EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR-A AND ITS RECEPTORS IN UTEROPLACENTAL TISSUES OF THE NORMOTENSIVE AND HYPERTENSIVE PREGNANT RAT

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MASTER OF SCIENCE

by

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PREFACE

I hereby certify that this thesis contains no material which has been accepted for the award of any other degree in any university and, that to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made in the text of this thesis.

The study presented in this thesis was completed by the author at Victoria University of Technology under the supervision of Dr. Kerry Dickson and Dr. Sarah Fraser.

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Part of this work has been presented at the following conferences

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ABBREVIATIONS

aa	Amino acid
A_2	Thromboxane
H_2O_2	Hydrogen peroxide
ANGIS	Australian National Genomic Information Service
Ang-1	Angiopoietin 1
Ang-2	Angiopoietin 2
β-FGF	β fibroblast growth factor
bFGF	Basic fibroblast growth factor
BSA	Bovine serum albumin
cDNA	Complementary deoxyribonucleic acid
CT	Cytotrophoblast
DDT	Dithiothreitol
DEPC	Diethylpyrocarbonate
DIG	Digoxigenin
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dNTPs	Equimolar mixture of dATP, dCTP, dGTP, dTTP
EB	Ethidium bromide
ECM	Extracellular matrix
EDTA	Ethylenediaminotetra-acetic acid
EGF	Epidermal growth factor
EP	Epithelial cell

Flk-1	Fetal liver kinase (rat)
Flt-1	c-fms-like tyrosine kinase
G3PDH	Glyceraldehyde-3-phosphate dehydrogenase
HCG	Human chorionic gonadotrophin
HGF	Hepatocyte growth factor
IFNα	Interferon alpha
IL	Interleukin
IUGR	Intrauterine growth retardation
KDR	Kinase insert domain-containing receptor (human)
MMP	Metalloproteinase
mRNA	Messenger ribonucleic acid
NO	Nitric oxide
PA	Plasminogen activator inhibitors
PAI	Plasminogen activator inhibitors
PBS	Phosphate buffered saline
PDGF	Platelet derived endothelial cell growth factor
PGI_2	Prostacyclin
PIGF	Placental growth factor
RNase	Ribonuclease
RTK	Receptor tyrosine kinase
RT-PCR	Reverse transcriptase polymerase chain reaction
SHR	Spontaneously hypertensive rat
TGF-α	Transforming growth factor alpha
TIMP	Tissue inhibitor of metalloproteinase

TNFα Tumour necrosis factor alpha

TSP1 Thrombospondin-1

VEGF Vascular endothelial growth factor

VEGFR-1 Vascular endothelial growth factor receptor 1

VEGFR-2 Vascular endothelial growth factor receptor 2

VEGF-A Vascular endothelial growth factor A

VEGF-B Vascular endothelial growth factor B

VEGF-C Vascular endothelial growth factor C

VEGF-D Vascular endothelial growth factor D

VPF Vascular permeability factor

UV Ultraviolet

WKY Wistar Kyoto rat

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SUMMARY

Hypertension, is a common clinical complication of pregnancy, often leading to pre-eclampsia and fetal intrauterine growth retardation. Many studies have shown that vascular endothelial growth factor A (VEGF-A) is a potent angiogenic factor, that is, it is responsible for the formation of new blood vessels from existing vessels. VEGF-A has been shown to be expressed in uteroplacental tissues, particularly during implantation. Levels of VEGF-A mRNA have also been shown to be significantly lower in placental tissue from pre-eclamptic women compared with control women (Cooper *et al*, 1996). The first aim of this project, was to establish the use of RT-PCR (reverse transcriptase-polymerase chain reaction), Northern blot analysis, immunohistochemistry and *in situ* hybridization, to measure the expression and localization of VEGF-A, vascular endothelial growth factor receptor 1 (VEGFR-1) and vascular endothelial growth factor receptor 2 (VEGFR-2) in normal rat uteroplacental tissues at early, mid and late gestation. The second aim of this project was to measure expression of VEGF-A, VEGFR-1 and VEGFR-2 in uteroplacental tissues of the normotensive (WKY) and the spontaneously hypertensive rat (SHR) at 7, 11 and 19 days of gestation. Spontaneously hypertensive rats have been previously established as an animal model for pre-eclampsia.

The uterus containing the placenta and fetus was removed from normal Sprague Dawley, WKY and SHR rats over a range of gestational ages. After 11 days of gestation, the uterus and placenta were separated. Tissues were frozen immediately in liquid nitrogen and stored at -80°C, or fixed in 10% formaldehyde for immunohistochemistry. Total RNA was extracted using TRIZOL reagent and mRNA levels of VEGF-A and its receptors were examined using RT-PCR and Northern blot analysis. mRNA levels of VEGF-A and its receptors were compared with those of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (G3PDH).

We have successfully used RT-PCR to measure mRNA expression of VEGF-A, VEGFR-1 and VEGFR-2; Northern blot analysis to measure expression of VEGF-A, and immunohistochemistry to localize VEGF-A protein in rat uteroplacental tissues. We have shown that VEGF-A and its receptors were expressed in uteroplacental

tissues of the normal rat. We found no major differences in the expression levels of VEGF-A and VEGFR-1 in uteroplacental tissues at early, mid and late gestation. We showed that VEGF-A_{164} was the predominant isoform found in uteroplacental tissues. Finally, we found no major differences in the expression levels of VEGF-A, VEGFR-1 and VEGFR-2 in uteroplacental tissues in normotensive rats (WKY) compared with hypertensive rats (SHR).

We conclude that VEGF-A and its receptors, VEGFR-1 and VEGFR-2, were expressed in uteroplacental tissues of pregnant rats. Furthermore, mRNA levels of VEGF-A and its receptors were comparable in normotensive and hypertensive rats. These results suggest that VEGF-A and its receptors may have an important role in the development of the fetal and maternal portions of the placenta, but that they are not the primary factors involved in the aetiology of pre-eclampsia.