HDL Attenuates Palmatic Acid-induced H9C2 Cardiomyoblast Lipotoxicity And Apoptosis

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Abstract

Elevated free fatty acids can cause generation of reactive oxygen species (ROS) , leading to cardiomyocyte apoptosis. High-density lipoprotein (HDL) has been reported to possess key atheroprotective properties, including cellular cholesterol efflux capacity, anti-oxidative and anti-inflammatory activities. However, the underlying mechanisms are still largely unknown. Therefore, the aim of the present study is to test whether HDL could protect against palmitic acid (PA)-induced cardiomyocyte injury and to explore the possible mechanisms.Our results showed that HDL protected PA-induced cell death in a dose-dependent manner. PA increased ROS generation and induced the phosphorylation of JNK which in turn activated NF-κB-mediated inflammatory proteins expression. We also found that PA increased phosphorylation of P53 which in turn disturbed the balance of Bcl-2 family proteins, destabilized mitochondrial membrane potential, and triggered subsequent cytochrome c release into the cytosol and activation of caspase 3. These detrimental effects were ameliorated by HDL. Results from this study may provide insight into a possible molecular mechanism underlying HDL suppresses the free fatty acid-induced cardiac dysfunction.

Results

FIG.1

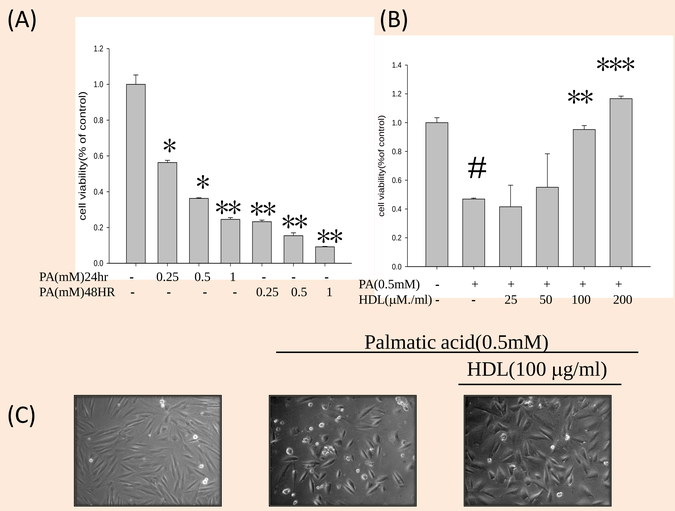


Fig.1. Effects of HDL on palmatic acid-induced H9C2 cells death. (A)H9C2 cells were incubated with PA at different concentrations as indicated for 24 or 48 hrs.(B)PA treated H9C2 cells were incubated with HDL at different concentrations as indicated,viability was determined via MTT assay. The values represent means±SEM from three separate experiments. #P< 0.01 vs. control; *P<0.05 vs. palmatic acid alone treatment. (C) photomicrographs were from phase-contrast microscopy.

