INVESTIGATION INTO THE EFFECTS OF IL-17 AND LAURIC ACID ON FARNESOID X RECEPTOR (FXR) EXPRESSION IN HUMAN HEPG2 CELLS

By

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ABSTRACT

INVESTIGATION INTO THE EFFECTS OF IL-17 AND LAURIC ACID ON FARNESOID X RECEPTOR (FXR) EXPRESSION IN HUMAN HEPG2 CELLS

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Farnesoid X receptor (FXR) acts as a ligand-modulated transcription factor and is a member of nuclear receptor family. FXR highly expressed in liver, kidney, intestine and adipose tissue. It is involved in bile acid metabolism, lipid metabolism and glucose metabolism. The involvement of FXR in various metabolisms makes it a promising candidate as a therapeutic target. IL-17 is a proinflammatory cytokine which promotes inflammatory response in mammalian immune system. While lauric acid, a saturated medium-chain fatty acid, is shown to have anti-inflammatory properties. This study was designed to investigate the effect of IL-17 and lauric acid in FXR expression in human HepG2 cells. Different concentrations of IL-17 at 1 ng/mL, 10 ng/mL and 100 ng/mL were used to treat HepG2 cells. The FXR mRNA expression was evaluated using qRT-PCR. IL-17 alone was able to repress the FXR mRNA expression in dose-response manner to 0.40-fold in 10 ng/mL of IL-17. Hence, 10 ng/mL of IL-17 was selected for subsequent treatment with lauric acid. HepG2 cells were co-treated with IL-17 and

different concentrations of lauric acid to evaluate if lauric acid displayed anti-inflammatory properties. Surprisingly, the FXR mRNA expression was further repressed to 0.07-fold, 0.19-fold and 0.30-fold with the addition of 5 μM, 10 μM and 20 μM of lauric acid respectively. However, the FXR mRNA expression was abrogated in lauric acid dose-respondent manner in 24-hour incubation. Protein analysis of FXR expression using western blot showed discrepancies between FXR mRNA and protein, indicating the possibilities of post-transcriptional or post-translational modification. In conclusion, this present study shows that IL-17 and lauric acid act synergistically in repressing FXR mRNA expression but lauric acid in higher concentration is able to augment IL-17 repressed FXR mRNA expression in a dose-dependent manner.

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DECLARATION

I hereby declare that the project is based on my original work except for quotations and citations which have been duly acknowledge. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

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Khoo Yie Woon

APPROVAL SHEET

The project report entitled "**INVESTIGATION INTO THE EFFECTS OF IL-17 AND LAURIC ACID ON FARNESOID X RECEPTOR (FXR) EXPRESSION IN HUMAN HEPG2 CELLS"** was prepared by KHOO YIE WOON and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Biomedical Science at Universiti Tunku Abdul Rahman.

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PERMISSION SHEET

It is hereby certified that **KHOO YIE WOON** (ID No: **13ADB04398**) has completed this final year project entitled "**INVESTIGATION INTO THE EFFECTS OF IL-17 AND LAURIC ACID ON FARNESOID X RECEPTOR (FXR) EXPRESSION IN HUMAN HEPG2 CELLS**" under the supervision of Dr. Chew Choy Hoong from the department of Biomedical Science, Faculty of Science.

I hereby give permission to the University to upload the softcopy of my final year project in pdf format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

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(KHOO YIE WOON)

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AF-1	Activation function 1
AhR	Aryl hydrocarbon receptor
ApoAI	Apolipoprotein A1
ApoC-III	Apolipoprotein C3
apoC-ll	Apolipoprotein C2
APS	Ammonium Persulfate
Asbt	Apical sodium dependent bile acid transporter
BCP	1-Bromo-3-Chloropropane
BSEP	Bile acid export pump
C/EBP	CCAAT-enhancer-binding protein
CA	Cholic acid
CDCA	Chenodeoxycholic acid
CO_2	Carbon dioxide
C_q	Threshold cycle
CYP7A1	7α-hydroxylase cytochrome P-450
-d (RFU)/ dT	Rate of change of relative fluorescent units with time
DBD	DNA binding domain
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
ED50	Half maximal effective dose
EDTA	Ethylenediaminetetraacetic acid
EG	Ethanol: Glycerol

EMSA	Electrophoretic mobility shift assay
FBS	Fetal bovine serum
FGF-15	Fibroblast growth factor-15
FoxO1	Forkhead box O1
FXR	Farnesoid X receptor
g	Acceleration of gravity (~9.8 m/s^2)
G6Pase	Glucose-6-phosphatase
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GEG	Guanidine hydrochloride: Ethanol Glycerol
GPBAR1	G protein–coupled bile acid receptor 1
GPR84	G protein-coupled receptor 84
GST	Glutathione-S-transferase
HDL	High-density lipoprotein
HNF4α	Hepatocyte nuclear receptor 4 alpha
HRP	Horseradish peroxidase
hsp90	Heat shock protein 90
IBD	Inflammatory bowel disease
IC50	Half maximal *inhibitory* concentration
IFNγ	Interferon gamma
IκB	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor
IKKi	Inducible IκB kinase
IKKα	Inhibitor of nuclear factor kappa-B kinase subunit alpha
IKKβ	Inhibitor of nuclear factor kappa-B kinase subunit beta

