Egypt. J. Chem. **Vol. 65**, No. 5 pp. 179 - 186 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Cardioprotective Effect Of Cyanidin-3-O-Glucosidein Ischemic Heart Is Mediated Via Inhibition Of Autophagy

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Abstract

It is elevated proves that the polyphenolic compounds of natural origin, such as anthocyanins, being cyanidin-3-O-glucoside (Cy3G) one of the most widely distributed anthocyanins, may control ischemia/reperfusion (I/R) injury although how much sheltering occurs is not obvious. Here, the role of cyanidin-3-O-glucoside pretreatment of cardiac ischemia and dysfunction was studied. Cyanidin-3-O-glucoside in 3 doses (5, 10, and 15 mg/kg body weight), was introduced in vivo 5 min before a 45 min closure of the left anterior descending artery, followed by a 120 min reperfusion in male Wistar rats. Blood samples and cardiac tissue specimens were obtained for studying the cardiac markers and the expression of lncRNA-CTC-448F2.4, miR1273a, and mRNA-DCN (Decorin), Specimens of left ventricles were obtained and prepared for histopathological criteria. Specimens of pretreated rats showed minimal cardiac edema, hemorrhage, cellular inflammatory infiltration, and fibrosis in cardiac tissue samples. Notably, this protective administration of cyanidin-3-O-glucoside also produced a dose-dependent downregulation in lncRNA- CTC-448F2.4 and mRNA-DCN (Decorin) with elevation in miR-1273a expressions. Cyanidin-3-O-glucoside pretreatment leads to improvements in cardiac function tests, enzymes, and myocytes. In conclusion, Cyanidin-3-O-glucoside is promising for the protection of the heart from injury following ischemia-reperfusion in the rat examination.

Keywords: Cyanidin-3-O-glucoside; epigenetic ;ischemia/reperfusion (I/R) injury; Rat model

1. Introduction

One of the leading causes of morbidity across the world is acute myocardial infarction (AMI). Myocardial reperfusion is known to be an effective therapeutic choice against AMI [1]Dramatic reduction in mortality from acute myocardial infarction has occurred during the last decades. However, there is still an increasein the incidence of post-infarction heart failure[2].In cardiovascular diseases, ischemic heart disease is the main cause of death, and it is believed that the blood supply restoration, such as reperfusion therapy, is the most effective treatment of cardiac ischemia [3] Nevertheless, an irreversible damage may be caused or even exacerbated during reperfusion of ischemic myocardium with oxygen and substrate-richblood, leading to a deterioration of heart function, which is called an ischemia–reperfusion (I/R) injury[4].Following (I/R)treatment, an occurrence of myocardial ischemia/reperfusion (I/R) injury usually shows in patients with cardiovascular diseases, such as strokes, coronary heart diseases and myocardial infarction[5]. During myocardial (I/R)injury , metabolic disorders and vascular structural damage may present in patients who suffer from (I/R) injury [6] (I/R) injury is induced by reperfusion of blood flow through different complex processes, including disruption of mitochondrial membrane potential, ion accumulation, the formation of reactive oxygen species, etc. Autophagy is one of the processes

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Receive Date: 13 September 2021, Revise Date: 20 September 2021, Accept Date: 26 September 2021
DOI: 10.21608/ejchem.2021.95695.4494

