



4TH ANNUAL AUB BIOMEDICAL RESEARCH DAY

DRUG DISCOVERY

FROM BENCH TO BEDSIDE

West Hall, Saturday, February 15, 2014

9:00 am - 2:00 pm

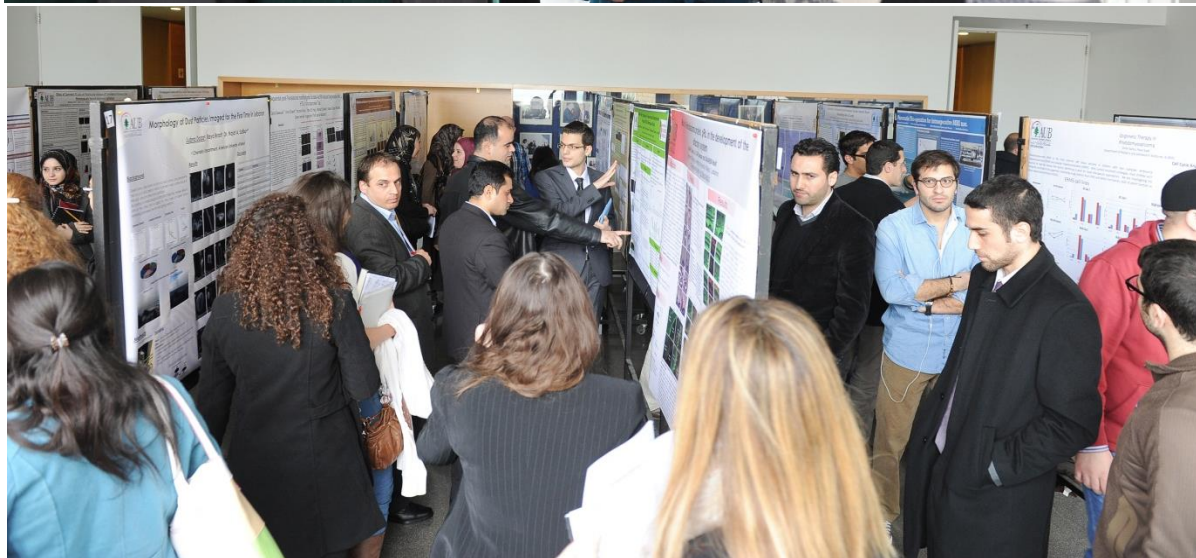
Organizing Committee

Chairperson

Ayad Jaffa, Assistant Dean of Graduate Studies & Interdisciplinary Programs, FM, Department of Biochemistry and Molecular Genetics

Members

- Hala Muhtasib, FAS, Department of Biology
- Kamal Bouhadir, FAS, Department of Chemistry
- Marwan Sabban, FM, Department of Anatomy, Cell Biology and Physiological Sciences
- Nahla Hwalla, FAFS, Dean
- Zaher Dawy, FEA, Department of Electrical and Computer Engineering
- Nadine Darwiche, FM, Department of Biochemistry and Molecular Genetics
- Assaad Eid, FM, Department of Anatomy, Cell Biology and Physiological Sciences
- Soha Yazbek, FHS, Medical Laboratory Sciences Program
- Nada Melhem, FHS, Medical Laboratory Sciences Program
- Yumna Maalouf, FM, Medical Dean's Office
- Ali Nabbouh, FM, Graduate Student Affairs



Student Awardees of the 2013 AUB Biomedical Research Day

- **Anita Barikian**, FM: *Success of Endoscopic Laser Cyclophotocoagulation after Prior Transscleral Cycloablation*
- **Jihane Soueid**, FM: *Autism Susceptibility Genes in the Lebanese Population*
- **Elia El Habre**, FAS: *Connexin43 over-expression induces partial Mesenchymal-Epithelial Transition in MDA-MB-231 breast cancer cells*
- **Antoine Hanna**, FM: *Assessment of oral health in elementary school children in Beirut: a comparison between private and public schools*

2013 Farouk Jabre Award Recipients

- **Dr. Noel Ghanem**, FAS and **Dr. Raya Saab**, FM: *Investigating the influence of neuro-developmental stage on brain tumorigenesis upon in-vivo disruption of the tumor suppressors RB and p53 in neural precursor cells*
- **Dr. Fadi Karameh**, FEA and **Dr. Ziad Nahas**, FM: *Simulation and prediction of seizure generation and propagation under electroconvulsive therapy*
- **Dr. Assaad Eid**, FM and **Dr. Kamal Bouhadir**, FAS: *Synthesis and biological study of novel carbocyclic nucleoside analogs*

2013 Program Projects in Biomedical Research Award Recipients

- **Dr. Rosemary Boustany**, FM: *Autism in Lebanon*
- **Dr.'s George Nemer and Firas Kobeissy**, FM, **Dr.'s Zaher Dawy and Hazem Hajj**, FEA, and **Dr. Pierre Karam**, FAS: *The identification of the "Compensatory Septal Proteome" (CSP) and Tll1 substrates involved in cardiac septation: potential for cardiac defect biomarkers*
- **Dr.'s Mona Nabulsi and Haya Hamadeh**, FM: *A complex breastfeeding promotion and support intervention in a developing country: a randomized clinical trial*
- **Dr.'s Marwan Refaat, Georges Nemer and Wassim Abou Kheir**, FM, and **Dr. Diana Jaalouk**, FAS: *Cardiomyopathies at AUBMC: Behind the genetics, clinical and bio-translational perspectives*

2013 Innovative Biomedical Research Grants Recipients

- **Dr. Omar Obeid**, FAFS: *Glycemic index of bread: fiber or minerals? This is the question*
- **Dr.'s Georges Daoud and Wassim Abou Kheir**, FM: *Establishment of in vitro model for studying placental development and pregnancy-related diseases using trophoblast stem cells isolation and characterization*
- **Dr. Hala Ghattas**, FAFS: *The role of dietary phosphorus in the development and progression of NAFLD in rats*

List of jury members for the 3rd Annual Biomedical Research Day

Faculty of Medicine

1. Rihab Nasr
2. Georges Daoud
3. Asad Zeidan
4. Chantal Farra

Faculty of Arts and Sciences

5. Zakaria Kambris (Biology)
6. Heinrich Burggraf Zu Dohna-Schlobi (Biology)
7. Noel Ghanem (Biology)
8. Raghida Abu Merhi (Biology)
9. Pierre Karam (Chemistry)
10. Patra Diagambara (Chemistry)
11. Bilal Kaafarani (Chemistry)

Faculty of Engineering and Architecture

12. Joseph Zeaiter (Chemical Engineering)
13. Mariette Awad (Biomedical Engineering)

4TH ANNUAL AUB BIOMEDICAL RESEARCH DAY

DRUG DISCOVERY

FROM BENCH TO BEDSIDE

Schedule of events

9:00 am - 9:30 am	<p>Welcome note</p> <p>Dr. Ayad Jaffa, Assistant Dean for Graduate Studies and Interdisciplinary Programs</p> <p>2014 Farouk Jabre Award Presentation <i>Provost Dallal, Dean Sayegh, Trustee Jabre</i></p>
9:30 am - 10:30 am	<p>Keynote speaker to be introduced by Dr. Samia Khoury, Associate Dean for Clinical and Translational Research</p> <p>Dr. Fadlo Khuri, Chair of the International Advisory Council to AUB FM / AUBMC and Professor and Chair of Hematology and Medical Oncology, Adjunct Professor of Medicine, Pharmacology and Otolaryngology, Roberto C. Goizueta Distinguished Chair in Translational Cancer Research, Deputy Director, Winship Cancer Institute, Emory University School of Medicine</p> <p><i>Title: Targeting survival signaling in lung and aerodigestive cancers</i></p>
10:30 am – 11:00 am	Coffee break
11:00 am – 12:00 pm	<p>CNRS-L</p> <ol style="list-style-type: none">1. Dr.'s Charles Tabet (CNRS) and Rabih Talhouk (FAS, AUB) - <i>"Scholarship programs of the Lebanese National Council for Scientific Research"</i>2. Ms. Rula Atweh (CNRS) - Horizon 2020 program
12:00 pm – 2:00 pm	Poster viewing followed by lunch, award presentation for the top 4 posters and closing

Objectives

- serve as a platform to bring together the research community of different AUB faculties and to showcase the biomedical research performed at AUB
- provide an intellectual environment for scientific exchange among the various researchers at AUB
- provide a platform for students, postdoctoral fellows and junior investigators to present their scientific findings and to foster collaboration within the AUB family of investigators

Eligibility

- Students
- Trainees
- Residents
- Research Assistants
- Fellows
- Post docs

Keynote Speaker

Dr. Fadlo Raja Khuri



Dr. Fadlo Khuri is Professor and Chair of Hematology and Medical Oncology, Deputy Director for the Winship Cancer Institute of Emory University, and Roberto C. Goizueta Chair in Cancer Research. He received his B.S. from Yale University and his M.D. from Columbia University, completing an internship/residency at Boston City Hospital, and a fellowship in hematology and medical oncology at Tufts University/New England Medical Center. He began his career at the MD Anderson Cancer Center in Houston Texas. He has received an American Cancer Society Career Development Award, numerous DoD and NIH/NCI grants, and funding from the State of Georgia.

