

The impact of the environmental hazard, acrylamide, on the male reproductive tract and transgenerational phenotype

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Table of Contents

Declaration	vii
Acknowledgements	viii
List of Figures	x
List of Tables.....	xiv
List of Abbreviations	xv
General.....	xv
Multigenerational treatment groups	xix
Units	xx
Prefixes.....	xx
Abstract.....	xxi
Chapter 1. Literature Review.....	1
1.1. Introduction	1
1.2. Environment and health	3
1.2.1. Physical factors affecting human health	4
1.2.2. Biological factors affecting human health	4
1.2.3. Chemical factors affecting human health.....	5
1.2.3.1. <i>Air</i>	5
1.2.3.2. <i>Water</i>	6
1.2.3.3. <i>Soil</i>	6

1.2.3.4. Food.....	7
1.3. Transgenerational transmission	8
1.4. Acrylamide.....	16
1.4.1. Acrylamide reactivity and toxicity	17
1.5. Acrylamide genotoxicity and reproductive toxicity	18
1.5.1. In vivo acrylamide exposure	21
1.6. CYP2E1	25
1.6.1. Metabolism of acrylamide	26
1.7. CYP2E1 Induction	27
1.7.1. Male reproductive tract	30
1.8. Concluding remarks	31
1.9. Hypothesis and aims	33
Chapter 2. Materials and Methods	35
2.1. Buffers and Reagents	35
2.2. Animals	35
2.3. In vivo experimental design	36
2.3.1. Acute xenobiotic administration	36
2.3.1.1. Acrylamide	36
2.3.1.2. Ethanol.....	37
2.3.2. Multigenerational acrylamide exposure	38
2.4. Tissue fixation and embedding.....	43

2.5. Cell Culture	44
2.5.1. mECap18 cells	44
2.6. Gene Expression Analysis	44
2.6.1. RNA Extraction	44
2.6.1.1. <i>Extraction Procedure</i>	45
2.6.1.2. <i>DNase Treatment of RNA</i>	45
2.6.1.3. <i>Determination of Nucleic Acid Concentration</i>	46
2.6.2. Polymerase Chain Reaction (PCR)	46
2.6.2.1. <i>Reverse Transcription (RT)</i>	46
2.6.2.2. <i>PCR</i>	47
2.6.2.3. <i>Real-Time PCR</i>	47
2.7. Protein Analysis	48
2.7.1. Protein extraction and quantification	48
2.7.1.1. <i>SDS extraction</i>	48
2.7.1.2. <i>Microsome and mitochondria extraction and purification</i>	49
2.7.1.3. <i>Protein Quantification</i>	50
2.7.2. Immunoblotting	50
2.7.2.1. <i>SDS-PAGE, Western Transfer and immunoblotting</i>	50
2.7.3. Immunofluorescence	52
2.7.3.1. <i>Immortalised cell</i>	52
2.7.3.2. <i>Tissue section</i>	54

2.7.3.3. <i>Fluorescence quantification</i>	55
2.8. DNA damage Analysis	55
2.8.1. Somatic/Immortalised cell	55
2.8.2. Spermatozoa	56
2.8.3. Quantification.....	57
2.8.3.1. <i>Multigenerational acrylamide exposure</i>	58
2.9. Statistics	58
Chapter 3. Quantification of CYP2E1	59
3.1. Background	59
3.2. Aims	64
3.3. Results	65
CYP2E1 Immunoblot Optimisation	65
3.3.1. Optimisation of mitochondria and microsome extraction	69
3.4. Discussion	77
Chapter 4. Modulating CYP2E1 expression in the male reproductive tract	82
4.1. Background	82
4.2. Aims	85
4.3. Results	86
4.3.1. Acrylamide.....	86
4.3.2. Ethanol	96

