



The Role of Mitochondrial Energetics and Inflammasome NLRP3 in Diabetic Cardiomyopathy

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i42B32449

Editor(s):

(1) Dr. Triveni Krishnan, National Institute of Cholera and Enteric Diseases, India.

Reviewers:

(1) Makhlof Mohamed Mahmoud Bekhit, Benha University, Egypt.

(2) Ana Cristina García Ulloa, Instituto Nacional de Ciencias Médicas y Nutrición, Mexico.

Complete Peer review History: <https://www.sdiarticle4.com/review-history/72727>

Review Article

Received 20 June 2021
Accepted 26 August 2021
Published 02 September 2021

ABSTRACT

Diabetic Cardiomyopathy is the worldwide leading cause of lethal heart disorders burdening the healthcare systems. Mitochondrion is the key regulator of myocardial metabolism. It fuels the cardiocytes and regulates the pumping activity of heart. People living with diabetes have defected myocardial metabolism which may likely to cause ventricular dysfunction or other heart disorders due to mitochondrial DNA (mtDNA) mutation. Furthermore, the inflammatory injury due to inflammasome activation is a potent contributor to the cardiac injuries. Though the mechanism of inflammation is still poorly known. This review highlights the association of altered mitochondrial energetics and inflammasome activation with cardiomyopathies.

Keywords: *Diabetic cardiomyopathy; inflammasome; mitochondrial energetics; caspases-1; interleukin IL-1 β ; interleukin IL-18.*

1. INTRODUCTION

Diabetic Cardiomyopathy is the significant contributor to cardiac disorders which may include coronary heart disease, heart failure or other complications. It causes many molecular alterations in the heart [1]. Long term hyperglycemia is linked with micro vascular and macro vascular diseases that can forefront to blindness, nerve damage, kidney disease, visual impairment, heart diseases amputation, and strokes [2,3]. People living with diabetes are at the higher risk of heart diseases and it is worldwide leading cause of mortality and morbidity [4]. On the other hand for the normal functioning of the heart, a maintained redox balance and continual supply of mitochondrial ATP is required. In people living with diabetes, this mitochondrial redox balance is disturbed because excessive supply of glucose affects the normal mitochondrial functions [5] & related to monogenetic defects in Beta-cell functions [6-8]. This mitochondrial dysfunction is upstream defect imposed on the heart due to diabetes [9]. There occurs point mutations in mitochondrial DNA results in the release of proinsulin that is unable to convert into insulin and such characters are inheritable in autosomal dominant patterns [10-12].

Furthermore inflammatory process is another factor, thought to be the major modulator of diabetic cardiomyopathy [13-15]. This inflammatory reaction is initiated by reactive oxygen species (ROS) produced by mitochondrial oxygen [16]. Recent studies in mice models indicates that diabetes boosts up apoptosis in the heart cell that results in the serious injuries to the cardiocytes [16-18]. The sterile inflammatory response is mainly mediated by multi-protein complex known as inflammasome which is an initial biosensor of heart complications [19]. The NLR family of intracellular radars is a critical factor of the native immune system. Majority of NLR family adherents can form multiprotein centers, called inflammasomes, and are proficient of stimulating the cysteine protease caspase-1 in response to a wide range of incentives containing both microbial and self-molecules. Caspase-1 activation giving out the discharge of the proinflammatory cytokines interleukin-1b (IL-1b) and IL-18, which play key roles in host shield to infective insults. Deregulation of the inflammasome has also been associated to a variety of cardiac complications [8,14,20,21]. Cytokines and chemokines released by T-helper

cells are the key mediators of inflammatory process [13]. The chemokine CXCL10 actively participates in the heart damage initiation and progression. The extent of these cytokines accumulation is dependent upon the decline in cardiac functions [22]. The objective of this discussion is to highlight the inflammatory processes which are occurring in people living with diabetes and could become the reason for the lethal cardiac injuries. If scientists could identify the key factors of these inflammatory processes, there are possibilities to introduce effective anti-inflammatory therapies as a cure of cardiomyopathy.

2. MITOCHONDRIAL ENERGETICS

Mitochondrion is the main source which provides energy required for the normal functioning of the heart. Ca^{+2} ions regulate the mitochondrial function and ATP generated by cellular respiration in mitochondria is utilized for heart contractility. However, Ca^{+2} ions accumulation leads to reduced energy production and increased ROS specie level [23]. This creates oxidative stress leading to mitochondrial dysfunction and heart failure [24]. Diabetes type I contributes to impaired systolic and diasystolic function due to hyperglycemia. Recent studies on mice models have indicated the involvement of diabetes in altered mitochondrial energetics. People living with diabetes have reduced respiration in complex II and IV substrate, relatively low ADP phosphorylation rate and increased H_2O_2 emission, thus creating oxidative stress in cardiocytes. Thus therapies that correct the metabolically challenged diabetic heart would have potential to cure various diabetic cardiomyopathies [5,17,25-27].

