The effects of maternal cigarette smoke exposure on brain health in offspring

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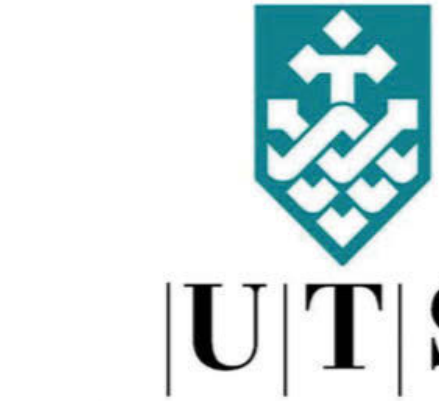
A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy



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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 part of the collaborative doctoral degree and/or fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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List of Abbreviations

(P)1	Postnatal day 1
ACEC	Animal Care & Ethics Committee
Akt	Protein kinase B
Ang II	Angiotensin II
ARNT	PER-arl hydrocarbon receptor nuclear translocator protein
Atg	Autophagy-related protein
BBB	Blood-brain barrier
CI, II, III, IV, V	Complex I, II, III, IV, V
Drp-1	Dynamin related protein-1
eNOS	Endothelial nitric oxide synthase
EPO	Erythropoietin
FADD	fas-associated death domain
Fis-1	Fission protein-1
H_2O_2	Hydrogen peroxide
HI	Hypoxic ischemic
HIE	Hypoxic ischemic encephalopathy
HIF-1α	Hypoxia inducible factor
ICAM-1	Intercellular adhesion molecule-1
IKKβ	IκB Kinase
IL	Interleukin
JNK	MAPK - c-Jun N-terminal kinases
L-C	L-Carnitine
LC3A/B-I/II	Microtubule-associated proteins light chain 1A and 1B
MAPK	Mitogen-activated protein kinase
MnSOD	Manganese superoxide dismutase
NADPH	Nicotinamide adenine dinucleotide phosphate
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NHS	Normal horse serum

NIK	NFκB - inducing kinase
Nox	NADPH oxidase
Nox	Nitric oxide
nSMase	Neuronal plasma membrane neutral sphingomyelinase
OHŸ	Hydroxyl radical
ONOOŸ	Peroxynitrate
Opa-1	Optic atrophy-1
OXPHOS	Oxidative phosphorylation
PBG	Phosphat buffered saline with goat serum
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline with Tween-20
PER	Period circadian protein
PI3-K	Phosphatidylinositol 3'-kinase
Pink-1	PTEN-induced putative kinase 1
PTEN	Phosphatase and tensin homolog
ROS	Reactive oxygen species
rtPCR	Real time polymerase chain reaction
SE	Smoke exposure
SELC	SE breeder, supplemented with L-Carnitine
SIM	Single-minded protein
src	sarcoma
Tdt	Terminal deoxynucleotidyl transferase
TNFα	Tumor Necrosis Factor α
TNFα receptor	(TNFR)1/2
TOM	Translocase of mitchondrial outer membrance proteins
TRADD	TNFR-associated dead domain
TRAF	TNFR-associated facotr
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor

Publications arising from this work

Chan, Y.L., Saad, S., Pollock, C., Al-Odat, I., Jones, N. and Chen, H. (2017) **Maternal L-Carnitine supplementation improves brain health in offspring from cigarette smoke exposed mothers**. *Frontiers in Molecular Neuroscience* 10(33).

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Chan, Y. L., Saad, S., Al-Odat, I., Zaky, A. A., Oliver, B., Pollock, C., Li, W., Jones, N. M., and Chen, H. (2016) **Impact of maternal cigarette smoke exposure on brain and kidney health outcomes in female offspring.** Clin. Exp. Pharmacol. Physiol.