4.4. Discussion	104
Chapter 5: Heritable sensitivity to acrylamide.....	113
5.1. Background	113
5.2. Aims	115
5.3. Results	116
5.3.1. DNA Fragmentation	118
5.3.2. CYP2E1.....	129
5.4. Discussion	133
5.4.1 Transgenerational reproductive phenotype	133
5.4.2 CYP2E1 alterations and DNA fragmentation following single generation exposure	134
5.4.3 Multi- and transgenerational sperm DNA fragmentation	135
5.4.4 Multi- and transgenerational regulation of CYP2E1 abundance	139
Chapter 6. Final Discussion	142
6.1. Overview	142
6.2. CYP2E1 Induction	144
6.2.1. Potential technologies to assess CYP2E1 induction pathways	147
6.3. Multigenerational outcomes of acrylamide exposure.....	150
6.3.1 Adaptive response to parental environmental insult	153
6.4. Mechanism of transgenerational transmission	155
6.4.1. Chromatin Modifications	155

6.4.1.1. DNA	156
6.4.1.2. Proteins.....	157
6.4.1.3. Assessing chromatin modifications	157
6.4.2. Small RNAs	158
6.5. Concluding remarks	160
References	162
Appendices	206
Appendix A.....	206
A.1. Buffers and Stock Solutions	206
Appendix B	211
B.1. Primer sequences	211
Appendix C	212
C.1. Immunofluorescence Controls	212
C.1.1. Immortalised cell	212
C.1.2. Tissue section	212
Appendix D	213
Appendix E	215

Declaration

I hereby certify that the work embodied in the thesis is my own work, conducted under normal supervision. The thesis contains no material which has been accepted, or is being examined, for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968 and any approved embargo.

Caitlin Chambers

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List of Figures

Figure 2.1. Acute acrylamide and ethanol exposure regimen.	36
Figure 2.2. Multigenerational exposure paradigm.	39
Figure 3.1. Expression of <i>Cyp2e1</i> in tissues of <i>Mus musculus</i> CD-1 strain.	60
Figure 3.2. CYP2E1 is present in the liver and the male reproductive tract.	66
Figure 3.3. CYP2E1 is present in the mouse reproductive tract.	68
Figure 3.4. Protocol for purification of mitochondria. All stages were completed on ice or at 4°C.	70
Figure 3.5. Purification of mitochondria.	71
Figure 3.6. Secondary protocol for purification of mitochondria.	72
Figure 3.7. Purification of mitochondrial protein from liver.	73
Figure 3.8. Liver microsome and mitochondria purification.	74
Figure 3.9. Liver microsome and mitochondria purification.	75
Figure 3.10. Altering the number of homogenisation strokes affects liver microsome and mitochondria purification.	76
Figure 3.11. The ratio of CYP2E1 detected in microsomes and mitochondria purified from each respective sample.	77
Figure 4.1. Acute acrylamide exposure leads to DNA damage in spermatozoa <i>in vivo</i>	87
Figure 4.2. <i>Cyp2e1</i> gene expression is not altered by acrylamide exposure <i>in vivo</i>	89
Figure 4.3. Acute acrylamide exposure results in increased levels of CYP2E1 protein in the spermatocytes of male mice.	91
Figure 4.4. CYP2E1 is present in the cytoplasm of mECap18 cells.	93
Figure 4.5. Acrylamide exposure does not affect mECap18 cell number.	93

Figure 4.6. In vitro acrylamide exposure does not change <i>Cyp2e1</i> gene expression in mECap18 cells.	93
Figure 4.7. CYP2E1 is dose-dependently elevated in mECap18 cells following acrylamide exposure.	95
Figure 4.8. DNA damage is elevated in mECap18 cells following acrylamide exposure.	96
Figure 4.9. Acute ethanol exposure does not lead to DNA damage in spermatozoa <i>in vivo</i>	97
Figure 4.10. <i>Cyp2e1</i> gene expression in testis is not altered by ethanol exposure <i>in vivo</i>	99
Figure 4.11. Acute ethanol exposure results in increased levels of CYP2E1 protein in the spermatocytes of male mice.	100
Figure 4.12. Ethanol at 343 mM reduced mECap18 cell number.	101
Figure 4.13. <i>In vitro</i> ethanol exposure leads to an increase in <i>Cyp2e1</i> gene expression in mECap18 cells.	102
Figure 4.14. CYP2E1 is dose-dependently elevated in mECap18 cells following ethanol exposure.	103
Figure 4.15. DNA damage is elevated in mECap18 cells following ethanol exposure.	104
Figure 5.1. Grand-paternal acrylamide exposure results in decreased testis to body weight ratio.	117
Figure 5.2. Paternal acrylamide exposure elevated DNA fragmentation in spermatozoa of unexposed progeny (EO), while grand-paternal acrylamide exposure results in increased DNA fragmentation in spermatozoa irrespective of subsequent exposure.	119

