Abstracts

Abstracts of Theses Approved for the MSc and PhD Degrees at the Faculty of Medicine, Health Sciences Center, Kuwait University, Kuwait

MSc Degree

1 Placental Growth: Possible Link between Sex Steroid Hormone Levels, Estrogen Receptors Expression and the Tumor Suppressor Gene p53
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Estrogen is essential for initiation and maintenance of pregnancy in all animals. However, high levels of estrogens during pregnancy may have a specific growth-retarding effect on the placenta. Consequently, there has to be a control mechanism that enables the placenta to proliferate regardless of the otherwise high inhibitory level of circulating estradiol. This may partially be mediated through a decrease in estrogen receptors (ERs) and a parallel decrease in p53 expression, as a link between the expressions of these genes has been reported. Therefore, we hypothesize that during pregnancy, there is a decrease in placental ERs and p53 gene and protein expressions or translocation of these proteins between the nuclear and cytosolic compartments. Rat placentae were collected at 16, 19, and 21 days of gestation (dg). Gene and protein expressions of ERα, ERβ, and p53 were studied using real-time PCR (RT-PCR) and Western blotting followed by immunodetection. Placental weight increased significantly between 16 versus 19 dg and 16 versus 21 dg, while there was no significant increase between 19 dg versus 21 dg. ERα, ERβ, and p53 were detected at both the gene and protein levels as early as of 16 dg. In addition, the proteins were identified in both the cytosolic and nuclear compartments. Parallel expression patterns were shown by ERα, ERβ, and p53 at both gene and protein levels indicating a positive link between ERs and p53. The expressions of ERα, ERβ, and p53, at the gene level and protein level, decreased at 19 dg allowing the placenta to increase in weight, while at 21 dg, the expression of the proteins increased leading to suppressed placental growth. This was reflected in the placental weights obtained. There was a trend for these proteins to decrease in the cytosolic compartment, while increasing in the nuclear compartment of the cell with gestation, indicating a possible translocation of these proteins between cytosolic versus nuclear compartments. This could be the limiting factor for pregnancy progression, whereby pregnancies may not be allowed to progress beyond the expected delivery time. Other complex mechanisms controlled by different factors in addition to the pathway we studied may also be involved in protecting developing placenta and fetus from the effects of high estrogen levels.

Male Dawoud Al-Bader (Supervisor)

2 In vitro Screening of Enhydrazone Compounds for Anticonvulsant Activity
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Epilepsy is a globally recognized chronic disease. The pathophysiology of the epileptic seizure is complex, which makes it challenging to develop a treatment. There is still a need for developing new anticonvulsant agents. Anticonvulsant activity studies were performed on enamone E139, a compound structurally related to enhydrazones, and revealed that this agent can suppress in vitro...
seizures in the hippocampus [Ananthalakshmi, Edafiogho and Kombian, 2007]. In this study, 3 enhydrazones were screened for anticonvulsant activity by assessing their activity against neuronal excitation recorded in area CA1 of the rat hippocampus. The rank order of potency and efficacy for these compounds is EMP8 > EMP6 >> EMP10. EMP8 was used as a representative enhydrazzone to further characterize the effects of these compounds. EMP8 depressed evoked population spike (PS) recorded, an effect blocked by 50 μM bicuculline, 1 μM CGP 54626, and occluded by 500 μM vigabatrin. Zero Mg2+ buffer transformed a single PS into multiple PSs, and EMP8 reversibly decreased the number of these multiple spikes. This effect could be blocked by pretreatment with CGP 54626. Addition of picrotoxin to the perfusing buffer induced multiple spikes and the suppressive effect of EMP8 on this was significant. EMP8 inhibited zero Mg2+ and picrotoxin-induced spontaneous bursts, an effect that was blocked by CGP 54626. In the electrical model, stimulus train-induced burstings were also suppressed by EMP8. This study revealed that EMP8, and hence enhydrazones, can produce anticonvulsant effects through their ability to suppress action potential firing and excitatory synaptic transmission as well as suppressing chemically and electrically induced epileptiform activity.

