

Cell death, dysglycemia and myocardial infarction (Review)

XIAO-FANG TIAN¹, MING-XIA CUI¹, SHI-WEI YANG², YU-JIE ZHOU² and DA-YI HU³

¹Key Laboratory of Preclinical Study for New Drugs of Gansu Province, Department of Pharmacology, School of Basic Medical Sciences, Lanzhou University, Lanzhou, Gansu 730000; ²12th Ward, Department of Cardiology, Beijing An Zhen Hospital, Capital Medical University, Beijing Institute of Heart Lung and Blood Vessel Disease, The Key Laboratory of Remodeling-Related Cardiovascular Disease, Ministry of Education, Beijing 100029; ³Department of Cardiology, People's Hospital Affiliated to Peking University, Beijing 100044, P.R. China

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Abstract. Dysglycemia (hyper- and hypoglycemia) has been associated with higher mortality among patients suffering from myocardial infarction (MI). Moreover, dysglycemia may induce cell death. Cell death (necrosis, apoptosis and autophagy) is a ubiquitous process that characterizes the course of several diseases, including MI, and occurs in diverse forms varying in mechanism, pattern and consequence. Therefore, cell death is a potential pathway through which dysglycemia affects the outcome of MI and it is essential to regulate myocardial cell death in the treatment of patients with MI caused by dysglycemia. In this review, we summarized the mechanisms of MI at the cellular level and the regulatory effects of dysglycemia on myocardial cell death. The ability to modulate myocardial cell death may be a promising target of new treatments aimed at limiting MI caused by dysglycemia. However, further research is required to elucidate the mechanisms underlying cell death regulation in MI caused by dysglycemia.

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Correspondence to: Professor Yu-Jie Zhou or Professor Shi-Wei Yang, 12th Ward, Department of Cardiology, Key Laboratory of Remodeling-Related Cardiovascular Diseases, Beijing Anzhen Hospital, Capital Medical University, Ministry of Education, An Ding Men Wai, Chao Yang District, Beijing 100029, P.R. China
E-mail: azyj12@163.com
E-mail: jackydang@163.com

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1. Introduction

Cell death may be defined as an irreversible loss of plasma membrane integrity (1). Three types of cell death have been distinguished in mammalian cells according to morphological criteria: necrosis, apoptosis and autophagy. Cell death, either progressive or acute, is a hallmark characteristic of various cardiac diseases, including myocardial infarction (MI) (2). MI remains a leading cause of morbidity and mortality worldwide (3). MI occurs when myocardial ischemia (diminished blood supply to the heart) exceeds a critical threshold and overwhelms myocardial cellular repair mechanisms designed to maintain normal operating function and homeostasis (4). Dysglycemia (hyper- and hypoglycemia) is a strong predictor of mortality and increases the mortality risk among MI patients (5-7). Furthermore, previous studies reported a correlation between dysglycemia and cell death (8,9). Therefore, we may hypothesize that cell death is a potential pathway through which dysglycemia affects the outcome of MI. It is imperative to achieve a full understanding of the mechanism underlying cardiomyocyte death and investigate the mechanisms by which dysglycemia affects the outcome of MI at the cellular level.

2. Cell death

Cell death and cell proliferation are the two predominant physiological processes that regulate tissue homeostasis in the adult organism. They act to maintain cell numbers in tissues and organs at constant levels. Similar to cell proliferation, cell death is closely controlled in its activation and execution. Apart from biochemical processes occurring in the dying cell, execution ultimately involves the orderly removal of cell debris. Thus, cell death is an essential part of tissue renewal.

3. Classification and mechanisms of cell death

Necrosis. Type I cell death, most commonly known as necrosis, is often negatively perceived: death lacking the characteristics of programmed cell death and thus accidental and uncontrolled. However, previous studies demonstrated that necrosis is closely regulated. There are two main necrotic pathways: the death receptor pathway and mitochondrial pathway (10).