Figure 5.3. Paternal DNA fragmentation in spermatozoa correlated with DNA fragmentation in progeny following acrylamide exposure of the F1 generation.....	122
Figure 5.4. Paternal (F1) DNA fragmentation in spermatozoa correlated with DNA fragmentation in progeny (F2) in the absence of acrylamide exposure of the F1 generation.	124
Figure 5.5. Grand-paternal (F0) DNA fragmentation in spermatozoa negatively correlated with DNA fragmentation in progeny in the absence of acrylamide exposure of any generation.....	127
Figure 5.6. Chronic acrylamide exposure results in increased levels of CYP2E1 protein in the spermatocytes of male F0 mice.....	129
Figure 5.7. Chronic acrylamide exposure results in increased levels of CYP2E1 protein in the spermatocytes of male F1 mice, following paternal acrylamide exposure, but exposure decreased CYP2E1 in offspring of control males.....	130
Figure 5.8. Multigenerational exposure to acrylamide altered CYP2E1 protein abundance in the spermatocytes of male F2 mice.	132
Figure 5.9. Paternal and grand-paternal acrylamide exposure results in multi- and transgenerational reproductive phenotypes in progeny.....	141
Figure C.1. Goat anti-rabbit fluorescent conjugated secondary antibody (Alexa fluor 488) does not non-specifically bind on mECap18 cells.	212
Figure C.2. Goat anti-rabbit fluorescent conjugated secondary antibody (Alexa fluor 594) does not non-specifically bind on testis sections.....	212
Figure D.1. Morphological analysis of reproductive factors after acrylamide and ethanol treatment.	213
Figure D.2. Testis morphology is grossly unaffected by acrylamide and ethanol treatment in male mice.	214

Figure E.1. Acrylamide exposure does not alter reproductive outcomes in any generation.	223
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List of Tables

Table 1.1. Multi- and transgenerational phenotypes following parental exposure to environmental insult.	10
Table 1.2. Alteration of CYP2E1 expression and abundance in body tissues following substrate exposure.....	28
Table 2.1. Summary of total animal numbers, average weight and water consumption in each generation and treatment group.	43
Table 2.2. Immunoblotting conditions for detecting CYP2E1, SDHA, SERCA 1/2/3 and GAPDH on nitrocellulose membranes.....	52
Table 5.1. Paternal DNA fragmentation in spermatozoa correlated with DNA fragmentation in progeny following acrylamide exposure of the F1 generation.....	123
Table 5.2. Paternal (F1) DNA fragmentation in spermatozoa correlated with DNA fragmentation in progeny (F2) in the absence of acrylamide exposure of the F1 generation.	125
Table 5.3. Grand-paternal (F0) DNA fragmentation in spermatozoa negatively correlated with DNA fragmentation in progeny in the absence of acrylamide exposure in any generation.	128
Table 6.1. Adaptive response in offspring following parental environmental insult.	154
Table 6.2. RNA altered by paternal insult induces altered phenotype in offspring.	159
Table A.1. Buffers and stock solutions	206
Table B.1. PCR primer sequences.....	211
Table E.1. Mean weight, water consumption and daily acrylamide intake for each mouse.	215
Table E.2. Summary of Tail DNA percentage intensity for each unexposed (O) and exposed (E) lineage.	224