Charles I. Ezeamuzie (Supervisor)
Samuel B. Kombian (Co-Supervisor)

3 The Effect of Lapatinib, a Dual Inhibitor of EGFR and ErbB2 Phosphorylation, on NF-κB Signaling through eNOS in Diabetes-Induced Vascular Dysfunction
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Vascular dysfunction is a major complication of diabetes mellitus, but its signal transduction mechanism(s) are poorly understood. Diabetes-induced elevation in epidermal growth factor receptor (EGFR) signaling is an important early event in mediating vascular dysfunction. ErbB2 (a second member of the EGFR family) is the preferred partner for dimerization with EGFR. We propose that there is enhanced phosphorylation of EGFR and erbB2 followed by receptor heterodimerization, which leads to subsequent reduction in endothelial nitric oxide synthase (eNOS) activity and activation of the proinflammatory transcription factor NF-κB in diabetes-induced vascular dysfunction.

Thus, our aim in the present study is to determine the effect of acute and chronic treatment of streptozotocin-induced type 1 diabetic rats with lapatinib, a dual inhibitor of EGFR and erbB2 phosphorylation, on the vascular responsiveness of the mesenteric bed vasculature to vasoactive agents such as norepinephrine (NE) and carbachol (Carb). In addition, we studied whether dysfunctional eNOS and activated NF-κB, as measured by phosphorylation of inhibitory IKB-α protein (p-IKB-α), are involved in mediating EGFR/erbB2 receptor signaling in diabetes-induced vascular dysfunction; western blotting of vascular tissues for total and phosphorylated IKB-α was significantly prevented by chronic oral lapatinib treatment and by acute treatment with lapatinib (10⁻⁵ M), AG 1478 (10⁻⁵ M), and AG 825 (10⁻⁵ M). Moreover, it was found that diabetes-induced vascular reduction in the total and phosphorylated eNOS was also prevented by chronic oral lapatinib treatment and by acute treatment with lapatinib (10⁻⁵ M), AG 1478 (10⁻⁵ M), and AG 825 (10⁻⁵ M). In addition, we found that diabetes-induced vascular elevation in the phosphorylated IKB-α and reduction in the inhibitory total IKB-α was significantly prevented by chronic oral lapatinib treatment and by acute treatment with lapatinib (10⁻⁵ M), AG 1478 (10⁻⁵ M), and AG 825 (10⁻⁵ M). We used L-NAME (selective eNOS inhibitor) as a positive control in the studies and to determine the signaling sequence between eNOS and NF-κB. Also, we used Bay (11-7821) as a positive control for IKB-α.

In conclusion, diabetes induces vascular dysfunction due to diabetes-induced elevation in phosphorylation of EGFR and erbB2, reduction in eNOS activity, and increase in NF-κB activity (increase in p-IKB-α). Lapatinib (a dual inhibitor of EGFR and erbB2) prevents diabetes-induced elevation in EGFR and erbB2 phosphorylation and leads to subsequent attenuation of the reduced activity of eNOS, and enhanced activity of NF-κB. Collectively, data suggest that there are beneficial effects of administering EGFR/erbB2 inhibitors (especially lapatinib) in correcting the vascular dysfunction associated with diabetes.