The death receptor pathway of necrosis may be stimulated by tumor necrosis factor (TNF- α), Fas ligand (FasL) and TNF-related apoptosis-inducing ligand. Programmed necrosis initiated by the ligation of TNF receptor (TNFR) 1, which is expressed in the majority of cell types and contains a cytoplasmic death domain (DD), as opposed to TNFR2, has been extensively characterized; thus, the research on death receptor-mediated necrosis focuses primarily on this pathway. Receptor-interacting protein (RIP) 1 consists of an N-terminal kinase domain, a RIP homotypic interaction motif and a C-terminal DD (11). Among these, DD mainly controls cell death. Therefore, TNFR1 is important due to its binding to the DD of RIP1 (12), which ultimately leads to cell necrosis. The mitochondrial pathway is an alternative pathway of necrosis. Anaerobic glycolysis is activated during ischemia to provide ATP, leading to the accumulation of H⁺ and acidosis. H⁺ is pumped out of the cell by the Na⁺/H⁺ exchanger, which, in combination with the malfunctioning Na⁺/Ca²⁺ exchanger operating in reverse mode, results in increased concentrations of cytoplasmic Ca²⁺. Increase in Ca²⁺ in mitochondria induces Ca²⁺-dependent dehydrogenase activation, a decrease in NADH and electron flux through the electron transport chain, an increased production of reactive oxygen species (ROS) and a decrease in ATP levels. Moreover, ischemia results in ATP depletion and ROS generation, with the latter being further exacerbated during reperfusion. The long-lasting opening of the membrane permeability transition pore, which is regulated by cyclophilin D (CypD), is involved in this pathway (2,13).

Apoptosis. Type II cell death, also known as apoptosis, has been well-characterized and the molecular events involved in apoptotic cell death are extensively comprehended. There are two major apoptotic signaling pathways: the extrinsic (also involving death receptors) and the intrinsic (mitochondria-mediated) pathways (14). The extrinsic pathway, being necrotic, involves death receptors. This pathway is also activated by death ligands, such as FasL or TNF- α , which bind to cognate receptors on the plasma membrane with Fas or TNFR (15). However, numerous studies suggest that Fas, rather than TNFR, is the major mechanism underlying the activation of extrinsic apoptosis. In the extrinsic pathway, death receptor Fas is activated by FasL. This interaction leads to the recruitment of a DD (e.g., Fas-associated DD) and caspase-8 activation, which in turn activates caspase-3. These events ultimately lead to cell apoptosis. By contrast, the intrinsic pathway is activated by a wide variety of apoptotic signals, including growth factor deprivation, hypoxia, oxidative stress and DNA damage (16). The mitochondrion is the primary organelle involved in the mediation of the intrinsic apoptotic pathway. Cytochrome *c* (cyto-*c*), which is released from mitochondria, is a key factor in this process. The regulation of cyto-*c* release from mitochondria is modulated by the Bcl-2 protein family. The Bcl-2 proteins may be classified as anti-apoptotic (e.g., Bcl-2 and Bcl-xL) or pro-apoptotic (e.g., Bad, Bak and Bax) (14). In the intrinsic pathway, cyto-*c* is induced by the pro-apoptotic Bax and Bak proteins. Subsequently, cyto-*c* with apoptotic protein activating factor 1 (Apaf1) and caspase-9 form the apoptosome, with resulting activation of caspase-9. Caspase-9, in turn, activates caspase-3, leading to cell apoptosis. In addition to cyto-*c*, Smac/DIABLO, apoptosis inducing factor (AIF) and

endonuclease G (Endo G) are also released from the mitochondria. AIF and Endo G may directly induce apoptosis, in contrast to Smac/DIABLO.

Although the extrinsic and intrinsic are two different pathways of apoptosis, they are intrinsically linked. One of the pro-apoptotic Bcl-2 family members, Bcl-2-interacting protein (Bid), may regulate the interaction between extrinsic and intrinsic pathways. Bid is usually located in the cytosol; however, when it is cleaved to 'truncated Bid' (tBid) by the activated caspase-8, Bid translocates to the mitochondria and regulates cyto-*c* release (17).