Dr. Khuri and his colleagues were the first to show the effectiveness of oncolytic viral therapy in combination with chemotherapy for advanced cancers, the first to show that upregulation of COX-2 and RAR-Beta were associated with a worse prognosis for lung cancer, and the first to show paradoxical upregulation of cell survival pathways on treatment with mTOR inhibitors. His honors include induction into the American Society of Clinical Investigation, listing in Best Doctors in America, the Naji Sahyoun Memorial Award from the Middle East Medical Assembly in 2006, the Waun Ki Hong Award from Md Anderson in 2010, and the American Association for Cancer Research Rosenthal Award in 2013. Dr. Khuri has authored more than 300 articles for peer-reviewed journals, and his work has been cited almost 12,000 times with an h-factor of 55.

Dr. Khuri has been continuously federally funded since 1996, and has served as principal investigator of the Emory Lung Cancer P01, and as co-principal investigator of the Emory Head and Neck cancer SPORE and the Chemical Genomics Center. Dr. Khuri has served on the editorial board for a number of journals, is an active member of several cancer societies, and is presently Editor-in-Chief of the American Cancer Society journal, *Cancer*.

Dr. Rabih Talhouk

Rabih Talhouk, Professor of Cell Biology and Chairperson of the recently established Graduate Council at the American University of Beirut (AUB), obtained his PhD in 1988 at Ohio State University. He then held a joint post-doctoral position at the Lawrence Berkeley National Laboratory and University of California San Francisco before joining the Biology Department at AUB in October 1992. He has been there since. With more than 50 publications, current work in his laboratory focuses on two main lines of research. The first has to do with deciphering the mechanisms that regulate the interaction of the cell with its microenvironment. Namely, the role of the



transmembrane gap junction proteins, connexins, and their associated proteins, catenins and zonula adherens, in mammary epithelial cell (MEC) differentiation and transformation. Studies demonstrated that MEC differentiation is dependent on optimal gap junction communication and the assembly of a gap junction protein complex at the cell membrane. This complex includes catenin and ZO proteins in association with the connexins. In contrast, induced gap junction re-assembly in breast cancer cell lines partially reverted the cancer phenotype of these cells, in a context dependent manner, and was associated with recruitment of beta-catenin into the gap junction complex at the cell membrane. Current focus is on connexin-related mechanisms that trigger tumor initiation in normal breast cancer cells. The second line of research aims at identifying bioactive molecules with anti-inflammatory and anticancer properties in extracts of indigenous medicinal flora and marine fauna. Several 2D and 3D culture models are used for that.

Dr. Talhouk has mentored more than 45 graduate students, won a teaching excellence award and has been involved in campus wide strategic planning, and university accreditation. Talhouk has also served AUB as Chairperson of the Biology Department and has held leadership positions on several university committees. He is also a founding member of AUB's interdisciplinary Nature Conservation Center, and founding board member of the Society for Advancement of Science and Technology in the Arab World (SASTA), a USA-based NGO. He has held several consultancies on adoption of biotechnology in the MENA region, food security, GMO and biosafety. He also serves on the executive board of the International Breast Cancer and Nutrition (IBCN) project.

Dr. Charles Tabet

Earth Scientist

PhD	University of Alabama, USA	2006
MSc	American University of Beirut	1978
BSc	American University of Beirut	1976



1982-1994 Teaching at AUB-Geology Department

1988-1997 Seismologist, National Center for Geophysical Research (CNRS)

1998-Present:

Head, Scholarship Program

National Council for Scientific Research (CNRS)

ABSTRACTS

Biomedical Engineering / Chemistry

A simplified mathematical model for predicting cross contamination in office building air conditioned by displacement ventilation

Carine Habchi¹, Kamel Ghali^{1*} and Nesreen Ghaddar^{1*}

¹*Mechanical Engineering Department, American University of Beirut, P.O. Box 11-0236, Beirut 1107-2020, Lebanon,*

In indoor environments, the occupants constitute one of the main sources of particles from the ultrafine to the coarse mode. As a result, diseases can be transmitted through exhaled droplets nuclei produced by the different respiratory activities. The behavior of the particles depends largely on their weight and the flow field determined by the air conditioning system adopted. To insure good indoor air quality (IAQ) it is advisable to design a ventilation system covering the inhalable range of particle diameters [0.1 μ m-15 μ m]. Recently, displacement ventilation systems (DV) are widely used in offices and buildings for its effectiveness in providing high indoor air quality and its ability to carry contaminants from the lower occupied zone. The purpose of this work is to study cross-infection between occupants in typical internal offices by the development and validation of a simplified model that simulates active particle behavior in spaces ventilated by displacement ventilation system. The developed model incorporates the deposition of particles on walls and the gravitational settling effect on particles distribution within the space. The model was validated using data from literature revealing that the current simplified model is able of capturing the physics of the problem with significant reduction of the computational time cost. The model results showed that as the particle diameter increases from 0.1 μ m the effect of the gravitational settling increases reducing the stratification in concentration created by the DV system and thus increasing the particle concentration at the breathing level of the exposed person. This effect remains until reaching a diameter where deposition on the floor opposing the DV principle acts as a removal factor. To overwhelm the gravitational effect, higher ventilation air flow rates is needed to satisfy good indoor air quality but unfortunately at a higher energy cost. Alternatively, the IAQ can be satisfied at lower energy cost by altering the size of particles making them heavier favoring deposition when infected people are treated by special medication.

Keywords: Cross contamination – displacement ventilation – aerosols – particle distribution

Funding source: Shammass PhD fellowship

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Nesreen Ghaddar (farah@aub.edu.lb)

PhD student: Carine Habchi (chh05@mail.aub.edu)

Development of a Biomimetic multilayered blood vessel-like structure on a Microfluidic Platform

Anwarul Hasan^{a,b,c,*}, Arghya Paul^{b,c,d}, Ali Khademhosseinib^{b,c,d}

^a Biomedical Engineering and Department of Mechanical Engineering, American university of Beirut, Lebanon

^b Center for Biomedical Engineering, Brigham and Women's Hospital, Harvard Medical School, USA

^c Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, USA

^d Wyss Institute for Biologically Inspired Engineering, Harvard University, USA

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Abstract: Vascularization of large tissues is crucial for the survival of engineered tissues [1], and is one of the biggest challenges in tissue engineering [2-4]. Despite the significant progress over the last few decades, the current approaches of tissue engineering still lack the ability to make perfusable blood vessels with native blood vessel like tri-layer structure. Recently we have made significant progress toward the development of multilayered vascular structures. In the current work we aim to present a new technique for the development of perfusable multilayered vascular structures on a microfluidic chip using a two-step pre-vascularization method. The method uses two concentric hypodermic needles, e.g. 800 micron and 400 micron outer diameter respectively where the inner diameter of the larger needle is larger than the outer diameter of the inner needle. By pre-inserting the two concentric needles in a microfluidic device from the two opposite ends a fibroblast cell-laden hydrogel pre-polymer solution was introduced into the device and cross-linked with ultra violet (UV) light. The larger needle was removed leaving behind a tubular hole with the smaller needle at the center. A second gel solution laden with smooth muscle cells (SMC) was injected into the channel followed by a second-step of UV-cross linking. Finally the small needle was removed, and HUVECs were surface seeded into the lumen, generating a tri layer blood vessel like structure. The vascular structures, developed using the new technique, were grown for several days with and without the continuous perfusion of media through the lumen of the vessels. The cells remained viable under this condition. The HUVECs aligned along the direction of flow under continuous perfusion. The multilayer vessel will have multifaceted applications including development of in vitro models of various cardiovascular diseases such as atherosclerosis and hypertension.

Keywords: Vascularization, Blood Vessel, Microfluidics, Tissue Engineering

Echoes of Bio-Numbers: On the Use of Computational Fluid Dynamics Modeling to Predict Spatial Wall Shear Stress Redistribution in Human Diseased Arteries

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American University of Beirut

(*)PhD Student, Mechanical Engineering Department

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Abstract

Wall Shear Stress (WSS) constitutes a major parameter for assessing future atherosclerotic progression in human diseased arteries. Low WSS values contribute majorly to changing the behavior of endothelial cells at the innermost intima of the arterial walls, constituting a suitable environment for future atherosclerotic progression at different pre and post stenotic locations. In addition, high WSS values reported in this study lead in turn to determine the risk for thrombus formation and hemolysis. Therefore, accurate assessment of blood hemodynamics via numerical techniques, in particular WSS, can provide clinicians with important information for diagnoses, future intervention planning, and follow-up. This is the aim of the current investigation.

