

Cardioprotective effects of arjunolic acid in LPS-stimulated H9C2 and C2C12 myotubes via the MyD88-dependent TLR4 signaling pathway

ABSTRACT

Context: Arjunolic acid (AA) is a triterpenoid saponin found in *Terminalia arjuna* (Roxb.) Wight & Arn. (Combretaceae). It exerts cardiovascular protective effects as a phytomedicine. However, it is unclear how AA exerts the effects at the molecular level. Objective: This study investigates the cardioprotective effects of arjunolic acid (AA) via MyD88-dependent TLR4 downstream signaling marker expression. Materials and methods: The MTT viability assay was used to assess the cytotoxicity of AA. LPS induced in vitro cardiovascular disease model was developed in H9C2 and C2C12 myotubes. The treatment groups were designed such as control (untreated), LPS control, positive control (LPS þ pyrrolidine dithiocarbamate (PDTC)-25mM), and treatment groups were co-treated with LPS and three concentrations of AA (50, 75, and 100mM) for 24h. The changes in the expression of TLR4 downstream signaling markers were evaluated through High Content Screening (HCS) and Western Blot (WB) analysis. Results: After 24 h of co-treatment, the expression of TLR4, MyD88, MAPK, JNK, and NF- κ B markers were upregulated significantly (2-6 times) in the LPS-treated groups compared to the untreated control in both HCS and WB experiments. Evidently, the HCS analysis revealed that MyD88, NF- κ B, p38, and JNK were significantly downregulated in the H9C2 myotube in the AA treated groups. In HCS, the expression of NF- κ B was downregulated in C2C12. Additionally, TLR4 expression was downregulated in both H9C2 and C2C12 myotubes in the WB experiment. Discussion and conclusions: TLR4 marker expression in H9C2 and C2C12 myotubes was subsequently decreased by AA treatment, suggesting possible cardioprotective effects of AA.