Autophagy. Type III cell death, also known as autophagy, is a cell survival mechanism that involves the degradation and recycling of cytoplasmic components. Autophagy modulates cell death through excessive self-digestion and degradation of essential cellular constituents (18,19). The regulation of autophagy is a complex process. Numerous signaling pathways, including nutrient signaling, insulin/growth factor pathways, energy sensing, stress response and pathogen infection, are crucial to the regulation of autophagy (20). Firstly, the central factor in nutrient signaling pathways is TOR/mTOR (21). TOR acts as an efficient gatekeeper in autophagy, on which it also exerts an inhibitory effect. In the presence of growth factors and abundant nutrients, it is the major inhibitory signal that shuts off autophagy. The mTOR pathway is regulated by the 5'-AMP-activated protein kinase (AMPK) (22). Glycogen synthase kinase-3 (GSK-3) is another regulator of the mTOR pathway in cardiac cells (23). Similar to AMPK, GSK-3 β was also demonstrated to activate autophagy by inhibiting mTOR signaling (24). Secondly, both insulin/growth factor- and pathogen infection pathway-induced autophagy appears to be TOR-independent (25). Thirdly, the energy-sensing signaling pathway is also involved in autophagy regulation (26). Lum *et al* (27) reported that cellular ATP content may induce autophagy. In cultured cardiac myocytes, a decrease in ATP levels is often associated with an increase in AMP. AMPK, which activates autophagy by inhibiting mTOR signaling, is activated in response to elevations in the AMP/ATP ratio. Thus, autophagy may be upregulated by AMP through the activation of AMPK (28-30). Lastly, various extra- and intracellular stresses, which include ER stress (31,32), hypoxia (33) and oxidative stress (34), also potentially induce autophagy and it is important for organisms to adapt to or overcome unfavorable conditions. In the transcriptional regulation of autophagy, autophagy genes are regulated at the transcriptional level in response to stress. Forkhead box class O (FoxO) transcription factor was the first transcription factor demonstrated to be necessary and sufficient to induce autophagy in the *Drosophila* larval fat body (35). FoxO1 and FoxO3 are highly expressed in cardiac cells (36) and regulate autophagy by activating transcription of the Atg genes (37). Both FOX1 and FOX2 have been reported to induce expression of the Bcl-2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3) (38,39), which renders them potent inducers of autophagy.

Although the classification into different modes of death is useful, there is considerable overlap between the different mechanisms. For example, not all the characteristics of apoptosis are observed in all cell types and, in certain instances, the

apoptotic cell may undergo secondary necrosis. In addition, previous studies indicated that apoptosis and autophagy may involve complementary pathways and that autophagic degeneration may be a part of apoptosis, at least in certain types of cells (40,41).

Physiological and pathological roles of cell death. Although replacement of senescent cells with new cells requires programmed elimination of older, damaged or less functional tissue, an unexpected loss of cells accelerates growth of similar cells, in a healing process that is essential for functional homeostasis. However, abnormalities in the control of cell death contribute to a variety of diseases. Extensive retardation of cell death has been associated with human disease (42). For example, insufficient apoptosis may contribute to carcinogenesis. However, excessive cell death may be a component of the pathogenesis. These processes are significant in the pathogenesis of major diseases such as cancer, stroke, infection, inflammation and neurodegenerative disorders (43). In addition, a number of different types of cells undergo cell death in the cardiovascular system and may underlie several human heart diseases, including atherosclerosis, heart failure and MI (44). For example, the death of endothelial and vascular smooth muscle cells is involved in vessel injury and remodelling and in several vascular pathologies, such as atherosclerosis and aneurysm formation (45). Loss of cardiomyocytes is associated with ischemic and dilated cardiomyopathies, with the injury being attributed to ischemia/reperfusion (I/R), as well as with MI.