activated in response to (I/R)injury [7], It offers a representation of a highly evolutionary conserved process. Through this process, macromolecules and cytoplasmic material are degraded into lysosomes and recycled for biosynthetic or energetic purposes. There is an association between dysfunction of the autophagic process and the onset and development of many human chronic pathologies, such as cancer, as well as metabolic, cardiovascular, and neurodegenerative diseases[8]. Autophagy is the "double-edged weapon" in the pathology of (I/R)injury, and there is a controversy about whether autophagy is beneficial or detrimental. It was targeted as a therapeutic strategy by many studies based on the autophagy impact and regulation on myocardial (I/R)injury [1]. The role of decorin in cardiovascular diseases was recently reviewed by Vu et al., they summarized the role and function of decorin in cardiovascular diseases. Decorin interacting via cell surface receptor regulates plenty of cellular functions (reduces TGF-β dependent fibrosis, proliferation of myofibroblasts and endothelial cells, renal and neuronal protection from ischemic assaults) but these processes are dependent on cell types and pathological circumstances[9]. Myocardial adaptation to oxidative/reductive stress is an essential mechanism that has been evolved for the survival of heart from different disorders. Mitochondria targeting antioxidants have an impact indeed in preventing the detrimental effects of myocardial ischemia/reperfusion injury and in other rodent models of cardiovascular diseases [10]. Complex and variable changes in miRNA expression occur before, during, and after myocardial ischemia and reperfusion. Many studies have pinpointed miRNAs' significant role in the modulation of the cascade of myocardial ischemia-reperfusion (I/R)injury, and the effect of certain therapies on miRNA expression[11]. Competing endogenous RNAs (ceRNAs) form regulatory networks may be the orchestrator and the main factor in pathological processes. The miRNAs are the key factor of the ceRNA network and may contributes in control of the expression of lncRNA"[12]. Bioinformatics analysis of TargetScan and miRWalk databases predicted that miR-1273a had the matched binding base with DCN (Supplementary). In the present study, Cyanidin significantly decrease cardiac enzymes after (I/R)injury, It is accompanied with significant good histopathological appearance of heart muscles and significant protection of the cardiac myocytes destruction,Cyanidin-3-O-glucoside presents many properties, such as anti-inflammatory and anti-tumor effects[13]. Cyanidin-3-O-glucoside protects from the decrease in diameter of order two arterioles. Moreover, at the end of the time-period of blood flow recovery, higher dosage Cyanidin-3-O-glucoside induced a vasodilatationby 40.5% of baseline in order two arterioles, these vasodilatation were associated with preservation of capillary blood supply, significant reduction in venular leakage as well as in leukocyte adhesion to venular walls which may be due to the reduction in reactive oxygen compounds formation induced by Cyanidin-3-O-glucoside, because reactive oxygen compounds is a key player, play an important role in the regulation of vessel wall permeability and adhesion of leukocyte to venular walls[14].

2. Experimental.

2.1. Experimental animals.

Sixty male Wistar rats weighing 200−250 g were obtained from the animal house at Nile Pharmaceuticals Company, (Cairo, Egypt). In an animal room, animals were housed with a 12 h light/dark cycle and access to tap water and normal rat chow ad libitum. All animal procedures were conducted in accordance with the National Institute of Health guideline for the care and use of laboratory animals (NIH Publication No. 85-23, revised 1996), and had the approval of the Institutional Animal Ethics Committee at Ain Shams University, Faculty of Medicine (Approval no. 149/2020).

2.2. Induction of ischemia/reperfusion via ligation of anterior descending coronary artery.

Ligation

Male Wistar rats were anesthetized with urethane (1.2 mg/kg body weight). Using a rodent ventilator at 70–80 breaths/ min, intubation was established, followed by exposure of the heart through median sternotomy and placement of a ligature around the left anterior descending coronary artery with the use of 5-0 polyethylene suture. A noose around a rubber band that was placed flat on the myocardium was formed with the use of the free ends of the ligature. Coronary occlusion was induced by the noose tightening around the rubber band for 45 min [14] This was confirmed by immediate blanching of the infarcted area. Releasing the ligature for 120 min achieved the reperfusion. For confirming myocardial ischemia, Electrocardiogram tracing in lead II was obtained before and after ligation. The sham animals were exposed to the same procedure but without ligation.

2.3. Animal groups

There was a random assignment of animals to five groups (12 rats/group):

Sham group: coronary ligature was positioned without being tied, ischemia-reperfusion (I/R)injury,

pretreated groups in three doses;Cyanidin-3-O-glucoside-5mg/kg body weight (Cy3G-5), Cyanidin-3-O-glucoside-10mg/kg body weight (Cy3G-10), Cyanidin-3-O-glucoside-15mg/kg body weight (Cy3G-15)group. The Cyanidin-3-O-glucosidedoses were administered intraperitoneally, 5 min prior to coronary ligation. In each group, half of the rats were allocated for the measurement of cardiac function and molecular assay, while the other half were allocated for histopathological examination.

