A study of the effect of leptin on prostate cells: emphasis on estrogen metabolism

Thesis presented by

Christine Nathan Habib Demian

Bachelor Degree in Pharmaceutical Sciences (2010)
Demonstrator of Pharmacology and Toxicology,
Faculty of Pharmacy, Ain Shams University.

Submitted for partial fulfillment of Master's degree in Pharmaceutical Sciences to Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University.

Under the supervision of

Prof. Ashraf Bahi El-Din Abdel-Naim

Professor of Pharmacology and Toxicology,
Faculty of Pharmacy, Ain Shams University.

Prof. Amani Emam Khalifa

Professor of Pharmacology and Toxicology,
Faculty of Pharmacy, Ain Shams University.
Seconded to "The Holding Company for Biological Products & Vaccines" as the Consultant to the Chairman for Strategic Planning.

Dr. Ahmed Mohamed Mahmoud Al-Abd

Lecturer of Pharmacology and Toxicology, Medical Division, National Research Center.

Faculty of Pharmacy
Ain Shams University
(2014)

Pre-requisite Post-Graduate Courses

Besides the work presented in this thesis, the candidate has attended the following courses:

General courses:

1. Instrumental analysis.
2. Physical chemistry.
3. Computer skills.
4. Statistics.

Special courses:

1. Pharmacology.
2. Clinical pharmacology and therapeutics.
3. Neuropharmacology.
4. Molecular pharmacology.
5. Selected topics in pharmacology and toxicology.

She has successfully passed examination in these courses with general grade ***Excellent.***

Head of Pharmacology and Toxicology Department

Prof. Ebtehal El Demerdash Zaki

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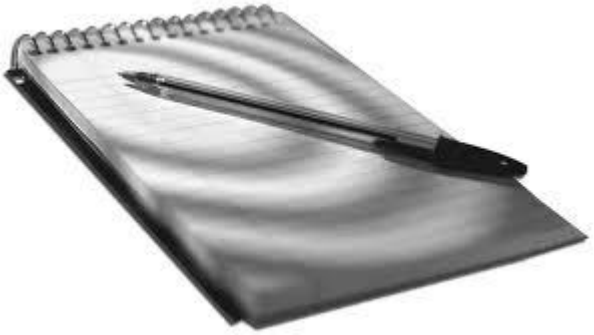
Christine Nathan Habib

Abstract



Obesity is recognized as an important risk factor for prostate cancer. Many obese individuals have leptin resistance associated with increased circulating plasma leptin. Previous *in-vitro* studies showed that leptin stimulated proliferation of human prostate cancer cells (DU145 and PC-3). Furthermore, estrogen and its metabolites are increasingly implicated in prostate cancer progression. Therefore, the present study was designed to investigate the effect of leptin on estrogen metabolism and whether it will shift estrogen metabolism to generate proliferative metabolites and/or decrease the formation of anti-proliferative metabolites in prostate cells. Malignant (PC-3) and benign (BPH-1) human prostate cells were treated with 17-β-hydroxy estradiol (1uM) alone or in combination with human recombinant leptin (0.4, 4, 40 ng/ml) for 72 h. Cell proliferation was determined by sulphorhodamine B assay and demonstrated that leptin caused significant growth potentiation in both cell lines. Immunocytochemical staining showed that leptin significantly increased the expression of estrogen receptor (ER) α and decreased that of ER-β in PC-3 cell line. Liquid chromatography-tandem mass spectrometry (LC-MS) revealed that leptin increased the concentration of the proliferative estrogen metabolite 4-hydroxyestrone and/or decreased that of the anti-proliferative metabolites (2-methoxyestradiol, 4-methoxyestradiol

and 2-methoxyestrone) in both cell lines. Interestingly, quantitative RT- PCR showed that leptin significantly upregulated aromatase and CYP1B1 expression, however downregulated that of COMT. Thus, these data indicate that leptin can influence prostate cell proliferation in relation to estrogen metabolism.

Keywords: Obesity; Leptin; Prostate; Estrogen metabolism.