Various parameters impact WSS distribution including plaque location, geometrical shape, arterial wall and plaque surface roughness, in addition to many other physiological and physical parameters resulting from arterial structural complexity, blood condition and many others.

Plaque formation on the arterial walls is associated with turbulent blood flow behavior. Accordingly, determining the proper mathematical constraints is important for accurately predicting the in vivo blood hemodynamics.

The current work reports on a parametric study conducted to investigate the effect of plaque shape and surface roughness on wall shear stress redistribution in human arteries using computational fluid dynamics (CFD) techniques. Simulations were also performed to assess the behavior of multiple plaques interference and its impact on blood hemodynamic distribution.

Rectangular, circular and trapezoidal plaque profiles with parameterized dimensions and surface roughness were constructed to model the plaque geometry in the diseased artery. Furthermore, interference between two similar rough rectangular shape plaque profiles was investigated by varying the distance separating the plaques and reporting on the changes at the level of WSS redistribution.

Steady state simulations were performed at the peak systole condition where the blood inlet velocity assumes a maximum value. Initial predictions have revealed a non-linear behavior of the WSS spatial distribution at pre and post stenotic regions for the three simulated plaque profiles (for smooth and rough plaque surfaces). Nonlinear WSS profiles accompanied with small size sharp gradients were also interestingly observed in regions located between multiple plaques showing discrepancies at the level of low and high WSS redistribution, not reported earlier.

Keywords: Wall Shear Stress (WSS), Computational Fluid Dynamics, Atherosclerotic Progression, Plaque, Hemodynamics, Human Diseased Arteries

Funding Source: FEA

Novel hydrazone-hydrazones derived from adenine for diabetic nephropathy

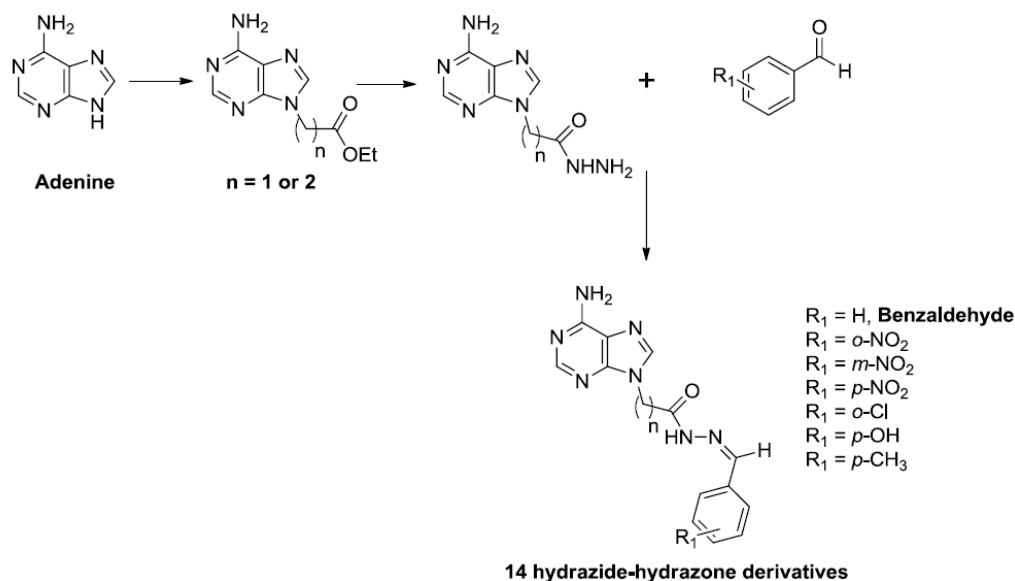
Rana Mezher¹, Nour Hassan¹, Ali Koubeissi¹, Assaad Eid² and Kamal Bouhadir^{1*}

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²Department of Anatomy, Cell Biology and Physiology, Faculty of Medicine, AUB.

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Carbocyclic nucleosides (carbanucleosides) displayed a wide range of biological activities up to date including antitumor, antibiotic, antimicrobial, antiviral, antimetabolite and herbicidal activities as well as inhibition of S-adenosyl-L-homocysteine hydrolase and picornavirus. One attractive feature in carbocyclic nucleosides is the replacement of the furanose ring with a cycloalkane ring that is resistant against phosphorylases which cleave the *N*-glycosidic bond in neutral nucleosides. In this study, we report the synthesis of a new series of carbocyclic adenine hydrazone-hydrazones to evaluate their effect on high-glucose (HG)-induced mesangial cells proliferation and HG-induced fibronectin expression. The synthesis of these hydrazones involves a coupling reaction between substituted benzaldehyde derivatives and 1-(2-hydrazidoethyl)adenine or 1-(hydrazidomethyl)adenine.



Key Words: Carbanucleosides, Adenine, Hydrazone-hydrazones, Diabetic nephropathy.

Rana Mezher and Nour Hassan are research assistants in chemistry.

The authors are grateful to the University Research Board (URB) at AUB and the Lebanese National Council for Scientific Research (LNCSR) for funding this project.

Synthesis and characterization of carbocyclic thymidyl, uridyl, cytidyl and adenosyl hydrazide-hydrazones for diabetic nephropathy

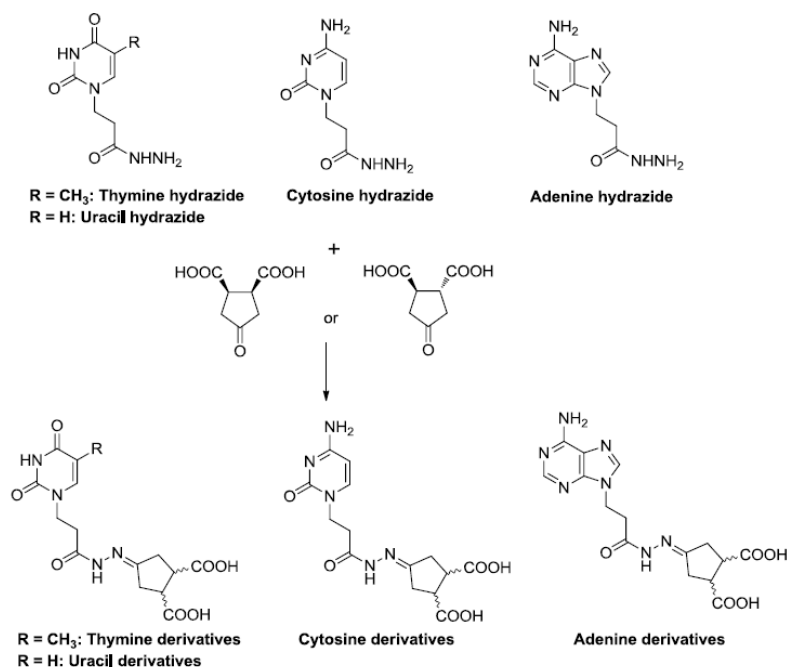
Mira Diab El-Harakeh¹, Fatima Mohsenz², Ali Koubeissi¹, Assaad Eid² and Kamal Bouhadir^{1*}

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Key Words: Carbanucleosides, Thymine, Uracil, Cytosine, Adenine, Hydrazide-hydrazones, Diabetic nephropathy.

Mira Diab El-Harakeh is a Master's student in chemistry.

The authors are grateful to the University Research Board (URB) at AUB, the Lebanese National Council for Scientific Research (LNCSR) and Farouk Jabre Biomedical Research Grant for funding this project.