FIG.2

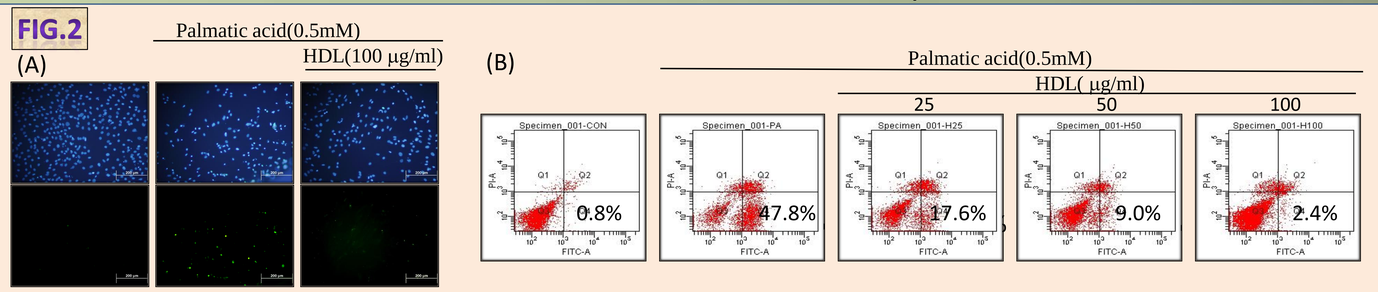


Fig2. Antiapoptotic effect of HDL on PA-induced H9C2 cells death. (A)Fluorescence images show the cells stained with 4,6-diamidino-2-phenylindole (DAPI)(upper panel) and stained using terminal deoxynucleotidyl transferase dUTP-mediated nick-end labeling (TUNEL) assay(bottom panel).(B) Flow cytometry profile represents Annexin-V-FITC staining in *x* axis and PI in *y* axis. The number represents the percentage of early apoptotic cells in each condition.

FIG.3

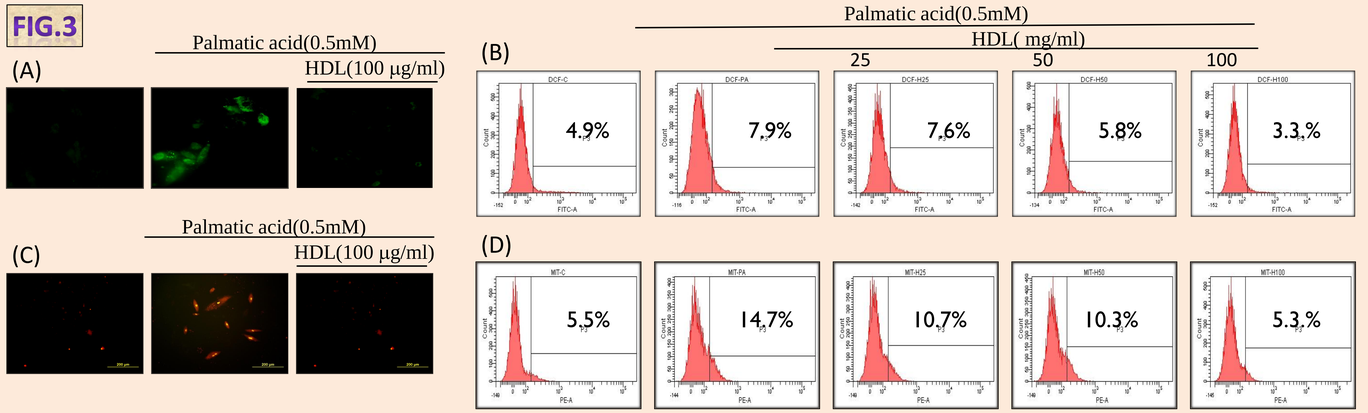


Fig.3 Inhibitory effect of HDL on palmatic acid induced ROS production in H9C2 cells. Fluorescence images showed the ROS level in H9C2 cells pretreated with HDL (100 μg/ml) for 2 hours followed by 0.5 mM palmatic acid for 24 hours incubation with (A) DCF-AM (10μM) and (C) Mit.sox(10 μM).Activation of (B) DCF-AM (10 μM) and (D) Mit.sox(10 μM) fluorescence intensity was examined using flow cytometry.