Saghir Akhtar (Supervisor)
Mariam Yousif (Co-Supervisor)

4 Investigation of Mn(III) N-Alkylpyridylporphyrins
Reducibility, Redox Cycling and Toxicity
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Imbalance between production of reactive oxygen species (ROS) and capacity of endogenous antioxidants to detoxify them results in oxidative damage to biological molecules and impairment in signaling pathways, a condition known as oxidative stress. Oxidative stress has been implicated in many diseases including cancer. One of the new approaches for cancer treatment is catalytic therapy. Catalytic therapy is based on the generation of ROS through administration of redox-cycling agents. A transition-metal complex can act as a redox drug. The resultant radical attack on critical cellular molecules ultimately leads to tumor growth suppression, apoptosis and necrosis. Mn porphyrins can act both as antioxidants and as pro-oxidants, and thus can be used for therapeutic purposes, including catalytic therapy. While the antioxidant...
properties of Mn porphyrins have been used successfully as a treatment in conditions where oxidative stress is a key damaging factor, their pro-oxidant activity has only recently started to be appreciated.

The aim of this study was to investigate the mechanisms of biological action of Mn porphyrins with defined charges, reduction potentials and lipophilicity, focusing on ROS production by redox cycling. The effect of natural reductants on the redox cycling ability of Mn porphyrins was investigated. We also investigated the effect of Mn porphyrins in combination with natural reductants on growth of prokaryotic cells (Escherichia coli), simple eukaryotic cells (yeast) and mammalian cells (cancer cell cultures). Upon combination with natural reducing agents, cationic Mn porphyrins with suitable redox potential participated as catalysts in redox cycling processes and showed pro-oxidant action resulting in H$_2$O$_2$-mediated cell killing. These effects were absent when cationic Mn porphyrins with positive redox potential were substituted with anionic Mn porphyrins that have either negative or positive redox potentials, suggesting that charge and redox potential play crucial roles in redox reactions and generation of ROS. Hydrophobicity also affected metalloporphyrin participation in redox reactions. The more hydrophobic meta-hexyl derivative showed higher efficiency in catalyzing respiration-dependent redox cycling and higher efficiency in causing cell death than shorter chain ethyl or methyl derivatives. The efficiency was also influenced by structural differences between ortho-, meta-, and para-isomers.

This study indicates that by suitable modifications, Mn porphyrins with appropriate charges, redox potential, hydrophobicity and shape can be designed, which fulfill the requirements for redox active therapeutics.

Ludmil Benov (Supervisor)
James Craik (Co-Supervisor)

5 Mitochondria-Targeting Properties and Photosensitizing Activity of Zn(II)
N-Alkylpyridylporphyrin-Based Photosensitizers
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Photodynamic therapy (PDT) is a clinically approved, minimally invasive therapeutic modality used for the management of a variety of cancers and benign diseases. The approach is based on the use of visible light to activate a light-absorbing compound (photosensitizer), which in the presence of molecular oxygen leads to the generation of singlet oxygen and other reactive oxygen species. These cytotoxic species damage and eventually kill target cells. Development of new photosensitizers with properties optimized for PDT applications is crucial for improvement in therapeutic efficiency of PDT.

Two classes of Zn(II) N-alkylpyridylporphyrins act as photosensitizers and kill cancer cells as well as antibiotic-resistant pathogenic bacteria. These zinc porphyrins are meso-substituted and chains are attached to the N-aryl moiety of the macrocycle. The aim of the study was to investigate how modifications at the periphery of ortho-, meta-, and para-Zn(II) N-alkylpyridylporphyrins affect their cellular uptake, subcellular distribution, biological effects, and photodynamic efficacy using an LS174T adenocarcinoma cell line as a model system.

Upon light activation, Zn(II) N-alkylpyridylporphyrins penetrate the cell membrane and are internalized by uptake mechanisms. Within the cell, they accumulate in the mitochondria, leading to mitochondrial structural protein (mitofilin) accumulation and PDT efficacy, and to identify potential mitochondrial targets.

Increasing the lipophilicity of the ZnP molecule by attaching an aliphatic chain of six carbons at the periphery of the porphyrin ring increased the efficiency of the PS. The amphiphilic hexyl ZnP analog was the most efficient in suppressing cellular metabolism and mitochondrial respiration. Notable mitochondrial targets for ZnPs were NADH and NADPH, a small mobile electron carrier (cytochrome c), a large transmembrane protein complex of the mitochondrial respiratory chain (cytochrome c oxidase), and a mitochondrial structural protein (mitofilin).