Synthesis and characterization of nucleic-based hydrazonesas potential antidiabetic drugs

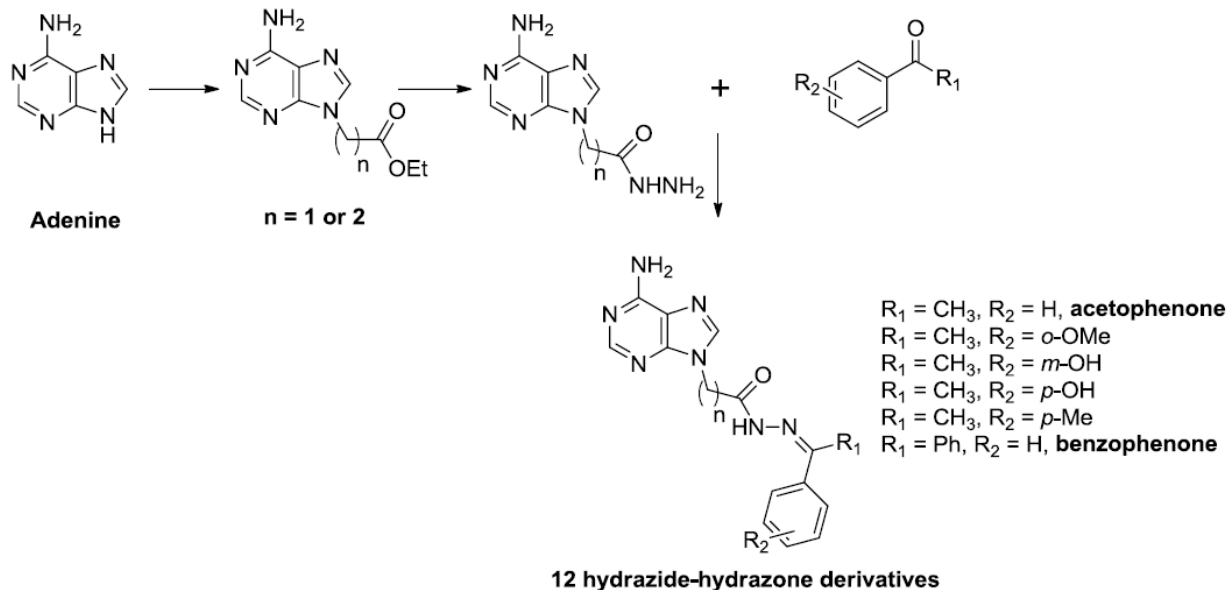
Sara Jaafari¹, Judy Hayek¹, Ali Koubeissi¹, Assaad Eid² and Kamal Bouhadir^{1*}

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Carbocyclic nucleosides (carbanucleosides) displayed a wide range of biological activities up to date including antitumor, antibiotic, antimicrobial, antiviral, antimetabolite and herbicidal activities as well as inhibition of S-adenosyl-L-homocysteine hydrolase and picornavirus. One attractive feature in carbocyclic nucleosides is the replacement of the furanose ring with a cycloalkane ring that is resistant against phosphorylases which cleave the *N*-glycosidic bond in neutral nucleosides. In this study, we report the synthesis of a new series of carbocyclic adenine hydrazide-hydrazones to evaluate their effect on high-glucose (HG)-induced mesangial cells proliferation and HG-induced fibronectin expression. The synthesis of these hydrazones involves a coupling reaction between substituted acetophenone derivatives or benzophenone and 1-(2-hydrazidoethyl)adenine or 1-(hydrazidomethyl)adenine.



Key Words: Carbanucleosides, Adenine, Hydrazide-hydrazones, Diabetic nephropathy.

Sara Jaafar and Judy Hayek are biology senior students.

The authors are grateful to the University Research Board (URB) at AUB and the Lebanese National Council for Scientific Research (LNCSR) for funding this project.

The Use of Upper Room Ultraviolet Germicidal Irradiation for Chilled Ceiling Mixed Displacement Ventilation System to Reduce Disease Transmission

Mohamad Kanaan¹, Nesreen Ghaddar^{1*}, Kamel Ghali¹, Georges Araj²

¹*Department of Mechanical Engineering, and* ²*Department of Pathology and Laboratory Medicine, American University of Beirut, Lebanon*

This work investigates the effectiveness of upper-room ultraviolet germicidal irradiation (UVGI) systems in disinfecting the air in the upper zone of rooms conditioned with chilled ceiling (CC) and displacement ventilation (DV) system. The CC/DV system is one of the air-conditioning systems that provide acceptable indoor air quality since microorganisms are transported by buoyancy to the upper zone of the space where they recirculate before entering the return duct. Even though mixing the return air with fresh air in the supply air stream offers a good opportunity to save energy, it results in high bacterial concentrations in the breathing zone. Therefore, the disinfection of air in the upper zone is highly recommended to allow recirculating a return fraction without violating the World Health Organization (WHO) requirement for bacterial count in the breathing zone to prevent occupants from cross-infection.

An analytical plume multi-layer zonal model is developed to investigate the effectiveness of upper-room UVGI mechanism for air disinfection when used with a mixed air CC/DV system. The model predicts the airborne bacteria concentration in the upper irradiated zone and in the occupied zone. The results from this model are compared with developed computational fluid dynamics (CFD) model that is validated using experimental velocity and temperature data for the same setup. Good agreement between model results and CFD predictions of bacteria concentration inside and outside the plume. The validated model is demonstrated in a case study where maximum allowable mixing ratio is determined to meet bacteria concentrations standard limits in the occupied zone.

Keywords: Indoor air quality – Mixed displacement ventilation – Upper-room UV germicidal irradiation – Reducing cross-infection

Funding source: Lebanese National Research Council, Beirut, Lebanon.

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Vision Based Compliant Gripper for Medical Tools

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Keywords: computer vision, kinematics, compliant gripper, biomedical engineering.

Abstract:

In the surgery room, miscommunication over tool handling between the nurse and the surgeon can cause delays and errors leading to unwanted negative consequences. To improve the efficiency of the surgery we seek to automate redundant tasks to allow nurses to focus on other high level skills. We propose to build a universal gripper that mounts on a robotic manipulator, grasps medical tools and hands them to the surgeon. The wide range of medical tools dictates the need of a robust yet compliant gripper. To achieve that we chose a gripper that relies on a flexible membrane that contains coffee granules. Using negative pressure, the gripper is able to generate interlocking, frictional, and suction forces that in turn are used to pick up the various tools.

We envision the system to automatically interpret the surgeon's needs who communicates with our proposed system via hand gestures. Cameras will be mounted on the robotic system to identify, using computer vision, the surgeon's hand gesture indicating required tool needed. Then after the tool is identified on the tool table, the manipulator will move the gripper, position it on top of the identified tool and activate the gripping scheme. Once positive gripping is achieved, the manipulator will move the gripped tool to the surgeon by determining a minimum time obstacle free trajectory.

For our experiments, we chose a readily available RHINO manipulator. An experiment was conducted to test the validity of the proposed system. The first prototype we built was successfully able to grip a pair of small eyebrow scissors having very similar dimensions, weight and surface roughness to the surgical scissors. The computer vision program that interprets the hand gestures of the surgeon as well as identifies and locates the designated tool is yet to be written. We also still need to optimize the design of the gripper and its assembly with the manipulator and cameras.

Position: Senior mechanical engineering students at the American University of Beirut (AUB)

Funding Source: Department of Mechanical Engineering at AUB

ABSTRACTS

Cancer

Anti-inflammatory and Anti-cancer Effects of the Ethanol and Purified Fraction of the Sea Cucumber *Holothuria Polii*

Kareh Mike^{1*}, Nahas Rana^{1*}, Al-Ghadban Sarah^{2*}, Al-Araj Lamis^{3*}, Saliba Najat^{3*}, El-Sabban Marwan^{2*}, Talhouk Rabih^{1*}

¹*Department of Biology*, ²*Department of Anatomy, Cell Biology, and Physiological sciences*, ³*Department of Chemistry Faculty of Arts and Sciences*, **IBSAR-Nature Conservation Center (NCC) for Sustainable Futures, American University of Beirut, Lebanon*

Corresponding Author: Rabih Talhouk; rtalhouk@aub.edu.lb

Keywords: Sea cucumber, Anti-cancer, Anti-inflammatory

Sea cucumbers are among the most important functional foods for their effectiveness against various diseases. Currently, we are investigating the presence of anti-inflammatory and anti-cancer bioactivities in *Holothuria polii*. Sea cucumber extract (SCE) inhibited the proliferation of mammary epithelial cells SCp2 by 65% without affecting the secretion of Endotoxin (ET) induced inflammatory markers, interleukin 6 (IL-6), Nitric Oxide (NO) and Matrix-Metalloproteinase9 (MMP9). Furthermore, SCE treatment did not affect ET-induced IL-1 β secretion from PMA activated THP-1 (monocytes). Treatment of breast cancer MDA-MB-231 cells with SCE inhibited proliferation by 60% in both, 2D (plastic) and 3D (matrigel) cultures. Cell cycle analysis showed 140% S-phase prolongation in SCE treated cells in 2D conditions. Moreover, SCE treatment favored spherical aggregate morphology over stellate outgrowths in 3D cultures of MDA-MB-231. Twenty-four hours post plating, SCE treatment decreased trans-well invasion of MDA-MB-231 cells by 30% (1:3 EHS dilution). RT-qPCR showed that SCE treatment decreased the mRNA levels of Vimentin in 2D and 3D cultures of MDA-MB-231 by 20% and 40% respectively. Twist expression was decreased by 60% in 3D but not in 2D cultures, in contrast to Zeb1 expression, which showed a 25% decrease in 2D but not in 3D conditions. Western blot analysis demonstrated a decrease in Vimentin expression in 2D and 3D cultures of SCE treated MDA-MB-231 and a downregulation in N-Cadherin expression in 3D conditions. Bio-guided fractionation showed that the aqueous (Aq) fraction among all the collected fractions retained the anti-proliferative activity detected in SCE. Interestingly, the Aq fraction decreased IL-6 secretion levels by 60%, NO by 50% and MMP-9 by 30% in ET-induced SCp2. In addition, Aq treatment inhibited the secretion of IL-1 β by 60% from THP-1. Moreover, Aq treatment decreased proliferation and invasion, prolonged S-phase, favored mesenchymal epithelial transition in MDA-MB-231. In conclusion, the Aq fraction holds a potential anti-inflammatory and anti-cancer activity. Currently, studies are underway to characterize the Aq fraction and determine its active component(s).

