Chinese Herbal Formulae and Non-alcoholic Fatty Liver Disease

A preliminary investigation in an experimental animal model

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"The Gods have put diagnosis before therapy- man must put careful observation and interpretation before diagnosis."

Franz Volhard (1876-1950)

CERTIFICATE OF AUTHORSHIP/ ORIGINALITY

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

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Abstract

Background: Non-alcoholic Fatty Liver Disease (NAFLD) embodies the hepatic manifestation of metabolic syndrome, commonly associated with obesity, hyperinsulinaemia, peripheral insulin resistance, diabetes, hypertension, hypercholesterolaemia and hypertriglyceridaemia (Sanyal, 2002). NAFLD is recently conceded to be a global epidemic burden, being ranked as the second leading cause of preventative death (Younossi, 2008). Since the pathogenesis of NAFLD remains controversial, no established pharmacological agent has been developed that effectively targets or prevents excessive fat accumulation in the hepatocytes and, as a result, current treatments are primarily symptomatic. Chinese herbal medicine has become a growing focus for Western medical research in the quest for more effective treatments since various Chinese herbal formulae are used in Asian countries for hepatic and metabolic syndromes (Chen et al, 2006, Hollander and Merchanick 2008).

Objective: To determine whether obesity related NAFLD could be induced in healthy rats by dietary manipulation alone and to investigate the effects of three Chinese herbal formulae in these animals on relevant variables on in vivo measures and on tissue analyses post mortem.

Design: The research comprised of two related studies. Study one was a between subjects equivalent group experiment with repeated measures, with the rats randomly divided among six intervention groups. Five groups were continuously fed a high fat diet (HFD) and one group fed a standard laboratory chow for 11 weeks until sacrifice. Study two entailed the collection of liver and adipose tissues immediately post mortem. These tissue weights were recorded and the livers were used to measure hepatic triglyceride content and for histological examination of the rat liver.

Participants and interventions: 56 male Sprague Dawley rats, six weeks old and weighing between 170 – 210g were randomly divided among six intervention groups. Five groups were continuously fed a HFD and one group, a standard laboratory chow for 11 weeks until sacrifice. After a five week induction period, the six week

intervention phase commenced with the allocated intervention administered daily by oral gavage. Blood collection occurred in the last week before sacrifice for subsequent analyses. The pharmacological interventions comprised Rosiglitazone (RSG); anti-diabetic medication, SK0504 (S4), SK0506 (S6) and a decoction of SK0506 (DS6) as well as water for the two control groups. Note: S4 combines Jiao Gu Lan (*Gynostemma pentaphyllum*), San Qi (*Panax notoginseng*), Huang Lian (*Rhizoma Coptidis*) and Dan Shen (*Salvia miltiorrhiza*) and S6 combines Dan Shen (*Salvia miltiorrhiza*) and Zhi Zi (*Fructus Gardenia Jasminodis*).

Main outcome measures: Bodyweight, food and energy food intake; biochemical and hepatic analyses (plasma glucose, Non-esterified Fatty Acids (NEFA), cholesterol, triglycerides (TG), High Density Lipoprotein-Cholesterol (HDL-C), Alanine Transaminase (ALT), Aspartate Transaminase (AST) and hepatic triglycerides); and histopathological staining (to observe hepatic morphology) were measured.

Results: The results for the five week induction phase suggest that despite the difference in diet (HFD or standard chow) the rats regulated their energy intake as there was no significant difference in the increase in mean weight for the two diets.

During the intervention phase there were further increases in body weight that plateaued after four weeks, quite independent of diet or intervention. However, there was no clear relationship between caloric intake and weight gain in that there were significant differences between the two S6 groups and the others and yet this did not equate to any difference in weight gain.

At the end of the intervention period there was no significant difference between most groups in blood glucose levels and typically animals were within the healthy range. Therefore high fat feeding alone, particularly in a short period of time of ten weeks did not cause hyperglycaemia.

Within the intervention groups there was evidence of marked hyperlipidaemia with the HFD as evidenced by significant increases in NEFA, TG and cholesterol. In general, this was attenuated by the interventions. Notably both forms of the S6 intervention had a cardioprotective effect illustrated by the elevation of levels of HDL-C.

