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

KHOO YIE WOON

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-responsive 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.
ACKNOWLEDGEMENTS

I am blessed to be given this chance to express my gratefulness to the person who I appreciate well. First and foremost, I would like to convey my deepest gratitude to my supervisor, Dr. Chew Choy Hoong. Her guidance, advice and knowledge are the one which helped me the most during the research as well as the thesis writing. This thesis would not have been possible without her persistent guidance.

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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.

____________________
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.

Approved by:

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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,

___________________

( KHOO YIE WOON)
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<tr>
<td>AhR</td>
<td>Aryl hydrocarbon receptor</td>
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<tr>
<td>ApoAI</td>
<td>Apolipoprotein A1</td>
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<tr>
<td>ApoC-III</td>
<td>Apolipoprotein C3</td>
</tr>
<tr>
<td>apoC-II</td>
<td>Apolipoprotein C2</td>
</tr>
<tr>
<td>APS</td>
<td>Ammonium Persulfate</td>
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<tr>
<td>Asbt</td>
<td>Apical sodium dependent bile acid transporter</td>
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<tr>
<td>BCP</td>
<td>1-Bromo-3-Chloropropane</td>
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<tr>
<td>BSEP</td>
<td>Bile acid export pump</td>
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<tr>
<td>C/EBP</td>
<td>CCAAT-enhancer-binding protein</td>
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<tr>
<td>CA</td>
<td>Cholic acid</td>
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<tr>
<td>CDCA</td>
<td>Chenodeoxycholic acid</td>
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<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
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<tr>
<td>Cₗ</td>
<td>Threshold cycle</td>
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<tr>
<td>CYP7A1</td>
<td>7α-hydroxylase cytochrome P-450</td>
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<tr>
<td>-d (RFU)/dT</td>
<td>Rate of change of relative fluorescent units with time</td>
</tr>
<tr>
<td>DBD</td>
<td>DNA binding domain</td>
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<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>DNase</td>
<td>Deoxyribonuclease</td>
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<tr>
<td>ED50</td>
<td>Half maximal effective dose</td>
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<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
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<td>EG</td>
<td>Ethanol: Glycerol</td>
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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 ($\sim 9.8 \text{ m/s}^2$)
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
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<td>PGC-1α</td>
<td>Peroxisome Proliferator-activated Receptor-γ Coactivator 1α</td>
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<td>PH</td>
<td>Partial hepatectomy</td>
</tr>
<tr>
<td>PPARα</td>
<td>Peroxisome Proliferator-activated receptor-alpha</td>
</tr>
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<td>PVDF</td>
<td>Polyvinylidene fluoride</td>
</tr>
<tr>
<td>qRT-PCR</td>
<td>Quantitative reverse transcription-polymerase chain reaction</td>
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<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RNase</td>
<td>Ribonuclease</td>
</tr>
<tr>
<td>RORα</td>
<td>RAR-related orphan receptor alpha</td>
</tr>
<tr>
<td>RORγτ</td>
<td>Retinoid-related orphan receptor gamma</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis</td>
</tr>
<tr>
<td>SHP</td>
<td>Small heterodimer partner</td>
</tr>
<tr>
<td>siRNA</td>
<td>Small interfering ribonucleic acid</td>
</tr>
<tr>
<td>SLE</td>
<td>Systemic lupus erythematosus</td>
</tr>
<tr>
<td>SREBP-1c</td>
<td>Sterol regulatory element-binding protein 1-c</td>
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<td>STAT3</td>
<td>Signal transducer and activator of transcription 3</td>
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<td>TBE</td>
<td>Tris-Borate-EDTA</td>
</tr>
<tr>
<td>TEMED</td>
<td>Tetraethylmethylene diamine</td>
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<td>TGF-β</td>
<td>Tumour growth factor-beta</td>
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<tr>
<td>Th17 cell</td>
<td>T helper 17 cell</td>
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<td>TLR4</td>
<td>Toll-like receptor 4</td>
</tr>
<tr>
<td>Tm</td>
<td>Melting temperature</td>
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<tr>
<td>TNF-α</td>
<td>Tumour necrosis factor-alpha</td>
</tr>
<tr>
<td>TRAF6</td>
<td>Tumor necrosis factor receptor associated factor 6</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>v/v</td>
<td>Volume per volume</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very low-density lipoprotein</td>
</tr>
<tr>
<td>w/v</td>
<td>Weight per volume</td>
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<tr>
<td>γδT</td>
<td>Gamma delta T cell</td>
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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; Salvatore, 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<sub>12</sub>H<sub>24</sub>O<sub>2</sub>. 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.
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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Graph above shows the standard curve of bovine serum albumin (BSA) standards with concentration of 0.2 mg/mL, 0.5 mg/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.