UNIVERSITY OF TECHNOLOGY SYDNEY

University of Technology Sydney -UTS

The Effects and Mechanisms of Saponins of *Panax Notoginseng* on Glucose and Lipid Metabolism in 3T3-L1 Cells

Master of Science by Research 2008

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Declaration

This thesis titled 'The Effects and Mechanisms of Saponins of *Panax Notoginseng* on Glucose and Lipid Metabolism in 3T3-L1 Cells" is of original work. The work presented in this thesis was carried out under the supervision of A/Prof Xianqin Qu at University of Technology, Sydney. The thesis has not been submitted by the candidate for the award of any other degree.

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Jane Jung Yeon Kim

2009

Abstract

Type 2 diabetes is a metabolic disorder which has been posed as a serious health problem in our present society. In light of the escalating prevalence of the disease and widespread evidence of progression to cardiovascular complications, there is a need to discover new agents to successfully treat this multifacet disorder.

Resistance to insulin-mediated glucose uptake and deregulation of lipid metabolism are early and major hallmarks in the development of type 2 diabetes. The aim of this thesis was to determine the effects and mechanisms of saponins of *Panax notoginseng* (SPN) on glucose and lipid metabolism. 3T3-L1 adipocytes was utilized to study whether this naturally occurring agent, which has been used in the treatment of ischemic cardio-cerebral vascular disease throughout China for years, improves insulin-mediated glucose uptake and lowers lipid levels *in vivo*.

3T3-L1 adipocytes were cultured and treated with 100 nM insulin alone or with concentrations of 10, 50, 100 and 200 μg/ml SPN, respectively. [^{3}H]2-deoxyglucose glucose uptake, GLUT4 immunofluorescence imaging and glycogen synthesis assay were carried out to determine the effects of SPN on glucose metabolism. In addition, lipid staining and lipolysis assay were carried to study the effects of SPN on lipid metabolism.

The results in Chapter 2 indicate that SPN consist of properties that improve glucose metabolism in 3T3-L1 cells. SPN significantly increased insulin-mediated glucose uptake

in a dose-dependent manner. Immunofluorescence imaging and analysis showed that SPN increased GLUT4 in the plasma membrane. Furthermore, glycogen synthesis augmented, whereby the incorporation of D-[U-^{14}C] glucose into glycogen was enhanced with SPN treatment in 3T3-L1 cells. These findings suggest that SPN may have a direct effect upon lowering glucose levels in type 2 diabetes.

Furthermore, results in Chapter 3 of this thesis illustrates that SPN also exert effects that regulate lipid metabolism. Lipid staining showed SPN significantly decreased lipid content 3T3-L1 adipocytes. In addition, SPN significantly inhibited lipolysis in vitro, indicating that this naturally occurring agent may decrease free fatty acid delivery to the liver thereby subsequently reducing hepatic glucose production.

In effort to discover a new anti-diabetic agent that achieves both treatment of type 2 diabetes and prevention of diabetic complications, this study supports that SPN is a potential agent that is capable of directly lowering blood glucose and lipid levels in type 2 diabetic patients. Further research with both in animal models and clinical trials, as described in Chapter 4, is needed to establish that SPN has high potential to be used as an agent for diabetes and its vascular complications. These findings may prove to be highly valuable to our society.

Acknowledgements

I would like to express my gratitude to my supervisor A/Prof Xianqin Qu for all her support and guidance throughout my study.

A big thank you to Yi Tan for all the help you have given me especially in the laboratory and to fellow students John Shim and Christine Kim for your on-going encouragement. Thank you for always listening and keeping me company.

Many thanks to Mike, Phil, June and Yean for all the technical advice and assistance given to me in the laboratory. Thank you for always offering to help me out when I had problems and questions about equipment, orders, reagents etc. I would like to also acknowledge the post-graduate students on Level 6.

Finally, I would like to thank my family and friends for all their love, concern and company. This would not have been possible without you all.

Publications and Communications

Publication

Kim, J., Xiao, H., Tan, Y., Shim, J., Kim, C., Seale, J. and Qu, W. (2008)
The effects and mechanism of saponins of *Panax notoginseng* on glucose metabolism in 3T3-L1 Cells. Diabetes, Obesity and Metabolism. (Submitted and under review)

Communications

Tan, Y., Kim, J. and Qu, X. (2007) Investigation of Chinese Herbal Medicines for Metabolic Syndrome and Type 2 Diabetes. The 4th Interational Congress of Traditional Medicine 2007, Suntec, Singapore. Pg 636-639.

Kim, J., Tan, Y., Shim, J., Kim, C and Qu, X. (2008) The Effects and Mechanisms of Notoginsenosides on Glucose and Lipid Metabolism in 3T3-L1 Cells. Annual Scientic Meeting of Australian Diabetes Society (ADS) Melbourne, Australia.

Shim, J., Kim, C., Tan, Y., Kim, J. and Qu, X. (2008) Investigation of Chinese Herbal Medicine in Treatment of Metabolic Syndrome. Annual Scientic Meeting of Australian Diabetes Society (ADS) Melbourne, Australia.

List of Figures

Abbreviations

ADRAC	Australian Adverse Drug Reactions Advisory Committee
AGI	Alpha-glucosidase inhibitors
AMPK	AMP-activated protein kinase
ARR	absolute risk reductions
BMI	Body mass index
CHM	Chinese Herbal Medicine
EGIR	European Group for the Study of Insulin Resistance
FFA	free fatty acids
GLUT1	glucose transporter 1
GLUT2	glucose transporter 2
GLUT3	glucose transporter 3
GLUT4	glucose transporter 4
HDL	high-density lipoprotein
HSL	hormone-sensitive lipase
IDF	International Diabetes Federation
IFG	impaired fasting glucose
IGT	impaired glucose tolerance
IL-6	interleukin-6
IRS	insulin receptor substrate
ISDN	isosorbide dinitrate
LDL	low-density lipoprotein
MAPK	mitogen-activated protein kinase
MCP-1	monocyte chemotactic protein-1
MI	myocardial infarction
MLC	myosin light chain
NCEP	National Cholesterol Education Program
NEFA	nonesterified free fatty acids
PDH	pyruvate dehydrogenase
PFK	phosphofructokinas
PI3-K	phosphoinositide 3-kinase
PKC	protein kinase- C
PPARγ	peroxisome proliferator activated receptor-γ
PVD	peripheral vascular disease
SPN	saponins of *Panax notoginseng*
T2D	Type 2 diabetes
TG	triglycerides
TNF-α	tumor necrosis factor α
TZD	thiazolidinedione

WHO	World Health Organisation
WTH	Waist-to-Hip

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