Investigation of diabetic cardiomyopathy and its treatment by copper chelation

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Abstract

Diabetes mellitus is estimated to affect approximately 7% of the populations living a western lifestyle. Of the multiple etiologies associated with diabetes, heart failure is the most common cause of death. A specific type of heart disease called diabetic cardiomyopathy is thought to be partially responsible. At this time, no one specific treatment is available for diabetic cardiomyopathy due to the wide variety of possible complex molecular changes, including metabolic disturbances, myocardial fibrosis, LV hypertrophy, and increased ROS production.

Abnormal copper metabolism in diabetes has been proposed to form part of the pathway that leads to diabetic cardiomyopathy. Our group have shown that treatment with the copper (Cu^{II}) chelator, triethylenetetramine, ameliorates the effects of diabetes on the heart at both the functional and molecular level.

This thesis aimed to further these studies by increasing our understanding of the mechanism of triethylenetetramine action on the diabetic heart. This was primarily achieved through the use of microarray technology but included the use of a range of molecular experimental techniques.

During this investigation it was determined that the most suitable microarray platform for our studies was the Affymetrix GeneChip® system. Using this system we identified more than 1600 gene changes associated with diabetes in the left ventricle wall. A disproportionate number of significant messenger RNA transcript changes were associated with the mitochondria and further investigation of these genes revealed changes associated with perturbed lipid metabolism and increased oxidative stress.

A second study investigated the molecular mechanisms underpinning improved cardiac function in the left ventricle of the heart from diabetic and sham animals treated with triethylenetetramine. There was an observed decrease in diabetic cardiac tissue triglyceride towards normal, possibly through improvement of the structure and stability of the mitochondria. Only a small number of changes in gene expression were detected after triethylenetetramine treatment using microarray technology, and none were detected using real time-quantitative PCR.

The final aim of this thesis was to understand the absorption and excretion of triethylenetetramine by both sham and diabetic animals. Our study found differences in the ability of diabetic animals to absorb and metabolise triethylenetetramine compared to sham animals. Also, the length of exposure was found to be an influencing factor in triethylenetetramine metabolism.

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Abbreviations

ACC	Acetyl-CoA carboxylase
AGE	Advanced glycation end products
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
Angptl4	Angiopoeitin-like protein 4
ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
AP-1	Activating protein 1
AST	Aspartate aminotransferase
AT II	Angiotensin II
ATP	Adenine triphosphate
ATP7A	Cation-transporting P-type ATPase 7A
ATP7B	Cation-transporting P-type ATPase 7B
ATX1	Anti-oxidant protein 1
BSA	Bovine serum albumin
BW	Body weight
^{o}C	Temperature (degrees celsius)
CAD	Coronary artery disease
CCCP	Carbonyl cyanide *m*-chlorophenylhydrazone
CHAPS	3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate
cmH_2o	Centimeters of water (pressure)
CoA	Coenzyme A
COX	Cytocrome oxidase
Cox17	Cytochrome oxidase assembly, subunit17
CPT	Carnitine palmytoyl transferase
cRNA	Complementary ribonucleic acid
CS	Citrate synthase
CTGF	Connective tissue growth factor
CTR	Copper transport protein
Cu	Copper
CV	Coefficient of variation
CVD	Cardiovascular disease
Cy-3/5	Cyanine-3/5
Cytb5	Cytochrome b5 (mitochondrial)
Da	Peptide mass (Dalton)
DAG	Diacylglycerol
DAT	N_1, N_{10}-diacetyltriethylenetetramine
DCM	Diabetic cardiomyopathy
DEPC	Diethylpyrocarbonate
DTT	Dithiothreitol
dNTP	Deoxyribonucleotide triphosphate
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
ECM	Extracellular matrix
EDTA	Ethylenediamine tetraacetic acid
ES	Enrichment score
ET	Endothelin
FA	Fatty acid
FAO	Fatty acid oxidation
FAT	Fatty acid transporter
FDR	False discovery rate
Fe	Iron
FFA	Free fatty acid
FMOC	9-Fluorenylthoxycarbonyl chloride

