THE ROLE OF ZINC IN PREVENTING FETAL DYSMORPHOLOGY AND BRAIN INJURY MEDIATED BY MATERNAL EXPOSURE TO INFECTION IN PREGNANCY

A Thesis Submitted For the Degree of Doctor of Philosophy

by

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ABSTRACT

Maternal exposure to viral and bacterial infection during pregnancy is associated with fetal dysmorphology and neurodevelopmental disorders including schizophrenia, cerebral palsy, autism and mental retardation. Previous studies in our laboratory using an established mouse model of endotoxin-induced fetal dysmorphology have led to the hypothesis that birth defects caused by infections during pregnancy are the result of fetal zinc deficiency resulting from the induction of a zinc-binding protein, metallothionein (MT) in the maternal liver as part of the maternal inflammatory response. Thus, we predicted that zinc deficiency would exacerbate the negative fetal outcomes caused by bacterial endotoxin lipopolysaccharide (LPS) and that zinc supplementation would protect against LPS-mediated teratogenicity. This premise was investigated herein and was extended to investigate underlying molecular mechanism, including the identification of markers of neurodevelopmental damage following LPS administration in early and late pregnancy, and to determine the influence of zinc treatment on any changes in expression of these markers.

In Chapter 2 it was demonstrated that prenatal exposure to LPS on gestational day (GD) 8 resulted in the development of physical birth defects including exencephaly, microcephaly, cleft lip and or palate, and micrognathia in GD 18 fetuses. Dietary zinc supplementation throughout pregnancy was found to prevent the LPS-related abnormalities. Furthermore, low dietary zinc and LPS exposure were found to be synergistic on teratogenicity. In addition, an inverse linear relationship was observed

between the concentration of zinc in the diet and teratogenicity with a reduction in the incidence of birth defects observed with increasing concentration of dietary zinc, a finding suggesting that even small increments of zinc above normal dietary intake are likely to have a beneficial impact on teratogenicity.

Maternal infection during late pregnancy has also been linked with prenatal brain damage. A major causal link underpinning this relationship is thought to be the cytokines released following a maternal inflammatory response to infection. In Chapter 3, the presence of cytokines released in response to LPS given on GD 16 was demonstrated by an increased number of tumour necrosis factor-alpha (TNF-α)-reactive cells and astrogliosis accompanied by extensive apoptotic cell death in GD 18 fetal brain. Recently our laboratory has reported that dietary zinc supplementation throughout pregnancy, prevented impairments in object recognition memory in offspring from dams exposed to prenatal LPS on GD 8. The question arises as to whether zinc is protective against LPS-exposure in late pregnancy. In Chapter 3, it is further demonstrated that LPS-induced brain injury was prevented by concurrent zinc treatment at the time of LPS exposure.

In Chapter 4, the expression of activity-dependent neuroprotective protein (ADNP) mRNA was identified as a marker of changes occurring in the fetus as a result of LPS exposure in early pregnancy. ADNP has been found to be essential for organogenesis and is a sensitive indicator of brain injury. Here it was demonstrated that LPS caused a rapid increase in embryonic ADNP expression, which was highly significant 24 hours after exposure. Whether the elevation in ADNP expression is in response to inflammatory damage or is induced by cytokines released by the maternal inflammatory

response is not clear. However, a major finding of the study is that concomitant zinc treatment prevented the LPS-induced increase in ADNP activity. The mechanism of protection by zinc is presumed to be centred on preventing the fall in plasma zinc and associated fetal zinc deficiency caused by LPS induction of MT, but may also include MT-independent actions of zinc including prevention of apoptosis and oxidative damage, or enhance tissue repair processes.

Taken together the findings in this thesis support earlier evidence that maternal MT-mediated transient fetal zinc deficiency in early pregnancy underpins LPS-induced teratogenicity. This is the first study to demonstrate that this mechanism may also apply to LPS-induced neurodevelopmental damage in early and late pregnancy. However, further studies are warranted to discriminate between the influence of MT and that of other inflammatory reactants (e.g. cytokines) on LPS-mediated damage late in pregnancy. The major finding of the thesis is that zinc treatment (either given subcutaneously with LPS or as dietary zinc supplementation throughout pregnancy) prevents the negative fetal outcomes including neurodevelopmental damage caused by prenatal exposure to LPS. This finding highlights the importance of zinc nutrition in pregnancy and the benefits that might be gained as a potential prophylactic treatment to minimise fetal damage caused by infections during pregnancy.

DECLARATION

This 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 this copy of my thesis, when deposited in the University Library, being made available in all forms of media, now or hereafter known.

Joanne Chua Sing Cheng

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Signature Date

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ABBREVIATIONS

ADNP	Activity Dependent Neuroprotective Protein
BBB	Blood-brain Barrier
BV	Bacterial Vaginosis
Cg	Cingulate Cortex
CMV	Cytomegalovirus
CNS	Central Nervous System
CP	Cerebral Palsy
CWM	Central White Matter
DG	Dentate Gyrus
GD	Gestational Day
GFAP	Glial Fibrillary Acidic Protein
H	Hippocampus
IL	Interleukin
LPS	Lipopolysaccharide
MT	Metallothionein
Poly I:C	Polyriboinosonic-polyribocytidilic Acid
PVL	Periventricular Leucomalacia
SCx	Subventricular Cortex
TNF-α	Tumour Necrosis Factor-alpha
TUNEL	Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling
UTI	Urinary Tract Infection