UNIVERSITI TEKNOLOGI MARA

MECHANISM OF URSODEOXYCHOLIC ACID (UDCA) CARDIOPROTECTION AGAINST HYPOXIA

ANIS SYAMIMI BINTI MOHAMED

MSc

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AUTHOR'S DECLARATION

I declare that the work in this thesis/dissertation 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 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.

Name of Student	:	Anis Syamimi Binti Mohamed
Student I.D. No	:	2012842542
Programme	:	Master of Science (Medicine) - MD780
Faculty	:	Medicine
Thesis Title	:	Mechanism of Ursodeoxycholic Acid (UDCA) Cardioprotection against Hypoxia
Signature of Student	:	.....
Date	:	August 2016

ABSTRACT

Ursodeoxycholic acid (UDCA) is the most hydrophilic bile acid and is widely used to treat liver diseases especially in cholestasis. Recently, UDCA is shown to have a potential effect in treating a cardiovascular disease which is an alternative to the current drugs that contains severe side effect. Studies have demonstrated that UDCA action is mediated by the sphingosine-1-phosphate (S1P) receptor in hepatocytes, however, not in heart. Furthermore, the mechanism of UDCA cardioprotection is still poorly understood. Therefore, this study was conducted to elucidate the mechanism of UDCA cardioprotection by using an *in vitro* hypoxic cardiomyocytes (CMs) model. CMs were isolated from newborn rats (0-2 days) and hypoxia was induced using chamber and $CoCl_2$. Cells were treated with UDCA (pre-UDCA and post-UDCA) and co-treated with either FTY720 (S1P receptor agonist) and PTX ($G\alpha_i$ inhibitor). The treated cells were subjected to proliferation assay (MTS assay), gene expression, protein expression (Western blot and immunofluorescence) and calcium imaging (loaded with Fluo-4). The data were analyzed by using the Sample Paired/Unpaired T-Test and One-way ANOVA. The findings of study show UDCA abolished the effect of chamber (Mean ± SEM; 55 % ± 7, $p < 0.05$) and $CoCl_2$-induced hypoxia (49 ± 4%, $p < 0.05$) on cell viability [UDCA-chamber (106% ± 2) and UDCA-$CoCl_2$ (94% ± 2, $p < 0.05$)]. No significant differences were observed in pre-UDCA and post-UDCA in hypoxic CMs ($p > 0.05$). The protective effect of UDCA was also observed in HIF-1α and p53 protein expression (UDCA-$CoCl_2$: 0.5387 ± 0.0087 vs $CoCl_2$ only: 0.8180 ± 0.0454, $p < 0.05$) where both HIF-1α and p53 protein were downregulated. Apart from that, no involvement of *HIF-1α, eNOS, NF-κB, FXR* and *BNIP3* gene were observed in this study. In addition, UDCA (0.95 ± 0.04) was shown to inhibit the alteration of $[Ca^{2+}]_i$ observed in $CoCl_2$-induced hypoxia cells (0.28 ± 0.03, $p < 0.05$). Treatment with PTX ($G\alpha_i$ inhibitor) partially inhibits the effect of UDCA on cell survival, but full inhibition of UDCA effect was observed in $[Ca^{2+}]_i$. The inhibition of $G\alpha_i$-coupled receptor pathways on HIF-1α and p53 protein expressions by PTX seems to further activate the effect of UDCA. Meanwhile, FTY720 shows the similarity of action with UDCA in cell viability, $[Ca^{2+}]_i$, HIF-1α and p53 protein expression. In conclusion, the current data suggest that UDCA could partially be mediated by $G\alpha_i$-coupled receptor-dependent and $G\alpha_i$-coupled receptor-independent pathways in cardioprotection mechanism against hypoxia. Furthermore, this study provides an insight of UDCA mechanism for cardioprotection in treating heart disease.

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