g	Mass (gram)
g	Acceleration due to gravity
γGCS	γ-glutamylcysteine synthetase
GAPDH	Glyceraldyhyde-3-phosphate dehydrogenase
GF-AAS	Graphite furnace - atom absorption spectroscopy
GFAT	Glutamine:fructose-6-phosphate amidotransferase
GEO	Gene expression omnibus
GlcNAc	UDP-*N*-acetylglucosamine
GLUT	Glucose transporter
GO	Gene ontology
GSEA	Gene-set enrichment analysis
GSH	Glutathione
GST	Glutathione S-transferase
HAD	Hexamethylenediamine dihydrochloride
hCTR	Human copper transport protein
HDL	High density lipoprotein
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HF	Heart failure
HNO_3	Nitric acid
H_2O	Water
H_2O_2	Hydrogen peroxide
HOAD	L3-hydroxyacyl CoA:NAD^+ oxidoreductase
HPLC	High performance liquid chromatography
hr	Time (hour)
HW	Heart weight
HW/BW	Heart weight/body weight ratio
ICP-MS	Inductively coupled plasma-mass spectrometer
IDH	isocitrate dehydrogenase
IgG	Immunoglobulin G
IL-1β	Interlukin-1β
KEGG	Kyoto encyclopaedia of genes and genomes
LC-MS	Liquid chromatography-mass spectrometry
LPL	Lipoprotein lipase
LV	Left ventricle
MAT	N_1-acetyltriethylenetetramine
MCD	Malonyl-CoA decarboxylase
MES	Maximum enrichment score
µg	Mass (microgram)
mg	Mass (milligram)
MIAME	Minimum information about microarray experiments
min	Time (minute)
M	Amount of substance per litre (molar)
mm	Length (millimetre)
µM	Amount of substance per litre (micromolar)
mM	Amount of substance per litre (millimolar)
µl	Volume (microlitre)
ml	Volume (millilitre)
MMLV	Moloney Murine Leukemia virus
mol	Amount of substance
mmol	Amount of substance
MMP	Matrix metalloproteinase
Mn	Manganese
mRNA	Messenger ribonucleic acid
MRI	Magnetic resonance imaging
MT	Metallothionein
mtDNA	Mitochondrial deoxyribonucleic acid
MVEC	Microvascular endothelial cell

NAD+	Nicotinamide adenine dinucleotide
NADPH	Reduced nicontinamide adenine dinucleotide phosphate
NCBI	National Center for Biotechnology Information
NF-κB	Nuclear factor-κB
nm	Length (nannometre)
NO	Nitric oxide
NOD	Non-obese diabetic mouse
NOS	Nitric oxide synthases
NTP	Nucleotide triphosphate
OCT	Optimum cutting temperature
O/N	Overnight
$ONOO^-$	Peroxynitrite
OXPHOS	Oxidative phosphorylation
PAI-1	Plasminogen activator inhibitor-1
PARP	Poly-(ADP-ribose) polymerase
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PDH	Pyruvate dehydrogenase
PDK	Pyruvate dehydrogenase kinase
PDP	Pyruvate dehydrogenase phosphatase
PEPK	Phosphoenolpyruvate carboxykinase
PET	Positron-emission tomography
PKC	Protein kinase C
PPAR	Peroxisome proliferator-activated receptor
RAGE	Receptor of AGE
REML	Restricted maximum-likelihood
RNA	Ribonucleic Acid
RNase	Ribonuclease
ROS	Reactive oxygen species
rpm	Revolutions per minute
RT	Room temperature
RT-qPCR	Real time quantitative polymerase chain reaction
s	Time (second)
SAPE	Streptavidin-phycoerythrin
SCO	Synthesis of cytochrome oxidase
SDS	Sodium dodecyl sulphate
SHR	Spontaneously hypertensive rat
snRNP	Small nuclear ribonucleoprotein
SOD	Superoxide dismutase
STZ	Streptozotocin
TBS	Tris buffered saline
TCA	Tricarboxylic acid cycle
TEM	Transmission electron microscope
TETA	Triethylenetetramine
Tfam	Transcription factor A, mitochondrial
TG	Triglyceride
TGF-β1	Transforming growth factor - β1
TIE	TGF-β1 inhibitory elements
TIGR	The Institute for Genomic Research
TOC	Tri-functional β-oxidation complex
TNF-α	Tumour necrosis factor-α
TXN	Thioredoxin
UCP	Uncoupling protein
UV	Ultraviolet
VLDL	Very low-density lipoprotein
yCCS	Yeast copper chaperone for superoxide dismutase
ZDF	Zucker diabetic fatty rat
Zn	Zinc