The consequences of paternal acrylamide exposure and potential for amelioration

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B. Biotech (Hons Class I)

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Declaration

Statement of Originality

The thesis contains no material which has been accepted 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 in the text. 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.

Statement of Authorship

I hereby certify that the work embodied in this thesis contains a published paper/s/scholarly work of which I am a joint author. I have included as part of the thesis a written statement, endorsed by my supervisor, attesting to my contribution to the joint publication/s/scholarly work.

Thesis by Publication

I hereby certify that this thesis is in the form of a series of published papers of which I am a joint author. I have included as part of the thesis a written statement from each co-author, endorsed by the Faculty Assistant Dean (Research Training), attesting to my contribution to the joint publications.

Collaboration

I hereby certify that the work embodied in this thesis has been done in collaboration with other researchers. I have included as part of the thesis a statement clearly outlining the extent of collaboration, with whom and under what auspices.

Signed…….

Aimee Lee Katen

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Publications and awards arising from work in this thesis

1. Publications

Chapter 1

Katen, A. L., and Roman, S. D. (2015). The genetic consequences of paternal acrylamide exposure and potential for amelioration. *Mutat. Res.- Fund. Mol. Mech. Mut*., 777: 91-100. **Published**

Chapter 2

Katen, A. L., Stanger, S. J., Anderson, A. L., Nixon, B. and Roman, S. D. (2016). Chronic acrylamide exposure in male mice induces DNA damage to spermatozoa; Potential for amelioration by resveratrol. *Reprod. Toxicol.*, 63:1-12. **Published**

Chapter 3

Katen, A. L., Chambers, C. G., Nixon, B. and Roman, S.D. (2016). Chronic acrylamide exposure in male mice results in elevated DNA damage in the germline and heritable induction of CYP2E1 in the testes. *Biol. Reprod*., 95:1-15. **Published**

Chapter 4

Katen, A.L., Sipilä, P., Mitchell, L.A., Stanger, S.J., Nixon, B. and Roman, S.D. (2017). Epididymal CYP2E1 plays a critical role in acrylamide induced DNA damage in spermatozoa and paternally mediated embryonic resorptions. *Biol. Reprod*. Accepted for publication 28th March. DOI: 10.1093/biolre/iox021. **Published**

2. Statements of contribution

I attest that the Research Higher Degree candidate Aimee Katen has contributed upward of 50% towards data collection/analysis and manuscript preparation for all the publications included in this thesis for which I am a co-author.

Dr. Shaun Roman
Date. 9/01/17

Prof. Brett Nixon
Date. 20/12/2016

Simone Stanger
Date 14/12/2016

Amanda Anderson
Date. 6/12/2016

Caitlin Chambers
Date. 15/12/2016

Dr. Lisa Mitchell
Date 16/12/2016

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Dr. Petra Sipilä

A/Prof. Frances Martin (ADRT)
Date. 09/01/2017

2. Conference Proceedings

Katen, A. L., Chambers, C. G., Nixon, B. and Roman, S. D. Consequences of chronic paternal acrylamide exposure on the health of offspring. 20th Annual RHD conference. Newcastle, Australia. November, 2015.

Katen, A. L., Nixon, B. J., Stanger, S. J., Nixon, B. and Roman, S. D. Dominant lethal effects of acrylamide linked to the Cyp2e1- mediated conversion to glycidamide within the epididymis and spermatocytes of male mice. 48th Annual Meeting of the Society for the Study of Reproduction. San Juan, Puerto Rico, USA. June, 2015.

Katen, A. L., Nixon, B. J., Stanger, S. J., Nixon, B. and Roman, S. D. The role of glycidamide and oxidative adducts in acrylamide induced DNA damage in male germ cells. 45th annual conference of the Society for Reproductive Biology. Melbourne, Australia. August 2014.