This study demonstrated that modifying the periphery of a positively charged ZnP by attaching aliphatic chains with appropriate length produced efficient, amphiphilic, mitochondria-targeting PS.

James Craik (Supervisor)
Ludmil Benov (Co-Supervisor)

6 Cellular Uptake and Distribution of Zn(II)
N-Alkylpyridylporphyrin-Based Photosensitizers – Effect of Hydrophobicity
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Photodynamic therapy (PDT) is an approved, minimally invasive therapeutic modality used for the management of a variety of cancers and benign diseases. The approach is based on the use of visible light to activate a light-absorbing compound (photosensitizer), which in the presence of molecular oxygen leads to the generation of singlet oxygen and other reactive oxygen species. These cytotoxic species damage and eventually kill target cells. Development of new photosensitizers with properties optimized for PDT applications is crucial for improvement in therapeutic efficiency of PDT.

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This study demonstrated that modifying the periphery of a positively charged ZnP by attaching aliphatic chains with appropriate length produced efficient, amphiphilic, mitochondria-targeting PS.

James Craik (Supervisor)
Ludmil Benov (Co-Supervisor)

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enced by structural differences between ortho-, meta-, and para-isomers. The amphiphilic hexyl derivatives of Zn(II) porphyrins were found to localize to membranous organelles including mitochondria, the endoplasmic reticulum and lysosomes, as well as the plasma membrane.

These results indicate that Zn(II) N-alkylpyridylporphyrins have great potential as photosensitizers for PDT.

Ludmil Benov (Supervisor)
James Craik (Co-Supervisor)

PhD Degree

1

Effect of Ouabain on Arterial Blood Pressure Regulation

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Ouabain has been implicated in the pathogenesis of essential hypertension. However, reports regarding the hypertensive effect of long-term administration of exogenous ouabain are inconsistent. This study was designed to help settle this controversy and give insights into ouabain-induced alterations that could either promote or prevent hypertension. Ouabain (32, 63, 283, and 324 μg/kg/day) was administered subcutaneously for 6–11 weeks to Dahl (salt sensitive/resistant) and Wistar rats. Blood pressure and heart rate were assessed using radiotelemetry, and measures of cardiovascular variability and baroreflex sensitivity were derived. Real-time PCR and immunoassays were used to screen for alterations in blood pressure regulatory mechanisms that could be responsible for ouabain-induced hypertension.

Despite elevated ouabain plasma levels, arterial blood pressure was not increased during the 3 months of ouabain administration. Low frequency power of systolic pressure variability, urinary excretion of catecholamines, cardiovascular responses to restraint stress, and high salt diet as well as the responsiveness to α-1 adrenergic stimulation were all not elevated by ouabain administration suggesting that activity of the sympathetic nervous system was not increased. However, surrogate indices of cardiac vagal nerve activity derived from electrocardiogram and heart rate variability were elevated during the course of ouabain treatment. Peripheral remodeling in mesenteric arteries that could support hypertension development was not evident, i.e. ouabain did not increase the gene expression of Na+/Ca2+-exchanger and α2 isoform of Na+/K+-ATPase. Instead, plasma levels of vasodilatory calcitonin gene-related peptide (CGRP) significantly rose from 55 ± 10 to 89 ± 20 pg/ml in ouabain treated rats.

In conclusion, long-term administration of low-dose ouabain did not increase arterial blood pressure in rats. Unchanged sympathetic drive, augmented cardiac vagal activity, lack of increase in the expression of Na+/Ca2+-exchanger and α2 isoform of Na+/K+-ATPase in resistance arteries, and elevated plasma levels of CGRP could be responsible.

Marian Turcani (Supervisor)
Maie Al-Bader (Co-Supervisor)