2.4. Blood sample collections and measurement of cardiac markers

In order to obtain the Blood for the assessment of serum cardiac markers, cTnT,CK–MB, and LDH centrifugation of the retro-orbital blood samples was performed at 1200×g for 10 min. A measurement of serum LDH activities was conducted spectrophotometrically, with a quantification of serum cTnTand CK-MB by the use of a commercial ELISA kit, Rat CKMB (Creatine Kinase MB Isoenzyme) ELISA Kit, based on the manufacturer's instructions.

2.5. Tissue preparation for histological microscope analysis of cardiac tissues

Specimens from both ventricles were fixated in 10 % PBS- buffered formalin overnight, then placed in 70 % ethanol and processed using paraffin block. Routine hematoxylin-eosin (H&E) and Masson's trichrome stain for detecting collagen fibers, staining were performed on the 3 μm tissue sections, There was an examination of all the tissue sections for morphological evidence of cell death and fibrosis.

2.6. Molecular assays

Quantification of the RNA panel using real-time PCR (qPCR)

Total RNA and miRNA extraction and purification: 1. total and miRNAs were extracted using a miRNeasy Mini Kit; cat no: 217004 (Qiagen, Hilden, Germany) according to the manufacturer's protocol. 2. Reverse transcription: A synthesis of cDNA was conducted by reverse transcription reaction using miScript II RT Kit; cat no: 218161; (Qiagen, Hilden, Germany). 3. miRNA gene expression analysis: The quantification of miRNA-1273a level was performed by amplification , using Hs_miR-1273a miScript Primer Assay; cat no: 218300, ID: MS00031339 and the miScript SYBR Green PCR Kit, cat no: 218073. A normalization of the gene expression was conducted by the Hs_SNORD68_11 miScript Primer Assay, cat no:218300, ID: MS00033712 as a housekeeper gene 4. The quality of total RNA was detected b spectrophotometer A260 to A280 ratio and only specimens with RNA/protein ratio more than 2 included in this study.

The quantification of Lnc-CTC- 448F2.4 expression level was performed by amplification , using RT² qPCR Primer Assay for HumanCTC448F2.4, Primer Assay; cat no: 330701; ID: LPH25249A and the RT² qPCR SYBR Green/ROX Master Mix Kit, cat no:330520. A normalization of the gene expression was conducted by the Hs_ACTB_1_SG QuantiTect Primer Assay, cat no:249900, ID: QT00095431 as a housekeeper gene 5. mRNA gene expression analysis: The quantification of mRNA-DCN expression level was performed by amplification , using the Hs_DCN _1_SG QuantiTect Primer Assay, cat no:249900, ID: QT00032459 and the QuantiTect SYBR Green PCR Kit cat no: 204141 (Qiagen, Germany). There was a use of the Primer sequence Hs_ACTB_1_SG QuantiTect Primer Assay, cat no:249900, ID: QT00095431 as housekeeper gene. After amplification, melting curve analysis was performed to confirm the specificities of the amplicon for SYBR® Green dye–based PCR amplification according to its specific melting temperature(Tm).An analysis of all samples was conducted with the use of the 5 plex Rotor-Gene PCR Analyzer (Qiagen, Germany).Gene Expression Calculation: The 2ΔΔCt method was conducted for analyzing gene expression levels as an endogenous reference control for normalization purposes.(Livak, K. J., &Schmittgen, T. D. (2001) Cardiac markers results and molecular results in a supplementary excel sheet (Results sheet).

2.7. Statistical Analysis

Graphpad Prism version 7 was used for carrying out results' analysis, with data expressed as mean ± SD. The study groups were compared by using analysis of variance (ANOVA), followed by Tukey's test. The Spearman correlation test was used to conduct a correlation between Lnc-CTC-448F2.4, miR-1273a, and DCN. $P < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. Effects on cardiac enzymes, CK-MB, LDH and cTnT

Induction of ischemia in the second group; the (I/R)injury group cause a significant upregulation in cardiac markers cTnT, CK-MB,LDH when compared to the sham group, all the pretreated groups revealed a significant reduction in cardiac markers ;CK-MB,LDH compared to the (I/R)injury group, Study for dose dependence shows that while the Pretreated group with low dose; the (Cy3G-5mg/kg body weight) was unable to significantly down-regulate the cTnT level. The pretreatment groups with higher dose (Cy3G-10/kg body weight) and (Cy3G-15/kg body weight) significantly down-regulate the cTnT level in both groups. while there is no significant