Vivekanandarajah, A., **Chan, Y. L.**, Chen, H., and Machaalani, R. (2016) **Prenatal cigarette smoke exposure effects on apoptotic and nicotinic acetylcholine receptor expression in the infant mouse brainstem.** Neurotoxicology 53, 53-63

Al-Odat, I., Chen, H., **Chan, Y. L.**, Amgad, S., Wong, M. G., Gill, A., Pollock, C., and Saad, S. (2014) **The impact of maternal cigarette smoke exposure in a rodent model on renal development in the offspring.** PLoS One 9, e103443

Other Publications

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Conference Presentations

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Chan, Y.L., Saad S., Al-Odat I., Jones N., Chen H. **Effects of maternal cigarette smoke exposure on brain inflammation, hypoxia and oxidative stress in male offspring**. Federation of European Neuroscience Societies Forum 2014 (Milan, Italy)

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Abstract

There are approximately 1 billion smokers worldwide with three million in Australia. Cigarette smoking contributes to a number of chronic diseases such as cardiovascular and cerebrovascular diseases. Although it is well known that maternal cigarette smoke exposure (SE) is detrimental to the health of offspring, more than 20% of women still smoke when they are pregnant. Previous studies only focused on brain structure, sizes and aetiology of the offspring from the smokers. However, none have investigated the impact of maternal smoking on the markers of inflammation, oxidative stress and mitochondrial wellbeing in the offspring's brain, whereas maternal smoking during pregnancy is linked to brain hypoxia-ischemic injury in the neonates and resulting cerebral palsy and associated disabilities in children. Mitochondrial integrity may play a key role, as they are the major powerhouse of the cells and vulnerable to increased oxidative stress. Mitophagy is a selective removal of damaged mitochondria by autophagy facilitated by fission and fusion. The former divides mitochondrion into healthy and damaged fragments; while the latter combines the healthy fragments to re-generate new mitochondria. Abnormal level of mitophagy markers have been observed in neurological conditions, such as stroke.

Thus, this thesis aimed to study (1) the impact of maternal cigarette smoke exposure on brain markers of inflammation, oxidative stress, and mitophagy in both dams and offspring at different ages; (2) the gender differences in response to maternal SE; (3) the impact of maternal L-Carnitine (antioxidant) supplementation during pregnancy and lactation on brain mitophagy and autophagy markers in offspring, and; (4) the impact of maternal SE on hypoxic ischemic (HI) injury in male offspring.

Virgin female Balb/c mice (6 weeks) were exposed to cigarette smoke (SE) or air (SHAM) 6 weeks prior to mating, during gestation and lactation. They were mated with male Balb/c mice (8 weeks). The pups were sacrificed at postnatal day (P) 1, P20 and 13 weeks for Aims 1-3 and P45 for Aim 4. In aim 4, hypoxic ischemic injury was induced in half of the litters via left carotid artery occlusion. Behaviour tests (novel objective recognition test, error ladder, grip test, and elevated plus maze test) were carried out in offspring with HI injury at P40 to assess motor and cognitive functions. The dams were sacrificed when the pups weaned. The brains of both dams and offspring were analysed

by western blotting, immunohistochemistry, and real-time PCR for markers of inflammation, oxidative stress and mitochondrial wellbeing.

It was found that brain inflammatory markers were increased in adult male SE offspring at 13 weeks, but not changed in female offspring by maternal SE. Brain endogenous antioxidant was reduced in male offspring, which was increased in female offspring by maternal SE. Mitochondrial oxidative phosphorylation (OXPHOS) complexes I, III and V were increased by maternal SE in male offspring but all OXPHOS complexes (I-V) were increased in female SE offspring. Brain cell damage was increased in male offspring but not in female offspring by maternal SE. Maternal L-Carnitine supplementation partially reversed the above-mentioned impacts of maternal SE in offspring's brain, including brain cell injury. HI injury reduced motor and cognitive functional outcomes in both SHAM and SE offspring but maternal SE did not worsen it. However, HI injury increased brain inflammatory markers in SE offspring, as well as mitochondrial fission markers. Autophagy and mitochondrial fusion markers were reduced by HI injury in male SE offspring. Apoptotic markers were also increased in SE offspring with HI injury.

In conclusion, maternal SE had adverse impact on the brain health in offspring with more impact on male offspring than females. Maternal L-Carnitine supplementation seems to partially reverse such maternal impact. Maternal SE can worsen the cellular outcome in the offspring's brain. Interventions to improve mitochondrial function may be plausible to mitigate the adverse impact of maternal SE.