CARDIOMYOCYTES WITH HYPOXIA/REOXYGENATION TREATMENT INDUCED APOPTOSIS THROUGH THE INCREASE OF ROS PRODUCTION AND MITOCHONDRIAL FISSION

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Myocardial infarction is the leading cause of morbidity and mortality in the world. The timely and successful myocardial reperfusion with the use of thrombolytic treat or primary percutaneous coronary is the most effective method to reduce the infarct size and improve the clinical outcome in patients after an acute myocardial infarction. The therapy of restoring blood flow to the ischemic myocardium, however, can induce cardiomyocyte death. The phenomenon is called the myocardial ischemia/reperfusion (I/R)-induced injury and paradoxically reduces the beneficial effects of myocardial reperfusion. It can cause adverse reactions, including increased oxidative stress and myocardial apoptosis. Mitochondrial fission forms the fundamental control of mitochondrial quality, which may play a key role in the progression of cardiac I/R-induced injury. However, the precise mechanism is involved in I/R-induced injury remains unclear. We established a hypoxia/reoxygenation (H/R) model by using H9c2 cells in vitro to simulate myocardial I/R-induced injury.The present study was designed to determine the condition of H/R is optimal for the detection of the role of mitochondrial function in cardiomyocytes and to examine the effects of H/R on the cell viability, apoptosis, ROS, mitochondrial membrane potential, and mitochondrial fission.Cells were exposed to hypoxic condition for 6 h or 24 h. They were then exposed to normoxia for another 3 h, 12 h, or 24 h. H/R (6 h/12 h) treatment significantly reduced cell viability by MTT assay. H/R treatment increased thenumber of apoptotic cells by using the TUNEL and annexin V-FITC /PI double staining assays. In addition, H/R treatment significantly increased the expression of cleaved caspase 3, an indicator of apoptosis, compared to the control.H/Rincreasedmitochondrial ROS, cellular superoxide anion and cytoplasmic H_2O_2 by using MitoSox, DCFH and DHEreagents, respectively.H/R increased mitochondrial fission-associated protein Mff and p-Drp1 expression. NAC, a ROS inhibitor, decreased H/R-increased apoptosis and mitochondrial fission. Based on these findings, Cardiomyocytes with H/R treatment induced apoptosis through the increase of ROS production and mitochondrial fission

Keywords - Cardiomyocytes, Hypoxia/Reoxygenation, Apoptosis, ROS, Mitochondrial Fission.