Rabih Talhouk: Professor - Mike Kareh: Student

Funding source: MAREX is an EC FP7-funded program

Combination of Arsenic and Interferon- α Inhibits Expression of HHV8 Latent Transcripts and Synergistically Improves Survival of Mice with Primary Effusion Lymphomas

Hiba El Hajj¹, Jihane Ali², , Akram Ghantous³, Louna karam², Dana Hodroj², Ahmad Daher², Kazem Zibara⁴, Chloé Journo⁵, Zaher Otroock⁶, Ghazi Zaatari⁷, Renaud Mahieux⁵, Marwan El Sabban⁸, Ali Bazarbachi^{1*}, Raghida Abou Merhi^{2*}

¹Department of Internal Medicine, Faculty of Medicine, American University of Beirut, Beirut, Lebanon,

²Lebanese University, Rafik Hariri Campus, Faculty of Sciences, Biology Department, Hadath, Lebanon

³International Agency for Research on Cancer, Lyon, France

⁴Lebanese University, Faculty of Sciences, Biology Department, fifth section, Nabatieh, Lebanon,

⁵Equipe Oncogénèse Rétrovirale, INSERM U1111 - CNRS UMR5308, Lyon, France

⁶Leukemia Program, Cleveland Clinic Taussig Cancer Institute, Cleveland, Ohio, United States of America

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Primary effusion lymphoma (PEL) is a rare lymphoproliferative disorder, manifested by malignant effusions in the pericardial, pleural, or peritoneal serous body cavities. Human Herpes Virus type 8 (HHV-8) latent infections have been found to be the etiologic agent responsible for PEL. Several virus latent genes include LANA-1, LANA-2, viral cyclin and viral FLIP involved in transformation and oncogenesis. Since PEL is poorly responsive to standard conventional cytotoxic chemotherapy, new effective therapeutic drugs are needed. PEL cell lines infected with HHV8 have a tumorigenic potential in NOD/SCID mice result in efficient engraftment and formation of malignant ascites with notable abdominal distension, consistent with the clinical manifestations of PEL in humans. Using this preclinical mouse model, we demonstrate that the combination of As/IFN and AZT/IFN inhibits synergistically proliferation, induces apoptosis and downregulates the latent viral transcripts LANA-1, v-FLIP and v-Cyc in PEL cells derived from malignant ascites. Additionally, histopathology examination and PCR analysis for v-FLIP revealed infiltration of the spleen, liver, lung and peritoneum by HHV8⁺ PEL malignant cells. Furthermore, we demonstrate that As/IFN and AZT/IFN induce synergistically higher survival time and decrease the peritoneal volume of PEL mice in comparison to control untreated mice. Our results provide a promising rational basis for a future potent therapeutic use of combined As/ IFN, or AZT/IFN in PEL patients. Thus, it could lead to the generation of new improved anti-viral therapies and prevention of HHV-8 associated PEL.

Keywords: Primary Effusion Lymphoma, HHV-8, AS/IFN, AZT/IFN, LANA, *ex-vivo* ascites

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Communication between hematopoietic stem cells and mesenchymal stem cells under normal and pathological conditions

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Keywords: Hematopoietic stem cells, mesenchymal stem cells, niche, interaction, Leukemia, connexins.

A specialized microenvironment in the bone “the niche” composed of stromal cells, including mesenchymal stem cells (MSCs), support hematopoietic stem cells’ (HSCs) self-renewal and differentiation. Whereas the role of paracrine interaction through soluble factors in the niche has been extensively explored, intercellular communication *via* connexins is yet to be elucidated especially under pathological conditions. This project was developed to study the role of MSCs in modulating HSCs differentiation and to investigate their role in the progression of leukemic cells. We examined the pattern of expression of connexins in HSCs and MSCs. We then co-cultured these cells and evaluated hematopoiesis by studying differentiation markers’ profiles. Mononuclear cells (MNCs) were isolated from mobilized peripheral blood of deceased patients by using ficoll gradient centrifugation. Hematopoietic stem cells (CD34⁺) were isolated from MNCs using flow sorting and easySep selection. MSCs were isolated and characterized by their ability to adhere to plastic, form colonies and morphology as well as expression of specific markers. MNCs isolated from mobilized peripheral blood showed a high percentage of CD34⁺ (20%) and some MSCs (4% CD271⁺). Using cell surface sorting, we isolated HSCs by the expression of CD34⁺ as a marker. These cells will be used to study the interaction between MSCs and HSCs in Leukemic conditions. Preliminary studies showed that MSCs express at least Cx43 and Cx45, tight junction and adhesion molecules (N-cadherin and β -catenin), stemness markers (Oct-4 and Nanog), mesenchymal markers (SNAIL, TWIST) and genes involved in migration (VEGF and SDF-1). In the context of MSCs-Leukemic cells interaction, we show that a reciprocal interaction exist between MSCs and HuT-102 cells (ATL cell line) by affecting the proliferation, the expression of adhesion and communication markers and soluble factors like VEGF. Using live imaging study, a direct cell-cell interaction between MSCs and HuT-102 exist through gap junctions was observed. These preliminary findings demonstrate a potential function for Cxs in regulating hematopoiesis and controls HSC fate. This project will provide a better understanding of the relationship between MSCs and HSCs in regulating hematopoiesis and leukemogenesis.

Participant: Tala Kanson (Post-Doctoral fellow)

Effect of *Holothuria polii* Extracts on Prostate Cancer Cells

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Keywords: sea cucumber extract, prostate cancer, apoptosis, cancer stem cells, metastasis.

Despite progress in the treatment of Prostate cancer, incidence and mortality rates are still high. The aim of this study is to investigate the effect of the ethanolic sea cucumber extract (SCE) (*Holothuria polii*), from the eastern Mediterranean sea, and their fractionated material against the proliferation and metastasis of human prostate cancer cells. Their bioactivity is also assessed for targeting an enriched population of prostate cancer stem cells (CSCs). The effect of the crude SCE on the viability and proliferation was investigated using trypan blue exclusion, MTT and Real Time Cell Analysis (RTCA) assays. Effect on cell cycle progression was studied on DNA content using Propidium Iodide (PI) by flow cytometry. Immunofluorescence (IF), western blotting (WB) and Hoechst nuclear stain were used to assess apoptosis. SCE effect on invasion and migration (RTCA, zymography and wound healing) was assessed. Real-time PCR, WB and IF were performed to study the effect of SCE on the expression level and cellular localization of metastatic markers. Sphere-formation assay was conducted to study the effect of SCE and an aqueous fraction on the proliferation of prostate CSCs using sphere formation assay. SCE inhibited the proliferation of two prostate cancer cells in a time- and dose-dependent manner. Cell cycle analysis revealed that SCE causes G₀/G₁ arrest and an increase in Pre G₀/G₁ suggesting DNA fragmentation. Hoechst stain, WB and IF showed that apoptosis is the mechanism that SCE is inducing. SCE inhibited the migration and the invasion of prostate cancer cells. EMT and metastatic markers decreased upon treatment. SCE inhibited the proliferation of an enriched population of prostate cancer stem/progenitor cells indicating that SCE targets CSCs. Furthermore, preliminary data showed that the aqueous fractionated SCE inhibited the proliferation of prostate cancer cells in 2D and 3D cultures. Our data suggests that SCE inhibits the proliferation and metastasis of prostate cancer cells, presumably through targeting prostate cancer stem/progenitor cells.

Participant: Diana Kadi Bahri (MSc. Student)

Exosomes: vectors for bio-delivery and a novel “induced cell-to-cell communication” mechanism.

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Keywords: exosomes, intercellular communication, connexins, HTLV-1.