Katen, A. L., Nixon, B. J., Stanger, S. J., Nixon, B. and Roman, S. D. Resveratrol is a dual purpose inhibitor of DNA damage in pachytene spermatocytes. 12th International Symposium on Spermatology. Newcastle, Australia. August 2014.

Invited Speaker at DNA Damage and Repair Workshop. 12th International Symposium on Spermatology. Newcastle, Australia. August 2014.

Katen, A. L., Nixon, B. J., Stanger, S. J., Nixon, B. and Roman, S. D. Resveratrol treatment reduces acrylamide-induced DNA damage in male germ cells. 18th annual RHD conference. Newcastle, Australia. November, 2013

Katen, A. L., Stanger, S. J. and Roman, S. D Resveratrol treatment reduces acrylamide-induced DNA damage in male germ cells. 44th annual conference of the Society for Reproductive Biology. Sydney, Australia. August 2013.

3. Additional Publications

Nixon, B.J., **Katen, A.L.**, Schjenken, J.E., Nixon, B. and Roman, S.D. (2014) Mouse Spermatocytes Express CYP2E1 and Respond to Acrylamide Exposure. *PLOS ONE*, 9: e94904.

4. Awards

Awarded '2015 Lalor Foundation Merit Award'. 48th Annual Meeting of the Society for the Study of Reproduction. San Juan, Puerto Rico, USA. (2015).

Invited Speaker at DNA Damage and Repair Workshop. 12th International Symposium on Spermatology. Newcastle, Australia. (August 2014).

Finalist for a HMRI Emlyn and Jennie Thomas Postgraduate Medical Research Scholarship. (2014).

Winner of 'Excellence Award for First Year Presentation'. 18th annual RHD conference. Newcastle, Australia. (2013).

Winner of 'Michael Sheahan Award for Most Charismatic Presentation'. 18th annual RHD conference. Newcastle, Australia. (2013)

Faculty of Science and Information Technology Research Training Scholarship (2013).

Australian Postgraduate Award PhD Scholarship (2013).

Table of contents

Abstract

Male germ cells are vulnerable to a wide variety of substances, including hormones, drugs, environmental toxicants, heat and radiation. Acrylamide is a reproductive toxicant that is ubiquitous in human lives, with its formation occurring during the cooking of many foods such as breads, potatoes, cereals and coffee. This toxicant has well established neurotoxic effects in humans, and is recognised as a probable human carcinogen. Acute exposure in male rodents results in DNA damage, infertility, heritable translocations and embryo resorptions. Hence, prolonged acrylamide exposure in males has the potential to not only affect the individual, but may also have consequences for offspring. Studies have demonstrated one unique enzyme responsible for the phase I metabolism of acrylamide; the cytochrome P450 2E1 (CYP2E1). Via this enzyme, acrylamide is converted to the highly reactive epoxide glycidamide. Importantly, glycidamide adducts with DNA, and is responsible for the toxicities associated with acrylamide exposure.

The aims of this thesis were to assess the DNA damage induced in germ cells following chronic low dose acrylamide exposure, relevant to human exposure estimates. Importantly, we sought to determine if this damage could be ameliorated by utilising an inhibitor of CYP2E1. We aimed to determine if this DNA damage harboured in the male gamete had consequences for the offspring. The fourth aim was to assess CYP2E1 localisation within the male reproductive tract and to relate this to the consequences to offspring of acute high dose paternal acrylamide exposure.

Our results demonstrated that there are two major sites of CYP2E1 expression within the male reproductive tract; the testis and the epididymis. Importantly, the expression in the mouse was equivalent to that in the human, further validating the use of the mouse

model. We determined that the CYP2E1 of the epididymis plays a vital role in induction of dominant lethality following acute acrylamide exposure. However, of most critical importance to humans may be the expression within the spermatocytes of the testis, which respond to chronic low level acrylamide exposure by induction of this enzyme and resultant DNA damage within the germ cells. This induction was inherited in the untreated progeny and may render these mice more susceptible to direct acrylamide exposure.