4. Cell death under the conditions of MI

Necrosis and MI. Necrosis, which was believed to be unregulated, was largely ignored in MI. Although cardiac myocyte necrosis is considered to be the major pathological lesion in acute MI (AMI), its significance in the pathogenesis was not formally evaluated until recently. A significant proportion of necrosis appears to be regulated and plays an important role in the pathogenesis of MI (46,47). It has been demonstrated that the death receptor and mitochondrial pathways mediate cardiac myocyte necrosis and play a key role in MI. In the death receptor pathway, infarct size was markedly reduced in mice with Fas loss-of-function mutations (48,49). In addition, genetic manipulation of the TNF- α signaling axis was also reported to be significant in MI. When necrosis is activated by TNF- α with deletion of TNFR1 and TNFR2, infarct size is exacerbated and coronary artery occlusion becomes permanent (50). Conversely, overexpression of TNF- α (at low levels) on a wild-type background restores myocardial damage (51). These studies suggested that the repressed death receptor pathway may limit MI during I/R. The role of the mitochondrial pathway in MI is also important. Deletion of CypD markedly decreases infarct size during I/R *in vivo*. Moreover, there are reports that necrosis occurring predominantly in the central area is more common between 6-24 h following MI (45). The extent of necrosis peaks at 24 h after reperfusion and remains constant thereafter (52,53).

Apoptosis and MI. In the extrinsic pathway, mice that lacked Fas exhibited a decrease in cardiac myocyte apoptosis in

models of doxorubicin toxicity, as well as marked reductions in infarct size following I/R (48). Cardiac myocyte-specific overexpression of Bcl-2 significantly reduced infarct size, cardiac myocyte apoptosis and cardiac dysfunction following I/R (54). Bax deficiency reduced infarct size and cardiac dysfunction following I/R and MI in mice (55). Taken together, these results suggested that the intrinsic apoptotic pathway also plays a central role in MI. Apoptosis has been demonstrated to be involved in the acute and chronic loss of cardiomyocytes in MI. Animal and human studies demonstrated that apoptosis was present in the border zone of the infarcted myocardium in the early phase (56). Permanent coronary occlusion induced maximum cardiac myocyte apoptosis at 4.5 h and reperfusion accelerated the timing of apoptosis, compared to permanent occlusion (57).

Autophagy and MI. Autophagy has also been detected in a variety of human cardiomyopathies, including ischemic heart disease, Danon disease, MI and heart failure (58). There is increasing evidence that autophagy, as a mechanism for the degradation of damaged long-lived proteins and organelles, plays an important role in the process of MI (59,60). Further investigations are necessary, although current available reports indicate that autophagy exerts a predominantly cardioprotective effect during MI.

Of note, induction of autophagy by inhibition of mTOR with everolimus (RAD) prevents adverse left ventricular (LV) remodeling and limits infarct size following MI (61). Furthermore, autophagy promotes the survival of cardiomyocytes under extra- and intracellular stress (62). In addition, autophagy was shown to be cardioprotective (63). Brady *et al* (64) reported that preconditioning enhanced autophagy and inhibition of autophagy eliminated the cardioprotective effects of preconditioning. Moreover, autophagy may maintain LV function during starvation via functional FoxO (37). Induction of autophagy via these methods may be a novel therapeutic approach aimed at limiting infarct size, attenuating adverse LV remodeling, promoting cardiomyocyte survival and maintaining LV function.

5. Regulatory effects of dysglycemia on cell death

Regulatory effects of hyperglycemia on myocardial cell death. Numerous physiological studies (8,65-69) have demonstrated that hyperglycemia exerts a direct detrimental effect on ischemic myocardium through several mechanisms. Hyperglycemia exerts a regulatory effect on cell death. Malhotra *et al* (65) demonstrated that hyperglycemia is a potent activating signal for cardiac protein kinase C (PKC) isozymes and induces the apoptosis program in cardiac myocytes. The PKC ϵ translocation activator (ψ ERACK) eliminated hyperglycemia-induced apoptosis, strongly suggesting a cardioprotective role for PKC ϵ . Therefore, cardiac PKC isozymes modulate hyperglycemia-induced apoptosis and activation of cardiac PKC ϵ protects adult rat ventricular myocytes (ARVM) against the hyperglycemia-induced death signal. Capes *et al* (66) reported that hyperglycemia induces ROS generation and apoptosis in cardiomyocytes, which contributes to diabetic cardiomyopathy. Rac1 is pivotal in hyperglycemia-induced apoptosis in cardiomyocytes. The