Exosomes are membrane nano-vesicles secreted by a multitude of cells under both physiological and pathological conditions. Exosomes contain biologic information such as protein, mRNA and miRNA that can be transferred to other cells, implicating them in many patho-physiological processes. We studied the role of exosomes in intercellular communication by investigating if connexins (the gap junction proteins)-associated with exosomes can be transferred to recipient cells. In addition, we examined if Tax (viral oncoprotein)-containing exosomes from HTLV-I (human T-cell lymphotropic virus type I) infected cells deliver Tax to recipient cells. Exosomes were purified from culture supernatants of 293T cells transfected with Cx26- or Cx43-Dendra-fluorescent protein. Exosomes were examined by scanning electron microscopy, by RT-PCR to detect Cx-Dendra transcripts, and by western blotting to detect the exosomal marker CD63 and Cx-Dendra proteins. Fluorescence microscopy was performed to photo-convert Cx43-Dendra exosomes and to detect their transfer to recipient cells. In addition, we collected exosomes from HTLV-I negative and positive cell lines. Purified exosomes were examined by scanning electron microscope and by western blotting to detect the expression of the exosomal marker TSG-101 and Tax proteins. Finally, we investigated Tax expression in normal cell lines following co-culture with HuT-102 exosomes for 24h. Transcripts and proteins of Cx26-/43-Dendra were detected in the exosomes of 293T-Cx-Dendra cells. Cx43D-containing exosomes were fluorescent and irreversibly photo-converted from green to red. Following co-culture, Cx43D-containing exosomes were successfully transferred to MDA-MB-231 human breast cancer cells. We demonstrated the expression of Tax in the exosomes of HTLV-I positive cells, as compared to the negative cells, at the transcriptional and translational levels. We also showed that Tax-containing exosomes deliver Tax to normal cells including human endothelial, monocytic and mesenchymal stem cells. These findings demonstrate that, following co-culture, exosomal cargo can be delivered to recipient cells with implications for cell-cell communication and HTLV-I pathogenesis.

Participant: Jamal El Saghir (Research Assistant)

Exploration of exosomes isolated from rhabdomyosarcoma tumor cells to understand their paracrine signaling

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Rhabdomyosarcoma (RMS) is an aggressive childhood soft tissue tumor, with two distinct subtypes, alveolar (ARMS) and embryonal (ERMS) histologies. The alveolar subtype is characterized by a specific translocation PAX-FOXO, the protein product of which is thought to contribute to its aggressive and metastatic behavior. Exosomes are small membranous vesicles (30-100 nm in diameter) secreted into body fluids by multiple cell types, including tumor cells. Tumor exosomes contain intact and functional protein and microRNA that can alter the cellular environment to favor tumor growth.

We hypothesize that the PAX-FOXO fusion protein results in specific effects on exosome cargo and biology, contributing to the invasive and metastatic potential of ARMS cells.

In the present study, we examined the functional roles of RMS-derived exosomes on tumor cell proliferation, invasion, and motility. In addition, we examined the RNA cargo of RMS-derived exosomes using miRNA array profiling. We found that RMS-derived exosomes exert a positive effect on cellular migration and invasion, suggesting a possible direct role in metastatic potential of RMS cells. We also found unique miRNA expression signatures in ARMS-derived exosomes, identifying pathways that may contribute to paracrine signaling in tumor progression.

Current work is focused on further analysis of the identified pathways, and possible biomarkers for ARMS. In addition, work is ongoing for identifying PAX-FOXO-specific effects on RMS exosome cargo, and its role in ARMS paracrine signaling and ensuing biologic behavior.

Keywords: Rhabdomyosarcoma, Exosomes, paracrine signaling

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Expression of Connexin43 in MDA-MB231: Implications for cancer metastasis.

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Keywords: Gap junction, connexin43, metastasis, overexpression, down-regulation.

Hetero-cellular interaction between cancer cells and the surrounding stromal cells play a crucial role in disease progression. Mesenchymal stem cells (MSCs) are emerging as critical players in the acquisition of the metastatic phenotype. Furthermore, the overexpression of connexins in breast cancer cells, acts as a tumor suppressing mechanism. In this study, we investigated the role of paracrine and direct cell-cell interactions between MSCs and breast cancer cells (MDA-MB231). *In vitro*, in a transwell co-culture system, MSCs (insert) induced Epithelial to Mesenchymal transition (EMT) in MDA-MB231 (well) characterized by a decrease in cell proliferation and reduction of E-cadherin expression. Mesenchymal to Epithelial transition (MET) was observed in the reverse co-culture setting. MSCs and MDA-MB-231 were shown to communicate through gap junctions by communication assays and live imaging. *In vivo*, NOG mice injected with MDA-MB231 modulated by MSCs caused an early primary tumor onset, larger primary tumor volumes, lower survival rate and a higher infiltration of cancer cells to different organs tested. To further assess the role of gap junctions, connexin43 (Cx43) was overexpressed or down-regulated in MDA-MB231. Western Blot data showed that, connexin43 was down-regulated in cells transfected with a specific Cx43 shRNA-GFP, and overexpressed in cells transduced with Cx43-Dendra lentiviral vector. Transduced cells were sorted into low and high Cx43-Dendra expressing cells. Proliferation assays [trypan blue dye and real time cell analysis (RTCA)] were conducted on non-transduced, low and high expressing Cx43-Dendra cells, which have revealed no significant differences in their proliferation. Real time PCR, western blots and zymograms are performed to assess the expression of connexins (Cx45, Cx43, Cx26 and Cx30), MMPs and angiogenic factors (VEGF and HIF-1 α).

Participant: Jalal Kazan (MSc. Student)

Gallotannin is a DNA damaging compound that induces senescence independently of p53 and p21 in human colon cancer cells

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Colorectal cancer is one of the leading cancer killers worldwide and is strongly associated with environmental causes, such as smoking, heavy alcohol use, high intake of red meat and no physical activities.

The plant secondary metabolite Gallotannin (GT) is the simplest hydrolyzable tannin shown to have anti-carcinogenic properties in several cell lines and to inhibit tumor development in different animal models.

Here, we determined if GT induces senescence and DNA damage and investigated the involvement of p53 and p21 in this response. HCT116 colon cancer cells wildtype for p53⁺/p21⁺ and null for p53⁻ and p21⁻ were treated with GT and senescence was determined by real time cell analyzer, β -galactosidase assay and senescence associated heterochromatin foci formation. The involvement of the reactive oxygen species in the GT effect was investigated by flow cytometry.

Moreover the effect of GT on cell cycle was studied by PI staining, and western blotting for different cell cycle markers. While DNA damage and mitotic slippage after GT treatment was measured by immunofluorescence and flow cytometry.

We found that GT induces senescence independently of p21 and p53 and reactive oxygen species were partially involved in the senescence response. GT was able to induce S phase cell cycle arrest and mitotic slippage in all three HCT116 cell lines. The arrest by GT was irreversible and significant DNA damage occurred following treatment as evidenced by p-H2AX staining. Our findings indicate that GT is an interesting anti colon cancer agent which warrants further study.

Key words: (gallotannin, colon cancer, senescence, cell cycle, DNA damage)

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Investigating the Efficacy of Combining Thymoquinone with Clinical Drugs to Enhance Cell Death in Adult T-cell Leukemia

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Thymoquinone (2-methyl-5-isopropyl-1,4-benzoquinone, TQ) is a natural main constituent of the volatile oil of *Nigella sativa* seed, or the black seed. TQ has shown an antineoplastic potential in both *in vitro* and *in vivo* models, but its mechanism of action has not been yet clearly defined. Interestingly, normal cell counterparts proved to be resistant against the antiproliferative effects of TQ. In our previous studies, we have shown that TQ induces cell death in both HTLV-1 positive cells (HuT-102 and MT-2) and HTLV-1 negative cells (CEM and Jurkat) by generating reactive oxygen species (ROS). On the other hand, Arsenic trioxide (As) became a very promising targeted therapeutic agent against adult T-cell leukemia (ATL) when combined with interferon (IFN). This combination synergistically induces cell cycle arrest and apoptosis *in vitro*; it eradicates the leukemia initiating cell potential *in vivo*, and lastly, it prolongs ATL patients' survival when combined to zidovudine. However, the toxicity of arsenic remains the main drawback of this treatment. In this study, we provide evidence that the combination of As and IFN with TQ, at lower concentrations, sensitizes several resistant HTLV-1 positive cell lines and increases the efficacy of the clinically used drugs. Here we demonstrate that As/IFN/TQ induce apoptosis as evidenced by the cell cycle analysis, and by PARP cleavage, another marker of apoptosis. This combination was also found to down-regulate the key driving oncoprotein (Tax) and to up-regulate the tumor suppressor protein p53. These findings collectively suggest that the use of a combination targeting two independent pathways (NF- κ B and ROS), requires lower concentrations, results in a better efficacy and therefore underlies a lower toxicity. A more detailed study of the potential anticancer effects of the combination treatment is needed to assess their mode of action at the molecular and signaling level along with the possibility to translate their use to clinic.

