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A novel atypical PKC-iota inhibitor, echinochrome A, enhances cardiomyocyte differentiation from mouse embryonic stem cells

Key words: echinochrome A, cardiomyocyte differentiation, mitochondrial function, reactive oxygen species, stem cells, kinase activity

Echinochrome A (EchA) is a marine bioproduct extracted from sea urchins having antioxidant, antimicrobial, anti-inflammatory, and chelating effects which is the active component of the clinical drug histochrome. We investigated the potential use of Ech A for inducing cardiomyocyte differentiation from mouse embryonic stem cells (mESCs). We also assessed the effects of Ech A on mitochondrial mass, inner membrane potential ($\Delta\psi m$), reactive oxygen species generation, and levels of Ca^{2+}. To identify the direct target of Ech A, we performed *in vitro* kinase activity and surface plasmon resonance binding assays. Ech A dose-dependently enhanced cardiomyocyte differentiation with higher beating rates. Ech A (50 μM) increased the mitochondrial mass and membrane potential but did not alter the mitochondrial superoxide and Ca^{2+} levels. The *in vitro* kinase activity of the atypical protein kinase C-iota (PKCι) was significantly decreased by 50 μM of Ech A with an IC_{50} for PKCι activity of 107 μM. Computational protein-ligand docking simulation results suggested the direct binding of Ech A to PKCι, and surface plasmon resonance confirmed the direct binding with a low K_D of 6.3 nM. Therefore, Ech A is a potential drug for enhancing cardiomyocyte differentiation from mESCs through direct binding and inhibition of PKCι activity.

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