3. MITOCHONDRIAL GENETICS

Mitochondria play important role in the secretion of insulin from beta cells of pancreas [28]. Various pedigree analyses have shown that some forms of diabetes are closely related to mitochondrial DNA mutation. In some cases, there occurs a unique 10.4kb deletion in mitochondrial DNA. The deletion breakpoint of about 10,432 is between 4398bp in tRNA gene and 14822 in cytochrome b gene that affects the mitochondrial oxidative phosphorylation. This deletion exclusively passes on via maternal inheritance and is the main causes of some forms of inherited diabetes [29]. Sometimes a point mutation from adenine to guanine occurs at position 8296 in tRNA and inherits maternally

[28]. People living with diabetes are at higher risk of coronary heart disease cardiomyopathies as compared to other metabolic syndromes [30].

4. INFLAMMASOME NLRP3

Inflammasome is a protein complex consisting of NLRP3, ASC (apoptosis associated speck-like protein containing caspase domain) and procaspase-1 involved in inflammatory reactions. It responds to microbial infection [31] as well as intracellular damages higher level of reactive oxygen species (ROS), islets amyloid polypeptide [32], uric acid crystals [33] and β -amyloid peptide [34]. The LRR region of inflammasome NLRP3 is sensor domain which senses the danger signals resulting in the oligomerization of NLRP3 via NACHT domain. Its

PYD is effector domain that interacts with the PYD domain of ASC which recruits procaspase-1 via its own CARD domain. Procaspase-1 is activated into caspase-1 by autocatalysis [35] resulting in the maturation of various cytokines including IL-1 β and IL-18 [13]. These activated cytokines also elicits the cell death known as Pyroptosis [36]. NLRP3 is normally in ubiquitinated form, its LRR domain is first deubiquitinated by ROS species which activates it [37]. IL-1 β is a proinflammatory cytokine, secreted by immune cells i.e., macrophages, monocytes and dendritic cells which on proteolytic cleavage by caspase-1 converts into mature form [38]. Inflammasomes are not just restricted to immune cells but also activated in non-immune cells such as cardiocytes, podocytes etc. [13].

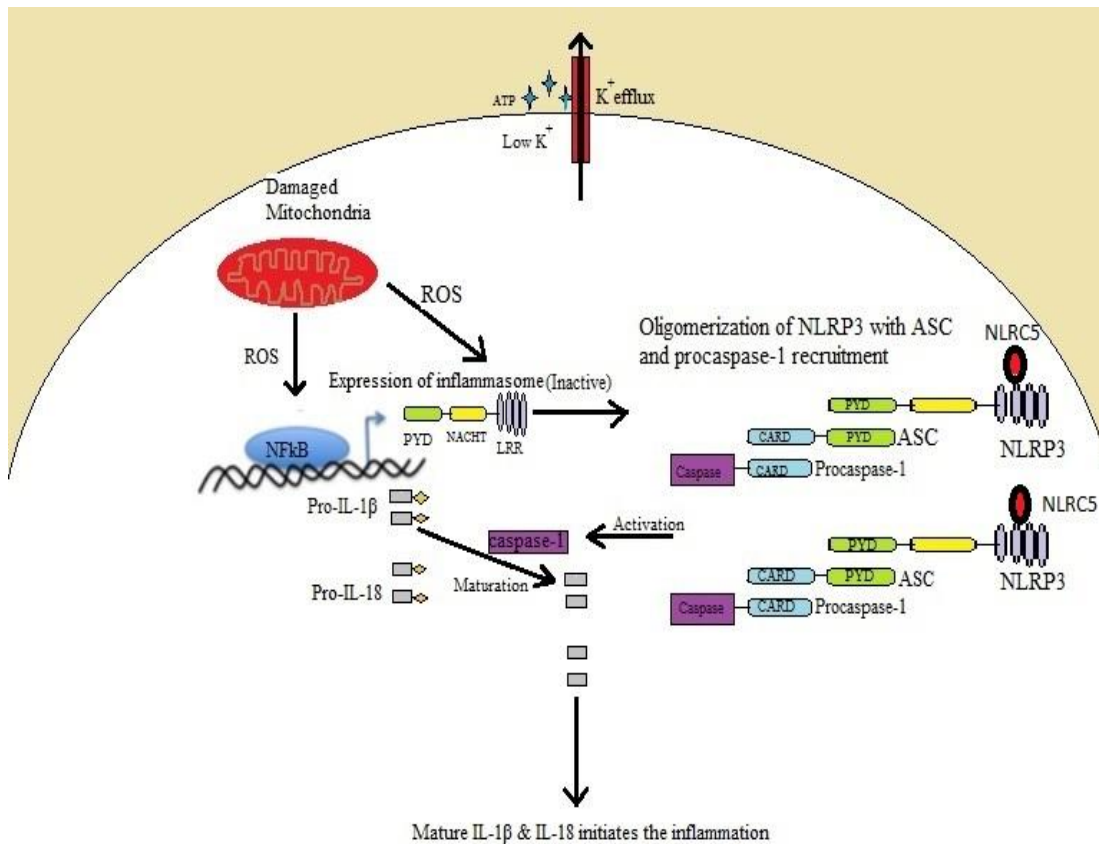


Fig. 1. Mitochondrial damage is occurring in diabetic patient which results in generation of excessive ROS that is sensed LRR region of inflammasome NLRP3. Then oligomerization of NLRP3 with ASC occurs via PYD domain and recruits procaspase-1. It gets activated into caspase-1 by autolysis and causes the maturation of interleukin IL-1 β and IL-18 that causes inflammation or pyroptosis