IL-17	Interleukin-17
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
IκB	Inhibitory kappa B
JAK-STAT	Janus kinase-signal transducer and activators of transcription
JNK	c-Jun N-terminal kinases
LBD	Ligand binding domain
LPL	Lipoprotein lipase
MAPK	Mitogen-activated protein kinase
MCFA	Medium-chain fatty acid
MEF	Mouse embryonic fibroblast
MEM	Minimum Essential Medium
MMP	Matrix metalloproteinase
NCoR	Nuclear corepressor
NEMO	NF-κB essential modifier
NF-κB	Nuclear factor kappa B
NHR	Nuclear hormone receptors
NK cell	Natural killer cell
NR	Nuclear receptor
NTCP	Na^+-taurocholate cotransporting polypeptide
Ost-α	organic solute transporter-alpha
PBS	Phosphate buffer saline
PEPCK	Phosphoenol-pyruvate carboxykinase

PGC-1α	Peroxisome Proliferator-activated Receptor-γ Coactivator 1α
PH	Partial hepatectomy
PPARα	Peroxisome Proliferator-activated receptor-alpha
PVDF	Polyvinylidene fluoride
qRT-PCR	Quantitative reverse transcription-polymerase chain reaction
RNA	Ribonucleic acid
RNase	Ribonuclease
RORα	RAR-related orphan receptor alpha
RORγτ	Retinoid-related orphan receptor gamma
SDS-PAGE	Sodium Dodecyl Sulfate -Polyacrylamide Gel Electrophoresis
SHP	Small heterodimer partner
siRNA	Small interfering ribonucleic acid
SLE	Systemic lupus erythematosus
SREBP-1c	Sterol regulatory element-binding protein 1-c
STAT3	Signal transducer and activator of transcription 3
TBE	Tris-Borate-EDTA
TEMED	Tetraethylmethylenediamine
TGF-β	Tumour growth factor-beta
Th17 cell	T helper 17 cell
TLR4	Toll-like receptor 4
Tm	Melting temperature
TNF-α	Tumour necrosis factor-alpha
TRAF6	Tumor necrosis factor receptor associated factor 6

v/v	Volume per volume
VLDL	Very low-density lipoprotein
w/v	Weight per volume
γδT	Gamma delta T cell

CHAPTER 1

INTRODUCTION

Farnesoid X receptor (FXR), with the gene symbol of NR1H4 is a member of nuclear receptor superfamily and acts as a ligand-modulated transcription factor. FXR was first discovered in year 1995, and is found abundantly in human liver, kidney, intestine and adrenals (Forman, et al., 1995; Li and Guo, 2015). Bile acids are the ligand which bind to FXR and leads to the activation of FXR. Hydrophobic bile acids chenodeoxycholic acid (CDCA) and cholic acids (CA) are the primary bile acids which bind to FXR most effectively (Wang, et al., 1999). The major function of FXR is to regulate the production of bile acids, in other word, FXR acts as the bile acids sensor in enterohepatic tissue. Bile acids level must be regulated as they are toxic and accumulation of bile acid will lead to hepatotoxicity (Fiorucci, et al., 2007). Besides bile acids homeostasis, activation of FXR will lead to other outcomes such as maintenance of cholesterol level by regulating its transport protein and biosynthesis enzymes (Watanabe, et al., 2004). Triglycerides and glucose metabolism are also affected by the regulatory mechanism of FXR. FXR is also responsible for liver regeneration, cholestasis, hepatic inflammation and hepatic fibrosis. FXR is studied extensively as it may be the therapeutic target in treating cholestasis, dyslipidemic disorders and insulin resistance patients. (Claudel, Staels and Kuipers, 2005; Fiorucci, et al., 2007; Wang, et al., 2008; Li, et al., 2010).

Interleukin-17 (IL-17) is a proinflammatory cytokine which is responsible for promoting host inflammatory response, auto-immunity, allergic and host defense. There are six members in this IL-17 family: IL-17A, IL-17B, IL-17C, IL-17D, IL-17E and IL-17F. Six of them are structurally similar. Among these six IL-17 members, IL-17A and IL-17F are the most common one as they have the highest degree of homology and they can form heterodimers or homodimer respectively (Wright, et al., 2007; Salvotore, et al., 2015). IL-17 is produced by different cells, mostly immune cells like natural killer cells, neutrophils, lymphoid-tissue inducer cells, gamma delta T cells, macrophages and dendritic cells (Cella, et al., 2009; Korn, et al., 2009; Takatori, et al., 2009; Passos, et al., 2010). IL-17 induces inflammatory response by triggering various pathways such as nuclear factor κB (NF-κB), mitogen-activated protein kinases (MAPKs) and CCAAT-enhancer-binding protein (C/EBP) cascades. Following these pathways, proinflammatory molecules will be synthesised. Cytokines like tumour necrosis factor- α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) produced from different cells will further enhance the inflammatory response. IL-17 will lead to the production of chemokines, recruiting immune cells to the site of injury. Besides cytokines and chemokines, matrix metalloproteinases (MMP), prostaglandin E2 will also be produced to degrade the extracellular matrix and induce vasodilation. All these combined to boost the inflammatory response induce by IL-17 (Chabaud, et al, 2000; Park, et al., 2005; Shen and Gaffen, 2008; Zhu and Qian, 2012; Song and Qian, 2013).