FIG.4

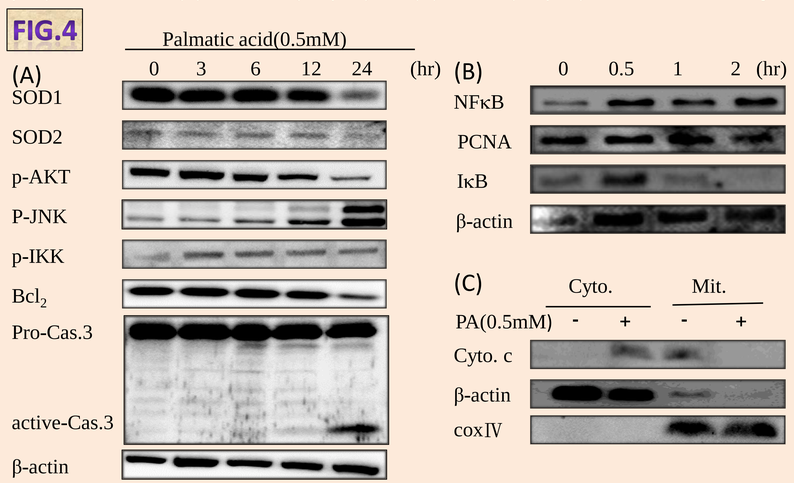


Figure.4. Palmatic acid-induced protein expression. (A) H9c2 cells were treated with palmatic acid (0.5 mM), and harvested at different time periods as indicated SOD1,SOD2,p-JNK,p-IKK,p-AKT,BCL_2,Caspase3,.(B)NFκB,IκB , and (C) cytrochrom C, proteins levels were examined by western blotting.

FIG.5

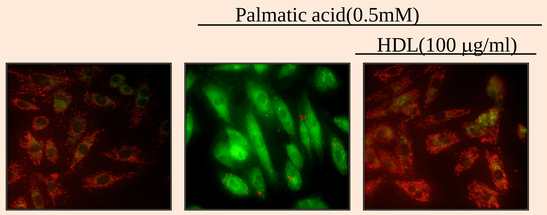


Fig.5. Effects of HDL on mitochondrial transmembrane permeability transition (△Ψm) in PA-treated H9C2 cells. △Ψm was assessed with the signal from monomeric (green) and J-aggregate (red) JC-1 fluorescence. Fluorescence images showed the activated JC-1 level in H9C2 cells pretreated with HDL (100 **μg/ml**) for 2 hours followed by 0.5 mM palmatic acid for 24 hours.

FIG.6

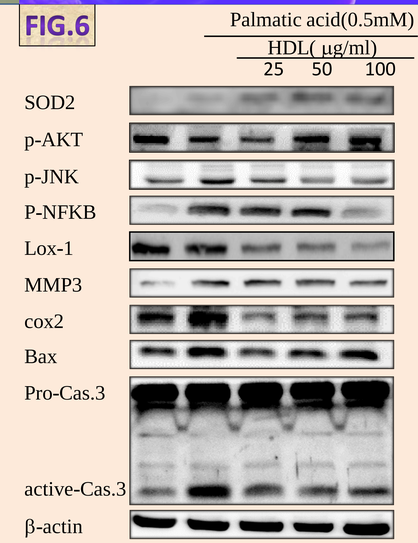
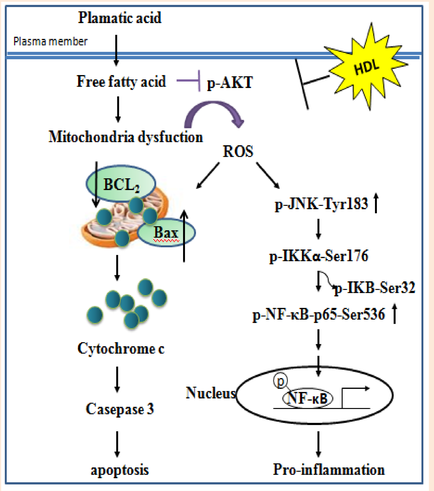


Fig.6. The levels of different proteins as indicated were analysed by western blotting. H9c2 cells were pretreated for 2 hours with HDL(25-100 μg/ml) for 2 hours and followed by palmatic acid (0.5mM) for 24 hours.

Summary



Our findings that HDL inhibits the Palmatic acid-induced oxidative damage and apoptosis in H9C2 cells , suggest that HDL may have clinical implications in the prevention of PA-induced cardiac injury.