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Pyrroloquinoline quinone inhibits oxygen/glucose deprivation-induced apoptosis by activating the PI3K/AKT pathway in cardiomyocytes

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Abstract The purposes of this study were to examine the protective effect of pyrroloquinoline quinone (PQQ) on oxygen/glucose deprivation (OGD)-induced injury to H9C2 rat cardiomyocytes and to investigate the mechanism. Using H9C2 cells cultured in vitro, we examined changes in cell viability with an MTT assay at 12, 24, and 48 h after injury induced by OGD. Various concentrations of PQQ (1, 10, and 100 μM) were added, and the effect of PQQ on cell viability after OGD was assessed using the MTT assay. Thus, the optimal concentration of PQQ for the protection of cardiomyocytes against oxygen and glucose deprivation injury was determined. We also used flow cytometry analysis to examine the effect of PQQ on H9C2 cells with OGD-induced injury. The molecular probe 2′,7′-dichlorofluorescin diacetate was used to label the H9C2 cells, and flow cytometry was used to detect the effect of PQQ on reactive oxygen species (ROS) content. After labeling the H9C2 cells using a mitochondrial green fluorescent probe (Mito-Tracker Green), we measured the change in the mitochondrial content of PQQ-treated H9C2 cells. Western blotting was used to examine the effect of PQQ on the phosphatidylinositol 3-kinase (PI3K)/Akt pathway in the H9C2 cells. The results of the MTT assay showed that 48 h of OGD significantly injured the H9C2 cells ($p < 0.01$) and that treatment with 100 μM PQQ effectively decreased the level of OGD-induced injury ($p < 0.01$). The results of the flow cytometry analysis showed that PQQ significantly reduced apoptosis in H9C2 cells subjected to OGD ($p < 0.05$). In addition, OGD significantly increased the ROS level in H9C2 cells ($p < 0.01$), and PQQ significantly inhibited this increase ($p < 0.05$). The results of the Mito-Tracker Green staining suggested that PQQ effectively inhibited the decrease in mitochondrial content caused by OGD ($p < 0.05$). Western blot analysis showed that PQQ partially reversed the decrease in Akt phosphorylation that was caused by OGD ($p < 0.05$). PQQ treatment dose-dependently protects H9C2 cells from OGD-induced injury by reducing apoptosis, decreasing intracellular ROS levels, and rescuing the OGD-induced decrease in mitochondrial content. The protective effect of PQQ may be related to its effects on the PI3K/Akt pathway.

Keywords Pyrroloquinoline quinone · Oxygen and glucose deprivation · Cardiomyocyte · Reactive oxygen species · PI3K/Akt

Introduction

Cardiovascular disease (CVD) is currently the leading cause of death in humans [1, 2]. In China, the morbidity and mortality of CVD have been increasing each year [3]. Ischemic heart disease constitutes a large subset of CVD, and thus is a serious threat to human health; thus, its pathogenesis and possible prevention strategies are hot topics in the medical research field [4, 5]. In the United States and many other industrialized countries, coronary heart disease is the main cause of death. Statistical data from recent years show that with economic development and the correlated changes in the standards of living, diet, and aging pattern in China, the morbidity and mortality of coronary heart disease in China are on the rise [6]. Coronary heart disease has a serious impact on quality of life,

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