Bai, F. J. Xiao, H. Wang, R. L. Ge, and L. S. Wang, “Hypoxia protects H9c2 cells against ferroptosis through SENP1-mediated protein DeSUMOylation,” International Journal of Medical Sciences, vol. 18, no. 7, pp. 1618–1627, 2021.
[13] X. Wang, J. Pan, D. Liu et al., “Nicorandil alleviates apoptosis in diabetic cardiomyopathy through PI3K/Akt pathway,” Journal of Cellular and Molecular Medicine, vol. 23, no. 8, pp. 5349–5359, 2019.
[14] D. Yan, Y. Cai, J. Luo et al., “FOXO1 contributes to diabetic cardiomyopathy via inducing imbalanced oxidative metabolism in type 1 diabetes,” Journal of Cellular and Molecular Medicine, vol. 24, no. 14, pp. 7850–7861, 2020.
[15] N. S. Dhalla, A. K. Shah, and P. S. Tappend, “Role of oxidative stress in metabolic and subcellular abnormalities in diabetic cardiomyopathy,” International Journal of Molecular Sciences, vol. 21, no. 7, p. 2413, 2020.
[16] S. J. Lee, P. Chandrasekran, C. H. Mazucanti, J. F. O’Connell, J. M. Egan, and Y. Kim, “Dietary curcumin restores insulin homeostasis in diet-induced obese aged mice,” Aging (Albany NY), vol. 14, no. 1, pp. 225–239, 2022.

[17] T. J. Park, J. H. Park, G. S. Lee et al., "Quantitative proteomic analyses reveal that GPX4 downregulation during myocardial infarction contributes to ferroptosis in cardiomyocytes," Cell Death & Disease, vol. 10, no. 11, p. 835, 2019.

[18] G. M. Abu-Taweel, M. F. Attia, J. Hussein et al., "Curcumin nanoparticles have potential antioxidant effect and restore tetrahydrobiopterin levels in experimental diabetes," Biomedicine & Pharmacotherapy, vol. 131, p. 110688, 2020.

[19] V. O. Gutierrez, R. P. Assis, C. A. Arcaro et al., “Curcumin improves the effect of a reduced insulin dose on glycemic control and oxidative stress in streptozotocin-diabetic rats,” Phytotherapy Research, vol. 33, no. 4, pp. 976–988, 2019.

[20] A. H. El-Far, Y. H. Elewa, E. Z. Abdelfattah et al., “Thymoquinone and curcumin defeat aging-associated oxidative alterations induced by D-galactose in rats’ brain and heart,” International Journal of Molecular Sciences, vol. 22, no. 13, p. 6839, 2021.

[21] H. Lv, H. Yang, Z. Wang et al., “Nrf2 signaling and autophagy are complementary in protecting lipopolysaccharide/d-galactosamine-induced acute liver injury by licochalcone A,” Cell Death & Disease, vol. 10, no. 4, p. 313, 2019.

[22] A. Uruno, D. Matsumaru, R. Ryoke et al., "Nrf2 Suppresses Oxidative Stress and Inflammation in App Knock-In Alzheimer’s Disease Model Mice,” Molecular and Cellular Biology, vol. 40, no. 6, p. e00467, 2020.

[23] W. Ye, J. Ma, F. Wang et al., "LncRNA MALAT1 regulates miR-144-3p to facilitate epithelial-mesenchymal transition of lens epithelial cells via the ROS/NRF2/Notch1/snail pathway," Oxidative Medicine and Cellular Longevity, vol. 2020, Article ID 8184314, 23 pages, 2020.

[24] C. Yu and J. H. Xiao, "The Keap1-Nrf2 system: a mediator between oxidative stress and aging," Oxidative Medicine and Cellular Longevity, vol. 2021, Article ID 6635460, 16 pages, 2021.

[25] S. Li, X. Wang, Y. Xiao et al., “Curcumin ameliorates mercuric chloride-induced liver injury via modulating cytochrome P450 signaling and Nrf2/HO-1 pathway,” Ecotoxicology and Environmental Safety, vol. 208, p. 111426, 2021.