There were potentially hepatotoxic effects indicated by elevation of liver enzymes associated with HFD feeding alone that were supported by histopathological changes in the liver.

Post mortem comparisons included weights of adipose tissues, hepatic tissue and hepatic morphology for evidence of NAFLD. With the epidydimal, subcutaneous and inguinal fat deposits, there was a significant degree of fat accumulation in the HFD group compared with the chow group. RSG showed no beneficial effect with regard to fat accumulation, which contrasts with the effects of the two herbal formulae S4 and S6. There was no evidence of hepatomegaly in any group.

High fat feeding resulted in excess hepatic triglyceride levels which was attenuated by RSG, S4 and S6, with S6 being the most effective. Note that no intervention completely prevented this high fat feeding accumulation.

The significant increase in hepatic TG accumulation and plasma biochemical analysis (NEFA, TG and cholesterol) with the high fat feeding in control groups was supported from the histological findings of macro- and microvesicular steatosis fed the HFD alone. These histological changes were absent in animals fed the other HFD based interventions (S6, S4 and RSG). There was no discernable fibrosis in any of the groups.

Conclusion: HFD feeding alone produced NAFLD as hypertriglyceraemia, hypercholesterolaemia and histological evidence of steatosis was observed. The three herbal formulae all attenuated the HFD's hyperlipidaemic effects in relation to plasma TG, NEFA, cholesterol, HDL-C and hepatic TG as no steatosis was formed. However lack of difference in weights between standard chow and HFD fed rats during the induction and intervention phases suggests the model failed to produce obesity which may be due to the short period of 11weeks of the dietary manipulation.

S4 and S6 both attenuated the effects on liver enzymes of the high fat diet, possibly indicating possible hepatoprotective effects with the values falling towards those for the Chow group. RSG by contrast elevated AST levels above those for HFD.

The two forms of S6; powder and decoction, compared in study one showed generally similar results.

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List of Abbreviations

ACC	Acetyl-CoA carboxylase
AGA	American Gasterology Association
ALP	Alkaline Phosphatise
ALT	Alanine Transaminase
AMPK	AMP activated protein kinase
AST	Asparate Transaminase
ATP	Adult Treatment Panel
BMI	Body Mass Index
CB	endocannabinoid receptor
CHM	Chinese Herbal Medicine
CVD	Cardiovascular Disease
CYP	cytochrome P4502
DM	Diabetes Mellitus
DNL	de novo lipogenesis
DS6	Decoction of SK0506
F	Female
FAS	fatty acid synthetise
FFA	Free Fatty Acid
GGT	Gama Glutamyl Transpeptidase
HCC	Hepatocellular Carcinoma
HDL-C	High Density Lipoprotein- Cholesterol
HFD	High Fat Diet
HNE	hydroxynonenal
HOMA	Homeostasis model assessment
IHCL	intrahepatic content of lipid
IDF	International Diabetes Federation
IL-6	interleukin-6
IR	insulin resistance
IRS	insulin receptor substrate
LDL	Low density Lipoprotein

LR	Liver
LXR-α	liver receptor
MDA	Malondialdehydre
MRI	Magnetic Resonance Imaging
M	Male
MS	Metabolic Syndrome
NAFLD	Non-alcoholic Fatty Liver Disease
NAS	NAFLD Activity Score
NASH	Non Alcoholic Steato-hepatitis
NECP	National Cholesterol Education Program
NEFA	Non Esterified Fatty Acid
OGTT	Oral Glucose Tolerance Test
PMN	polymorphonuclear
PPAR-α	peroxisome proliferators-activated receptors
ROS	reactive oxygen species
RSG	Rosiglitazone
S4	SK0504
S6	SK0506
SD	Sprague Dawley
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
SOCS	suppressor of cytokine signalling
SP	Spleen
SREBP	Sterol-regulatory element-binding protein
TG	Triglycerides
TNF-α	Tumour Necrosis Factor Alpha
TCM	Traditional Chinese Medicine
TZD	thiazolidineodione
UDCA	Ursodeoxycholic acid
VLDL	Very Low Density Lipoprotein
WHO	World Health Organisation
WR	Wistar Rat
ZOF	Zucker Obese Fatty