Table 1. List of table different molecules with their main action

Sr. no.	Molecules	Functions
1.	Caspase-1	Enzyme that involves in proteolytically cleaves of other proteins i.e. the precursors of the inflammatory cytokines interleukin 1 β and interleukin 18
2.	IL-18	Promote activation of Th1 cell and enhance the cytotoxic activity of CD8+ T cells and natural killer (NK) cells by upregulation of FasL
3.	CXCL10	They are involved in chemo-attraction for <u>monocytes/macrophages</u> , <u>T cells</u> , promotion of <u>T cell</u> adhesion to <u>endothelial cells</u> , <u>NK cells</u> and <u>dendritic cells</u>
4.	DAMPs	It activates the innate immune system by interacting with (PRR) pattern recognition receptors
5.	PAMPs	It activates innate immune responses and protect the host from infection

The NLRP3 inflammasome binds lysosomal content in the cytoplasm through cathepsin-B-dependent processing of an undeviating NLRP3 ligand (3). All DAMPs (Danger-associated molecular patterns) and PAMPs (Pathogen-associated molecular patterns) together with ATP and crystalline activators create the generation of reactive oxygen species (ROS). A ROS-dependent path prompts NLRP3 inflammasome compound formation. Caspase-1 clustering brings auto-activation and caspase-1-dependent progress and excretion of proinflammatory cytokines, such as interleukin-1 β (IL-1 β) and IL-18 (Schroder and Tschopp, 2010; Duewell, 2010). The NLRP3 inflammasome is triggered by mitochondrial apoptotic indicating a licensed assembly of interleukin-1 β (IL-1 β). NLRP3 secondary signal activators such as ATP prompted mitochondrial abnormality and apoptosis, consequential release of oxidized mitochondrial DNA (mtDNA) into the cytosol, where it bound to and stimulated the NLRP3 inflammasome [39].

Heart failure is allied with chronic cardiac inflammation which effects the normal functioning of heart. However the mechanism by which this inflammation occurs is still poorly known. Recent studies on mice models has indicated that Nlrp3 inflammasome is linked with heart diseases as it activates the proinflammatory cytokine IL-1 β resulting in systolic dysfunction. Blocking of this cytokine via antagonist receptor IL-1 reverses the injurious phenotype and can be a possible therapeutic approach in future to prevent heart failure [40]. Similarly myocardial infarction involves inflammatory process. It is regulated by many pattern recognition receptors (PRRs). One of the best known receptors is Toll-like receptors which get activated by ROS species (stressed condition). Activation of TLRs initiates the release of cytokines that activates the cytosolic

protein inflammasome. This leads to infarct size development in cardiocytes [41].

5. CASPASE INHIBITION

Apoptosis is the demarcated process of cell death in human body. This process is mostly regulated by the family of aspartate specific-cysteine proteases known as caspases. If this process is not regulated properly, it could cause some serious out comes such as heart failure. For therapeutic interest several studies on animal models have been done that indicates caspase inhibition can minimize the severity of heart diseases and hence can be cured. For this purposes, different pharmacological compounds such as N-benzyloxy carbonyl-Val-Ala-Asp-fluoromethylketone (z-VAD.fmk) [42] and VX-765 [43] were introduced in mice to prevent the effect mediated by caspases. Furthermore, *E. coli* endotoxin was also utilized to block the activity of caspases in *in vitro* studies [42]. Furthermore, many human proteins are antagonist to the activity of caspases. Out of them, one of the most effective proteins such as survivin was expressed in recombinant *E. coli* and its effect was tested in *vivo*. This protein proved to be very effective in inhibiting the activity of caspase 3 and 7 and critically halts the process of apoptosis of cells. These apoptotic inhibitor proteins are ubiquitously found in humans as well as viruses [44].

6. CONCLUSION

One of the leading cause of heart failure around the world is Diabetic Cardiomyopathy. Numerous genetic and metabolic alterations occur in diabetes that results in the malfunctioning of the heart processes which in turn might lead to cardiocytes death. mtDNA mutation is thought to be closely associated with diabetes that results in

metabolic changes in the activity of mitochondria. Due to high glucose level in the cells and defective functioning of mitochondria, a cellular oxidative stress is created. This oxidative stress mediates the activation of inflammasome that initiates inflammatory process and results in infarct size heart and cardiocytes injury. Thus inhibition of this inflammatory reaction can be the future therapeutic strategy of diabetic cardiomyopathies. *E.coli* endotoxin, survivin, VX-765 and z-VAD. fmk have potential therapeutical applications as pharmacological or biological inhibitors of inflammasome and caspases in near future. Moreover, this area holds room for the discovery of new potential and effective anti-inflammatory agents which could help in preventing this fatal cardiac condition.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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