Lauric acid is a saturated medium chain fatty acid with the molecular formula of $C_{12}H_{24}O_2$. It makes up 45%-53% of the entire fatty acid composition of coconut oil, and is said to be the effective compound in virgin coconut oil (Dayrit, 2015). In addition to coconut oil, palm kernel oil and laurel oil also contain lauric acid (Fife, 2013). Lauric acid has bactericidal activity and it can influence the host immune response (Martingez, Vahjen and Zentek, 2016). Lauric acid also has strong anti-microbial and anti-inflammatory activities against *Propionibacterium acnes* (Nakatsuji, et al., 2009). Combining all the findings above, it is therefore hypothesised that lauric acid can relieve the inflammatory effects induced by IL-17, leading to the up-regulation of FXR mRNA expression in HepG2 cells.

Therefore, the objectives of this study are:

i. To determine the dose response of IL-17 on FXR mRNA expression in HepG2 cells.
ii. To investigate the co-treatment of lauric acid and IL-17 on FXR mRNA expression in HepG2 cells using quantitative reverse transcription-polymerase chain reaction (qRT-PCR).
iii. To investigate if the FXR mRNA results are translated into its protein expression for both IL-17 dose response test and cell treatment with lauric acid and IL-17 using western blot.

CHAPTER 2

LITERATURE REVIEW

2.1 Nuclear Receptor Superfamily

Nuclear receptors (NRs) encompass a superfamily of proteins which is regulated by ligands. These NRs will regulate the expression of certain genes as they are one of the biggest groups of transcription factor in human body. The first NR was first discovered in 1960s by Elwood Jenson and his collaborators (Jensen, 1962). The actions of nuclear receptors contribute to development, physiology, metabolic homeostasis, reproduction, and diseases (Fattori, et al., 2014; McEwan, 2016).

Nuclear receptor family can be classified into nuclear hormone receptors (NHRs) and orphan nuclear receptors. NHRs are the nuclear receptor with its ligands already known. The ligands of the known-ligand nuclear receptor are mostly small lipophilic molecules such as fatty acids, hormones and steroids. The ligands of orphan receptors however, are still unidentified (Olefsky, 2001; Fattori, et al., 2014). All NRs share the common structure including the DNA binding domain and ligand binding domain. DNA binding domain recognises and binds to specific DNA sequences, it is linked to ligand binding domain by a hinge. Ligand binding

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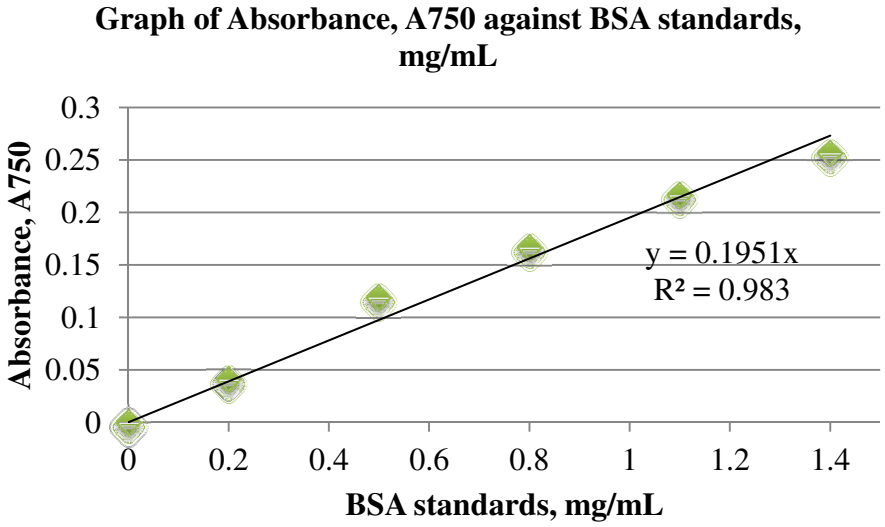
APPENDIX

APPENDIX A



Graph above shows the standard curve of bovine serum albumin (BSA) standards with concentration of 0.2 mg/mL, 0.5mg/mL, 0.8 mg/mL, 1.1 mg/mL and 1.4 mg/mL. The concentration of extracted protein was calculated based on the graph.