role of Rac1 is mediated through NADPH oxidase activation and associated with mitochondrial ROS generation. Deficiency of Rac1 reduced hyperglycemia-induced mitochondrial ROS production in cardiac cells, significantly inhibited apoptosis and slightly improved myocardial function. Furthermore, a previous study demonstrated that the levels of Bcl-2 and Bax (well-known proteins involved in apoptosis) were evaluated under consistently high glucose levels in human umbilical vein endothelial cells (67). Whether this theory is applicable to myocardial cell apoptosis remains to be elucidated. These findings suggested that hyperglycemia exerts an effect on apoptosis in patients with MI. There are also numerous ongoing investigations regarding the role of hyperglycemia in the autophagy of cell death. Eguchi *et al* (8) reported that diabetic mice exhibited a reduced infarct size and reduced apoptosis 24 h after reperfusion, as indicated by TUNEL analysis in cardiac sections. This may be explained by increased autophagy through the AMPK-mTOR pathway detected in diabetic mice hearts. Diabetic mice exhibit a smaller infarct area post-MI, which may be the result of upregulated autophagy. Therefore, we may be able to limit infarct size caused by dysglycemia via the activation of autophagy. Furthermore, higher glucose levels in patients with AMI are associated with higher free fatty acid concentrations. The cellular autophagosome-lysosomal complexes in autophagy degrade a large number of free fatty acids in order to maintain the mitochondrial energy supply and improve cell survival through autophagy degradation within a certain range (68,69). Thus, we may be able to protect cardiac myocytes against dysglycemia through the activation of autophagy. However, whether hyperglycemia is causally related to increased mortality due to a large infarct size and impaired LV function following AMI, or is simply an epiphenomenon of the severe disease conditions, remains to be elucidated; the mechanisms of the detrimental effect of hyperglycemia on the myocardium also require further investigation.

Regulatory effects of hypoglycemia on myocardial cell death. The pathophysiology of myocardial ischemia is an imbalance between myocardial oxygen demand and supply. Hypoglycemia may result in increased myocardial oxygen demand. In addition, hypoglycemia and coronary vasoconstriction limit the delivery of substrate (glucose and free fatty acids) to the myocardium, which further disrupts the balance between myocardial energy supply and demand (9). Therefore, the role of hypoglycemia in myocardial energy supply and demand at the cellular level is the focus of this review. Hypoglycemia also plays a role in the autophagy of cell death. Hypoglycemia causes a significant reduction in the levels of ATP. A decrease in ATP levels is accompanied by an increase in the AMP/ATP ratio, resulting in activation of the AMPK. Matsui *et al* (68) reported that glucose deprivation induced autophagy within a certain range via activation of AMPK in isolated cardiac myocytes. Decreased AMPK activity and subsequent reduction in cardiac autophagy are important events in the development of diabetic cardiomyopathy, as well as in MI. Therefore, we may be able to promote the survival of cardiomyocytes in the presence of dysglycemia by inducing autophagy.

6. Conclusion

Several studies have suggested that dysglycemia, including hyper- and hypoglycemia, is a strong predictor of mortality and significantly increases the mortality risk among MI patients. Furthermore, there are also studies (8,9,66-69) suggesting that dysglycemia induces myocardial cell death. Cell death (apoptosis, autophagy and necrosis) plays a significant role in MI. Thus, we hypothesize that cell death is a potential pathway through which dysglycemia influences the outcome of MI and that closely regulated myocardial cell death is essential in the treatment of patients with MI caused by dysglycemia. Due to the limited availability of clinical trial data suggesting that more efficient glucose control may improve patient outcomes following MI, in this review we aimed to summarize the mechanisms of MI at the cellular level, as well as the regulatory effects of dysglycemia on myocardial cell death. The ability to modulate myocardial cell death may be a promising therapeutic strategy in limiting MI caused by dysglycemia. However, the precise mechanisms of cell death in MI caused by dysglycemia, as well as the timing and manner of cell death modulation, remain to be elucidated.

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