UNIVERSITI TEKNOLOGI MARA

ANTI-APOPTOTIC MECHANISM OF URSODEOXYCHOLIC ACID (UDCA) ON HYPOXIC CARDIOMYOCYTES

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MSc

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Ursodeoxycholic acid (UDCA), the most hydrophilic bile acid is used as a therapeutic agent in liver related diseases and to eliminate hydrophobic bile acid induced apoptosis in liver. Recently, studies suggested the potential used of UDCA in treating heart related diseases. UDCA is reported cardioprotective against the development of ischemia. However, the mechanism of action in UDCA-cardioprotection is not clearly understood. Therefore, this study aimed to determine the anti-apoptotic mechanisms of UDCA on cardioprotection using an *in vitro* hypoxic model of neonatal rat cardiomyocytes. Rat heart from newborn (0-2 days old) was isolated for primary cell culture of cardiomyocytes. Hypoxia was induced by using $CoCl_2$ and hypoxic chamber. Cardiomyocytes were incubated with UDCA (pre-UDCA and post-UDCA) and co-incubated with FTY720 (S1P receptor agonist), PTX ($G\alpha_i$ inhibitor). The treated cardiomyocytes were subjected for proliferation assay (MTS assay), beating assessment assay, protein expression (aSMase and nSMase, Hif-1α, caspase-3 and caspase-9, ERK and Akt), ROS generation assay and gene expression (*Hif-1α, smpd1, smpd2, caspase-3, caspase-9*). The data were analyzed by using sample paired t-test and One-way ANOVA. MTS assay revealed that UDCA was not toxic to cardiomyocytes even at high concentration (250 μm). Results showed that $CoCl_2$ activates Hif-1α expression, reduces cell viability; reduce beating rate, upregulates nSMase protein, increases ROS production, downregulates ERK and Akt protein expression. Interestingly, pre-UDCA treatment significantly abolished the negative effects of $CoCl_2$ on cell viability, beating rate, ROS production, ERK and Akt expression in cardiomyocytes. Treatment with PTX partially inhibits the protection of UDCA against $CoCl_2$ negatives effects on beating rate of cardiomyocytes. Meanwhile, FTY720 showed similarity with UDCA action in downregulating *smpd1* gene expression and upregulates *caspase-9 gene* expression. In conclusion, the current data suggests that UDCA mechanism is mediated partially through $G\alpha_i$-coupled receptor dependent and independent pathways in protecting cardiomyocytes against hypoxia. This study provides an insight of anti-apoptotic mechanism of UDCA on hypoxic cardiomyocytes.

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TABLE OF CONTENT