List of Abbreviations

General

5mc	5-methylcytosine
8OHdG	8-oxo-2'-deoxyguanosine
APAP	acetaminophen
B2M	β 2 microglobulin
BEP	bleomycin, etoposide, cisplatin
BMI	body mass index
BPA	bisphenol A
BSA (solutions)	bovine serum albumin
BSA	body surface area
bw	bodyweight
cAMP	cyclic adenosine 3',5'-monophosphate
CD	control diet
cGMP	cyclic guanosine monophosphate
ChIP	chromatin immunoprecipitation
CTCF	corrected total cell fluorescence
CYP	cytochrome P450
DAPI	4'-6-diamidino-2-phenylindole
DBP	dibutyl phthalate
DEET	N,N-Diethylmeta-toluamide
DEHP	bis(2-ethylhexyl)phthalate
DEPC	diethylpyrocarbonate
DHT	5 α -Androstan-17 β -ol-3-one

DMEM	Dulbecco's Modified Eagle Medium
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
DTT	dithiothreitol
ECL	enhanced chemiluminescence
EDTA	Ethylenediaminetetraacetic acid
EM	EDTA/MOPS
ENU	ethylnitrosourea
ER	endoplasmic reticulum
ESTR	expanded simple tandem repeat
F0	parental
F1	first filial
F2	second filial
FBS	fetal bovine serum
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GPX	glutathione peroxidase
GusB	glucuronidase β
H ₂ O ₂	hydrogen peroxide
HED	human equivalent dose
HFD	high-fat diet
HMF	5-hydroxymethylfurfural
HPLC-UV	high-performance liquid chromatography-ultraviolet
HRP	horse radish peroxidase
I.P.	intraperitoneal

IgG	immunoglobulin G
JP8	jet propellant 8
LC	liquid chromatography
LIS	lithium diiododsalicyclate
mECap18	SV40-immortalised mouse caput epididymal epithelial cell line 18
mi	microsomal
Milli-Q® H ₂ O	Milli-Q® filtered water
miRNA	micro-RNA
MOPS	3-(N-morpholino)propanesulfonic acid
mRNA	messenger RNA
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MSG	monosodium glutamate
mt	mitochondrial
N3-GA-Ade	N3-(2-carbamoyl-2-hydroxyethyl) adenine adduct
N7-GA-Gua	N7-(2-carbamoyl-2-hydroxyethyl) guanine adduct
NOAEL	no-observed-adverse-effect-level
nt	nucleotide
PBS	phosphate-buffered saline
PBST	phosphate-buffered saline supplemented with 0.1% Tween-20
PCBs	polychlorinated biphenyls
PCR	polymerase chain reaction
piRNA	piwi-interacting RNA
pMAPK	phosphorylated mitogen-activated protein kinase

PND	postnatal day
RNA	ribonucleic acid
ROS	reactive oxygen species
RPE	retinal pigment epithelium
RT	reverse transcription
SDHA	succinate dehydrogenase complex, subunit A
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SE	standard error
sec	second(s)
SEM	sucrose/EDTA/MOPS
SERCA	sarco/endoplasmic reticulum
SGA	small for gestational age
siRNA	small-interfering RNA
sncRNA	small non-protein coding RNA
TAE	tris/acetate/EDTA
TBE	tris/borate/EDTA
TBHP	<i>tert</i> -butyl hydroperoxide
TBS	tris-buffered saline
TBST	tris-buffered saline supplemented with 0.1% Tween-20
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TPA	12-O-tetradecanoylphorbol-13-acetate
tRF	transfer RNA derived fragments
TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labeling

