ABSTRACT

Acute myocardial Infarction (AMI) is a major cause of premature mortality in developed countries and is largely associated with Ischemia/Reperfusion (I/R) injury, in which irreversible damage is caused to myocytes during infarction. Erythropoietin (EPO) is a hematopoietic cytokine, and its receptor (EPOR) is shown to be present in tissues outside blood, including the heart. The EPO also possesses a non-hematopoietic action, mediated through inhibition of apoptosis and appears to be essential for the tissue-protective effects. EPO is strongly inferred to protect the cardiomyocytes from the reperfusion injury and our aim is to elucidate the cardioprotective effect and exact mechanism behind the cardioprotection. H9C2 cells and Neonatal cardiomyocytes (NCM) were exposed to H/R (Hypoxia/Reperfusion) with or without pretreatment using 10, 15 and 20 U/ml of EPO. We determined apoptosis using the following assays. The cell viability was determined using MTT, apoptotic and necrotic nuclei by Acridine orange and Ethidium bromide, reactive oxygen species (ROS) by Dicholorofluoresin Diacetate (DCF-DA), Mitochondrial Membrane potential (Δψm) by Rhodamine-123, Calcium homeostasis by Fluo 4-AM, and activity of late apoptotic protease caspase-3 activity by caspase-3 assay. The expression of pAkt, pp38 MAPK, pBAD, XIAP and cytochrome c release were analyzed by western blot. Viability was increased from 53 % and 45 % in H/R injured H9C2 cells and NCM to 89% and 83.5% of (20 U/ml) EPO pretreated H9C2 and NCM respectively. Control and EPO pretreated samples showed bright green nuclei (normal nuclei) whereas H/R injured samples showed some early and late apoptotic/necrotic nuclei. In control and EPO treated myocytes, Rhodamine-123 colocalized with DCF fluorescence only in the perinuclear region. While in H/R induced samples, Rhodamin-123 colocalized with DCF fluorescence both in perinuclear and cytoplasmic
region. DCF fluorescence in samples subjected to H/R showed more fluorescence compared to control and EPO pretreated samples. EPO maintains Ca\(^{2+}\) homeostasis by maintaining Ca\(^{2+}\) both in the cytoplasm and in nucleus showed by Fluo-4 AM fluorescence throughout the cell compared to nuclear accumulation of Ca\(^{2+}\) in cells induced with H/R. EPO increases the phosphorylation of Akt, p38 MAPK, BAD and increased expression of XIAP compared to H/R. EPO pretreated samples showed decrease cytosolic release of cytochrome c and increased mitochondrial accumulation of cytochrome c but samples without EPO pretreatment showed increased cytosolic release of cytochrome c and decreased mitochondrial accumulation of cytochrome c. EPO prevented the caspase-3 activations induced by H/R. Further myocytes blocked with Wortmannin and SB203580 showed increase caspase-3 activity and thus abolishes the protective effect of EPO. Hence we conclude from this study for the first time that EPO maintains \(\Delta \psi_m\), calcium homeostasis and exerts both anti-apoptotic and anti-necrotic effect in I/R injured H9C2 cells and NCM through modulation of Akt, p38 MAPK and downstream effectors of Akt. Together, these findings support mechanistic evidence for the protective effect of EPO in cardiomyocytes to prevent H/R-induced cell death and possibly create new avenues for effective cardioprotective therapeutics.