Key words: thymoquinone, Arsenic, Interferon, ATL

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miRNA as Potential Biomarkers of Breast Cancer in the Lebanese Population and in Young Women: a Pilot Study

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Abstract

Relative to western populations, the percentage of women diagnosed with breast cancer at a young age in Lebanon is high. While the younger age of the Lebanese population compared to the West certainly contributes to this difference, potential genetic, reproductive and/or biological factors likely play an important role. The objective of this study is to investigate the contribution of five reported dysregulated (miRNAs) miRNAs miR-148b, miR-10b, miR-21, miR-221, and miR-155 in 20 normal and 57 cancerous breast tissues from Lebanese breast cancer patients. Results of the relative expression of these miRNAs determined by quantitative reverse transcription real time PCR were analyzed with respect to the patients' clinical and histopathology presentations. Compared to normal breast tissues, significant upregulation of miR-155, miR-21 and miR-148b, notable downregulation of miR-10b and non-significant expression of miR-221 were observed in tumor tissues. Moreover, miR-10b was significantly underexpressed in estrogen/progesterone receptor (ER/PR) negative tumors relative to ER/PR positive tumor tissues. miR-155 was also significantly overexpressed in postmenopausal patients and in those of age at diagnosis greater than 40 years old as well as in PR negative or in human epidermal growth factor 2 (Her2) positive tissues.

This study is the first one to report miRNA expression patterns in Lebanese breast cancer patients. We found that differential miRNA expression in breast cancer could be variable between Lebanese and Western populations. miR-10b was positively correlated with the ER and PR status and that miR-155 could be a noteworthy biomarker for the menopausal state, age at diagnosis, PR and Her2 status. Further studies will be performed to identify the role of the differentially expressed miRNA in different breast cancer cell lines.

Keywords

microRNA, RT-qPCR, breast cancer, biomarkers, Lebanon.

Position

Farah Nassar PhD student at the Biology Department

Funding Source

Medical Practice Plan

Molecular Mechanism of Action of the Synthetic Retinoid ST1926 in Imatinib-Sensitive and -Resistant Chronic Myeloid Leukemia

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Background and Aims:

Chronic Myeloid Leukemia (CML) is a clonal hematopoietic stem cell myeloproliferative disorder caused by the balanced translocation between chromosomes 9 and 22 and the formation of the BCR-ABL oncoprotein with constitutive tyrosine kinase activity. Imatinib, a tyrosine kinase inhibitor (TKI), is the first line of treatment for CML patients worldwide. Unfortunately, several patients develop resistance to imatinib. Retinoids regulate several crucial biological processes such as cellular proliferation, apoptosis, and differentiation, in particular of hematopoietic progenitor cells. The clinical usage of natural retinoids is hindered by undesirable side effects and acquired resistance. Therefore, synthetic retinoids, such as ST1926, which couple increased specificity and reduced toxicity were developed. We investigated the mechanism of action of ST1926 on i) the proliferation of human imatinib-sensitive (AR230, K562, and LAMA) and imatinib-resistant (AR230-r and K562-r) CML cell lines, ii) apoptosis induction, iii) the modulation of the DNA damage response, iv) the degradation of the BCR-ABL oncoprotein, and v) the longevity and tumor burden of CML mice.

Methodology and Results:

Using a well-established *in vitro* human CML model, we have shown that several tested CML cell lines, irrespective of their imatinib response, were sensitive to physiologically achievable micromolar concentrations of ST1926 using MTT cell proliferation and trypan blue exclusion assays. ST1926 induced apoptosis, as evident by PARP cleavage and TUNEL positivity, in all tested CML cells. Furthermore, ST1926 caused DNA damage as evidenced by the phosphorylation of H2AX (γ -H2AX) and the increase in the percentage of cells with DNA tailing using the COMET assay. Interestingly, using real time-PCR, we have shown that ST1926-induced downregulation of BCR-ABL oncoprotein is due to a reduction in the *bcr-abl* oncogene transcript levels. Most importantly, using a retroviral *bcr-abl* transduction murine CML model, we have shown that ST1926 prolonged the longevity of CML mice, reduced white blood cell counts, caused moderate reduction in spleen and liver weight, and resulted in apoptosis in the spleen of ST1926-treated CML mice.

Conclusion:

These results highlight the potential of ST1926 in CML targeted therapy in imatinib-sensitive and -resistant leukemic cells. We are currently investigating the effects of ST1926 on the eradication of leukemia-initiating cells using serial transplantation experiments.

Keywords: chronic myeloid leukemia, ST1926, apoptosis, DNA damage, BCR-ABL, survival.

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Potent Anti-tumor Activities of the Synthetic Retinoid ST1926 in Colon Cancer Cells with Different Genetic Background

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Background and Aims:

Globally, colorectal cancer ranks third in both incidence and fatality. Although current treatment of colon cancer can be aggressive, the cure index is still relatively low. Retinoids regulate several crucial biological processes such as cellular proliferation, apoptosis, and differentiation.

However, the clinical usage of all-*trans* retinoic acid (ATRA) is hindered by side effects and acquired resistance. Therefore, synthetic retinoids, such as ST1926, which couple increased specificity and reduced toxicity, were developed. In this study, we investigate the effects of ST1926 on the proliferation and cell death of several human colon cancer cell lines with different genetic background, and decipher the potential mechanisms involved.

Methodology and Results:

Using a panel of human colon cancer cell lines *in vitro*, we show that these tumor cells were resistant to ATRA while their proliferation was inhibited by ST1926, using MTT and trypan blue exclusion assays. Tumor cells were sensitive to pharmacologically achievable micromolar concentrations of ST1926, irrespective of their p53 status, while the “normal” colon cell line NCM460 was not affected at ten-fold higher concentrations. ST1926 induced apoptosis, as indicated by the accumulation of cells in the pre-G1 region of the cell cycle, as well as by TUNEL and Annexin-PI assays. It also led to S-phase cell cycle arrest, as assessed by DNA content flow cytometry analysis. Mechanistically, ST1926 increased the expression of the tumor suppressor proteins p53 and p21, and the Bax/Bcl2 ratio. Moreover, ST1926 caused extensive DNA damage documented by an increase in γH2AX expression and the Comet assay.

Conclusion:

These results highlight the potential therapeutic properties of ST1926 in colon cancer. We will now investigate the therapeutic activities of ST1926, alone or in combination treatment, in colon cancer mouse models.

Keywords: colon cancer; retinoids; ST1926; apoptosis; DNA damage.

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Retinoic acid and arsenic trioxide degrade mutated NPM-1 resulting in synergistic apoptosis of AMLs

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ABSTRACT

Nucleophosmin-1 (NPM1) is an essential gene that encodes a nucleolar shuttling protein. *NPM1* is the most frequently mutated gene in acute myeloid leukemia (AML), accounting for more than one third of all AML patients, leading to the ectopic accumulation of the NPM1 mutants in the cytoplasm of leukemic cells. In patients with normal diploid karyotype, NPM1 mutation, when present alone, confers a major survival advantage and lower risk of relapse. However, the presence of both NPM1 mutation and FLT3-ITD is associated with poor prognosis. A recent study suggested that addition of all Trans retinoic acid (ATRA) to chemotherapy improved survival only in AML patients harboring the NPM1 mutation in the absence of FLT3-ITD, but the basis for therapeutic benefit remains obscure. In acute promyelocytic leukemia (APL), both ATRA and arsenic trioxide induce degradation of PML-RARA chimeric protein and their combination cures APL patients. Here, we demonstrate that pharmacologically achievable concentrations of ATRA selectively and completely inhibited proliferation in AML cells with mutated *NPM-1* but not of those with wild type *NPM-1*. This was accompanied by caspase activation and apoptosis. Unexpectedly, arsenic had the same effects and, critically, low doses of arsenic and ATRA had a major synergistic effect to initiate apoptosis, again only in AML cells with mutated *NPM-1*. Mechanistically, both ATRA and arsenic induced proteasomal degradation of NPM-1 selectively in cells where this gene is mutated. Collectively, these findings imply that ATRA or arsenic-initiated NPM-1 degradation triggers apoptosis in mutant NPM-1-driven AMLs, strikingly reminiscent of PML/RARA degradation in APL. Our results therefore warrant clinical evaluation of this well-tolerated combination in NPM-1 mutant AML patients.

Key words: Nucleophosmin, ATRA, Arsenic.

Sea Cucumber (*Holothuria Polii*) Extracts Enhance Cell-Cell Communication and Decrease Cell Proliferation of Human Breast Cancer Cells *in vitro*.

