Wake Forest University
Graduate School of Arts & Sciences

Final Examination of

Ebba M. Alzayadneh

For the Degree of

DOCTOR OF PHILOSOPHY

COMMITTEE IN CHARGE

James C. Rose, Ph.D., Chairperson
Obstetrics and Gynecology

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Hypertension

Debra I. Diz, Ph.D.
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Wake Forest School of Medicine
Medical Center Boulevard
1st floor Hanes Conference Room 1064
Monday, July 21, 2014
1:00 p.m.
PROFESSOR(S) IN CHARGE OF RESEARCH
Mark C. Chappell, Ph.D.

FIELDS OF GRADUATE STUDY
Major Subject: Physiology and Pharmacology
Hypertension and Diabetic Nephropathy

SUMMARY OF DISSERTATION
A ROLE FOR ANGIOTENSIN-(1-7) TO ATTENUATE ADVANCED GLYcation END PRODUCT-INDUCED MYOFIBROBLAST TRANSITION IN THE NRK-52E RENAL PROXIMAL TUBULE CELL LINE

There is compelling evidence for a role of intracellular renin-angiotensin system (RAS) in cell signaling, function and cardiovascular pathologies. Diabetic nephropathy is a very common complication in which tubulointerstitial fibrosis may arise from epithelial to mesenchymal transition (EMT) of the proximal tubules cells. Advanced glycated end product (AGEs) including methylglyoxal-modified albumin (MGA) may contribute to the development and progression of diabetic nephropathy via activation of the receptors for AGE (RAGE) and the RAS. The RAS is functionally partitioned into the angiotensin II (Ang II)-angiotensin type 1 receptor (AT$_1$R) and the Ang-(1-7)-AT7-MasR pathways that generally oppose the actions of one another. Although AGE-RAGE activation may stimulate the Ang II-AT1R axis, the role of Ang-(1-7) in AGE-induced EMT in the kidney is not known. The present study characterized the intracellular expression of the RAS in renal proximal tubules utilizing NRK-52E tubular epithelial cell line. Furthermore, we examined the influence of AGE on Ang-(1-7)-AT7-MasR axis in MGA-exposed in NRK-52E cells. The RAS precursor protein angiotensinogen (AGT), and its processing enzyme renin were visualized by immunofluorescent (IFM) staining in the cell nuclei; we confirmed the protein expression in isolated nuclei by immunoblots. Renin activity (AGT to Ang I conversion) was demonstrated in the nuclei of NRK-52E cells and trypsin activation studies on the pro-form of renin revealed a prorenin to renin ratio of 3:1. Peptide IFM staining localized Ang II and Ang-(1-7) to the nucleus and immunoreactive peptide content averaged 59 ± 2 and 57 ± 22 fmol per mg protein (n=4), respectively. Peptide metabolism studies in isolated nuclei revealed a pathway for the direct processing of Ang I to Ang-(1-7) by the enzyme thimet oligopeptidase. We then assessed the impact of AGE on the intracellular RAS of NRK-52E cells. MGA exposure for 48 hours significantly reduced the intracellular levels of Ang-(1-7) by 50%; however, expression of Ang I or Ang II was not altered. The reduced cellular content of Ang-(1-7) by MGA was associated with increased metabolism of the peptide to the inactive metabolite Ang-(1-4) with no change in the conversion of Ang I to Ang-(1-7). Exogenous addition of Ang-(1-7) reversed the cellular effects of MGA on cellular hypertrophy and myofibroblast transition; Ang-(1-7) reduced immunostaining and protein expression of α-smooth muscle actin by 60% (α-SMA) [n=3, p<0.05]. Moreover, Ang-(1-7) abolished AGE-induced activation of the MAP kinase ERK1/2 to a similar extent as the TGF-β receptor kinase inhibitor SB58509. The inhibitory actions Ang-(1-7) were apparently mediated by the MasR as the antagonist D-Ala$^7$-Ang-(1-7) (A779) abrogated these effects. Collectively, we conclude that NRK-52E cells express RAS components that may contribute to the intracellular expression of the bioactive peptides Ang II and Ang-(1-7). Moreover, Ang-(1-7) may provide a novel therapeutic approach in addition to the conventional RAS blockade regimen to attenuate AGE-dependent diabetic renal injury.
SCHOLASTIC VITAE

EDUCATION
2014  Ph.D., Integrative Physiology and Pharmacology
      Wake Forest University, Winston-Salem, NC

2005  B.D.S. in Dentistry, The University of Jordan, Amman, Jordan

AWARDS
2010  Wake Forest University Travel Award

2009  The University of Jordan PhD Scholarship

RESEARCH AND TEACHING:
2009 – 2014  Graduate student in the laboratories of Mark C Chappell, PhD
             Hypertension and Vascular Research Center
             Department of Integrative Physiology and Pharmacology
             Wake Forest University

2011 – 2014  Visiting Lecturer on Pharmacology for Physical Therapist and Applied
             Human Physiology, Winston Salem State University, Winston Salem, NC

2007 – 2009  Teaching and Research Assistant, Department of Biochemistry and
             Physiology, School of Medicine, The University of Jordan

PROFESSIONAL MEMBERSHIPS:
2009 – Present  American Heart Association, Council for High Blood Pressure Research,
                Member

2011 – 2014  Visiting Lecturer on Pharmacology for Physical Therapist and Applied
             Human Physiology, Winston Salem State University, Winston Salem, NC
PUBLICATIONS

Manuscripts


**ABSTRACTS**


