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

Caitlin Gai Chambers, BSc (Hons)

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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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General

5mc	5-methylcytosine
8OHdG	8-oxo-2'-deoxyguanosine
APAP	acetaminophen
B2M	β2 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	5α-Androstan-17β-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_2O_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_2O	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	*tert*-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 (≥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 (≤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.