List of Abbreviations

AdipoR	Adiponectin receptor.
ADT	Androgen deprivation therapy.
Akt	Protein kinase B.
AMPK	5'adenosine monophosphate-activated protein kinase.
ANOVA	Analysis of variance.
AR	Androgen receptor.
ArKO	Aromatase knockout.
β-ERKO	Estrogen receptor-β knockout.
Bcl-2	B-cell lymphoma 2.
bFGF	Basic fibroblast growth factor.
BMI	Body mass index.
BPH	Benign prostatic hyperplasia.
cDNA	Complementary deoxyribonucleic acid .
COMT	Catechol-o-methyltransferase.
CYP1A1	Cytochrome P450 1A1.
CYP1B1	Cytochrome P450 1B1.
DAB	Diaminobenzidine.
dATP	Deoxyadenosine triphosphate.

dCTP	Deoxycytidine triphosphate.
dGTP	Deoxyguanosine triphosphate.
DHT	Dihydrotestosterone.
DMSO	Dimethyl sulfoxide.
DNA	Deoxyribonucleic acid.
Dns-Cl	Dansyl chloride.
dNTPs	Deoxy nucleoside triphosphates.
dTTP	Deoxythymidine triphosphate.
E2	17-β-Hydroxyestradiol.
EDTA	Ethylene diamine tetracetic acid disodium salt.
ELISA	Enzyme-linked immunosorbent assay.
ER-α	Estrogen receptor alpha.
ER-β	Estrogen receptor beta.
FBS	Fetal bovine serum.
FGF	Fibroblast growth factor.
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase.
GRB-2	Growth factor receptor bound protein-2.
HCl	Hydrochloric acid.
HPLC	High-performance liquid chromatography.
HRP	Horseradish peroxidase.
ICC	Immunocytochemical staining.

IGF-1	Insulin-like growth factor-1.
IL	Interleukin.
IRS1	Insulin receptor substrate 1.
JAK	Janus kinases.
kDa	Kilodalton.
K_2HPO_4	Dipotassium hydrogen phosphate.
LC-MS	Liquid chromatography-tandem mass spectrometry.
LEP	Leptin gene.
MAPK	Mitogen-activated protein kinases.
$MgCl_2$	Magnesium chloride.
MMPs	Matrix metalloproteinases.
MRM	Mixture reaction monitoring mode.
mRNA	Messenger ribonucleic acid.
ms	Millisecond(s).
MS/MS	Tandem mass spectrometry.
m/z	Mass/charge
NaCl	Sodium chloride.
NAD	Nicotinamide adenine dinucleotide.
Na_2HPO_4	Disodium hydrogen phosphate.
Nampt	Nicotinamide phosphoribosyltransferase.

NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells.
ng	Nanogram(s).
NIH	National Institutes of Health.
nm	Nanometer(s).
Ob	Obese gene.
ObR	Leptin receptor.
PAI-1	Plasminogen activator inhibitor-1.
PBEF	Pre-B cell colony enhancing factor.
PBS	Phosphate buffered saline.
PCa	Prostate cancer.
pg	Picogram(s).
PI3K	Phosphatidyl inositol 3-kinase.
RNA	Ribonucleic acid.
ROS	Reactive oxygen species.
RT-PCR	Reverse transcriptase- polymerase chain reaction.
SEM	Standard error of mean.
SHBG	Sex hormone-binding globulin.
SNP	Single nucleotide polymorphism.
SOCS3	Suppressor of cytokine signaling 3.
SRB	Sulforhodamine B dye.

STAT3	Signal transducer and activator of transcription 3.
TBE	Tris borate EDTA buffer.
TBS	Tris buffer saline.
TCA	Trichloroacetic acid.
TGF-β1	Transforming growth factor-β1.
TNF-α	Tumor necrosis factor-α
ug	Microgram(s).
ul	Microlitre(s).
uM	Micromolar(s).
uPA	Urokinase plasminogen activator.
UV	Ultraviolet.
v	Volt(s).
VEGF	Vascular endothelial growth factor.
WAT	White adipose tissue.
WHO	World health organization.
w/v	Weight/volume.

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