UTR	untranslated region
WHO	World Health Organisation
WT	wild-type

Multigenerational treatment groups

O	unexposed F0
E	exposed F0
OO	unexposed F1 progeny of unexposed lineage
OE	exposed F1 progeny of unexposed lineage
EO	unexposed F1 progeny of exposed lineage
EE	exposed F1 progeny of exposed lineage
OOO	unexposed F2 progeny of unexposed lineage
EOO	unexposed F2 progeny of unexposed F1 from exposed lineage
EOE	exposed F2 progeny of unexposed F1 from exposed lineage
EEO	unexposed F2 progeny of exposed F1 from exposed lineage
EEE	exposed F2 progeny of exposed F1 from exposed lineage

Units

°C	degrees Celsius
A	amps
Da	dalton
g (centrifugation)	gravity
g (weight)	grams
h	hour(s)
L	litre
M	molar
m	metre
min	minute(s)
sec	second(s)
U	units (enzymatic activity)
V	volts
wk	week(s)

Prefixes

k	kilo	10^3
c	centi	10^{-2}
m	milli	10^{-3}
μ	micro	10^{-6}
n	nano	10^{-9}

Abstract

The impact of exposure to environmental hazards on personal health and the health of our offspring has become of great importance over recent decades. The effect of gestational exposures across generations have been widely investigated, but what is less well understood is the impact of paternal preconception exposure. The research presented utilised the environmental hazard, acrylamide as a model to facilitate a greater understanding of paternal preconception exposure, and the multi- and transgenerational consequences. Throughout this research, the effect of acrylamide on the male reproductive tract was investigated, following single and multigenerational exposures, and the importance of the enzyme CYP2E1, known to modulate acrylamide-toxicity. CYP2E1 is a P450 metabolising enzyme, localised to the endoplasmic reticulum and/or the mitochondria throughout various tissues of the body, including in the pachytene spermatocytes of the testis and epithelial cells of the epididymis within the male reproductive tract. It was hypothesised that acrylamide would alter CYP2E1 protein abundance and DNA fragmentation in the male reproductive tract, and multigenerational exposure of the male germline would result in altered phenotypes in progeny.

An *in vivo* acute exposure model and *in vitro* cell culture were utilised to establish the effect of acrylamide on CYP2E1 in the male reproductive tract. Prior to the examination of acrylamide exposures, we performed immunoblotting analysis of CYP2E1 and optimisation of subcellular fractionation techniques to isolate and purify the components of the cell that harbour CYP2E1. Mitochondrial fractions from liver tissue were extracted and purified, while microsomal fractions from the endoplasmic reticulum require further optimisation, and thus this technique was not utilised for further analyses.

From the *in vivo* and *in vitro* exposure regimes it was determined that acrylamide increased the abundance of CYP2E1 in the spermatocytes of the testis (150% of vehicle) and mECap18 cells (130% of vehicle) and elevated DNA fragmentation in both the mECap18 cells (120% of vehicle) and mature spermatozoa ($\geq 120\%$ of vehicle). To postulate potential mechanisms of this induction comparison to the well-characterised CYP2E1 substrate ethanol was performed. Ethanol exposure also elevated CYP2E1 abundance in spermatocytes (130% of vehicle) and the mECap18 cells (150% of vehicle), in addition to *Cyp2e1* transcript expression in the mECap18 cells only ($\leq 500\%$ of vehicle).

To model environmental multigenerational exposure, a chronic regime of acrylamide exposure at a human-relevant dose following the paternal germline was employed. Paternal and grand-paternal acrylamide exposure modified the response to acrylamide in male offspring with significantly altered DNA fragmentation in mature spermatozoa and CYP2E1 abundance in spermatocytes with or without acrylamide exposure of the progeny. Additionally, acrylamide exposure at the human-relevant dose of ≈ 0.2 mg/kg bw/day resulted in the transgenerational phenotype of decreased testis to body weight ratio in the male F2 progeny following ancestral exposure to acrylamide (75-80% of unexposed lineage).

The experiments outlined herein demonstrate novel understanding of acrylamide and its effects on the male reproductive tract, and the impact of preconception exposure to the reproductive health of multiple generations. These data provide new insight into the transgenerational impact of an environmental hazard at under a human-relevant regime, following paternal preconception exposure, to expand our understanding of environmental health.