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Keywords: Sea cucumber extract, proliferation, invasion, connexin, fractionation

Extract from Sea cucumber (*Holothuria polii*) have diverse biological activities and represent a valuable source for anti-cancer molecules. In this study, we have prepared aqueous ethanolic extracts from sea cucumber (SCE), collected from the Lebanese East Mediterranean sea. The extracts were lyophilized, re-suspended in aqueous solution and analyzed for their biological and chemical characteristics and assessed for anti-tumor bioactive compounds targeting metastatic properties of cancer cells. Cell toxicity, proliferation, invasion and migration assays were studied in three breast cancer cells (MDA-MB-231, MCF-7 and MCF-10A). SCE treatment decreased cancer cell proliferation in a dose-dependent manner with no significant cytotoxicity. Further, SCE treatment increased the invasive properties of cancer cells, with no significant effect on cell migration. The expression of HIF-1 α and VEGF, determining factors of angiogenesis, were significantly increased in MDA-MB-231 and MCF-10A cells treated with SCE; however, no change in their expression was observed in treated MCF-7 cells. The expression of Cx26, a cell-to-cell communication protein thought to be a tumor suppressor protein was increased in MDA-MB-231 and MCF-10A cells upon treatment with SCE. The SCE extracts were lyophilized and re-suspended in H₂O/10%MeOH and subjected to a bio-guided fractionation. A liquid-liquid extraction using four different solvents with increasing polarity was performed as follows: petroleum ether, chloroform, ethyl acetate and butanol. Fractions were tested for their effect on proliferation and only the aqueous phase retained the activity. In conclusion, the aqueous fraction of SCE decreases cell proliferation and up-regulates gap-junction intercellular communication (GJIC) *in vitro*, suggesting a strong potential role of sea cucumber-derived molecules in the development of anti-cancer drugs.

Participant: Sara Al-Ghadban (Research Assistant)

Synthesis, Characterization and Assessment of the Anticancer Potential of Thymoquinone Pyrimidine Derivatives

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Background and Aims:

Thymoquinone (TQ) is the major active ingredient in *Nigella sativa* black seed oil. Isolated 50 years ago, it has shown promising antioxidant, anti-inflammatory and anticancer activities in both *in vitro* and *in vivo* models. Additionally, several studies have reported greater anticancer and antioxidant activities of TQ derivatives as compared to TQ. In this project, we aimed to modify the molecular structure of TQ in order to generate new TQ derivatives. We also characterized and investigated the derivatives' anticancer potential in a panel of cells that range from normal to less aggressive and highly aggressive cancer cell lines.

Methodology and Results:

The synthesis of the hydrazone-hydrazone derivatives involved a coupling reaction between TQ and 1-(2-hydrazidoethyl)thymine, uracil or cytosine. The characterization of the resulting compounds was performed by ¹H and ¹³C NMR as well as by MS spectrometry. Finally, MTT assay was performed to assess the anticancer potential of TQ and TQ derivatives against a panel of breast cells ranging from normal to highly aggressive breast cancer cell lines (normal MCF-10A, non-aggressive MCF-7, and highly aggressive MDA-MB231) as well as the normal colon cell line NCM460, and HCT-116 and HCT-116 p53^{-/-} colon cancer cells. At 24 h, 48 h and 72 h post treatment, our data indicate that all TQ derivatives were significantly more potent than TQ in inhibiting the different cancer cell lines while being less toxic to normal cells.

Conclusion:

These results highlight the anticancer therapeutic potential of TQ pyrimidine derivatives in different cancer cell lines. The project also provides a new approach for the enhancement of TQ anticancer activity. Our future goals are to investigate the effect of the structural modification on the cellular uptake *in vitro* as well as assess the derivatives' antitumor potential in xenograft models *in vivo*.

Keywords: Cancer; thymoquinone; hydrazone derivatives.

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Funding Source: University Research Board of the AUB

The Black Seed Extract Thymoquinone Inhibits Colon Cancer Stem Cells Alone and When Combined with 5-Fluorouracil

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Cancer relapse following all types of treatments remains a leading cause of death among humans worldwide. Colorectal cancer, after a potentially curative surgery, has the tendency to relapse after 5 years on average. It is believed that cancer stem cells (CSCs) are the main element in cancer relapse. CSCs are a subpopulation of cancer cells that retains the ability of self-renewal and differentiation into different mature cells. They play an important role in cancer homeostasis. Tumor-derived CSCs form spherical colonies *in vitro* when plated at clonal and low densities in serum-free defined media supplemented with growth factors, under anchorage-independent conditions. Most of the studies on CSCs, in the context of sphere-forming assays, are based on three broad parts: 1) if sphere formation occurs in the studied cell line, 2) if spheres will continue to self-renew and proliferate constantly, 3) if spheres will resist drug treatment in comparison to adherent cells. Although CSCs pose a significant problem for cancer relapse, it provides a target for drug therapy, namely, Thymoquinone or TQ (2-Isopropyl-5-methylbenzo-1,4-quinone). Work on TQ within the past decade in cellular and animal models of colon and pancreatic cancer cell lines has shown to be significantly effective in apoptosis induction, cell cycle arrest, tumor growth inhibition, tumor cell invasion reduction and angiogenesis inhibition. Here, we determined the effect of TQ on colon cancer stem cells with the crucial purpose of inhibiting cancer relapse. We also aimed to understand the therapeutic role of TQ and its potential mechanisms in cellular and animal models of colon cancer. HCT116 p53+/+ cells forming spheres as compared to HCT116 p53-/- showed less sensitivity to TQ treatment in a dose dependent manner. Both cell lines showed decreased sphere forming units (SFU) when exposed to TQ treatment alone and to a combination of TQ with the commercially common anticancer drug, 5-Fluorouracil (5-FU). These results show that sphere response to TQ treatment is p53 dependent. Moreover, these data show a potential synergism between TQ and 5-FU on sphere formation which implicates a promising inhibition of cancer recurrence.

Keywords: Colorectal cancer, Cancer stem cells, Thymoquinone, Sphere-formation assay, Cancer relapse

Funding source: University Research Board of the American University of Beirut.

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The effects of EAPB0503, a new imidazoquinoxaline compound, on Chronic Myeloid Leukemia

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Chronic myeloid leukemia (CML) is a myeloproliferative disorder that originates from a reciprocal translocation between chromosomes 9 and 22, resulting in the Philadelphia chromosome that harbors the bcr-abl oncogene. This fusion gene codes for a constitutively active tyrosine kinase, BCR-ABL that confers leukemogenesis. Tyrosine kinase inhibitors (TKI) have revolutionized the therapeutic management of CML. Imatinib, the first generation TKI, is highly effective in inducing remissions and prolonging the survival of CML patients. However, failure of this therapy occurs in almost one-third of the patients due to intolerance to treatment, lack of therapeutic response or resistance. Moreover, CML stem cells remain insensitive to this therapy, which almost inevitably leads to relapse upon treatment discontinuation. Imidazoquinoxalines are imiquimod derivatives with direct immunomodulatory effect and antitumor activity on melanoma and T-cell lymphoma, attributed to growth inhibition and induction of caspase-dependent apoptosis. We investigated the effects of EAPB0203 and EAPB0503, novel imidazoquinoxaline derivatives, on human CML cell lines. We showed that EAPB0203 and EAPB0503 inhibit cell growth of three CML cell lines in a dose- and time-dependent manner. Compared to the previously described EAPB0203, EAPB0503 has a more pronounced inhibitory activity on CML cells. We demonstrated that EAPB0503 induced a specific cell cycle arrest in mitosis, evidenced by increased phosphorylation of histone-3. This was accompanied by the direct activation of apoptosis as demonstrated by increased PreG₀ population, TUNEL-positivity, poly(ADP-ribose) polymerase cleavage and dissipation of mitochondrial membrane potential. The combination of EAPB0503 with imatinib synergistically inhibited CML cell proliferation and most importantly, EAPB0503 inhibited the proliferation of imatinib-resistant CML cells and downregulated levels of BCR-ABL oncoprotein, offering a promising therapeutic modality alone or in combination with TKI.

Keywords: Chronic myeloid leukemia, imidazoquinoxalines, apoptosis, EAPB0503, imatinib.

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The synthetic retinoid ST1926 as a novel therapeutic agent in rhabdomyosarcoma

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Rhabdomyosarcoma (RMS) is the most frequent soft-tissue sarcoma in children, and the third most common solid tumor in childhood. The alveolar subtype (ARMS) is frequently associated with a PAX3-FOXO1 fusion protein, and is associated with a poor prognosis and a 5-year survival rate of less than 30%. Despite multiple attempts at intensifying chemotherapeutic approaches to treatment, no improvements in survival have been made for patients with advanced stage disease over the past 10 years. E-3-(4o-Hydroxyl-3o-adamantylbiphenyl-4-yl) acrylic acid (ST1926) is a novel orally available compound belonging to the class of synthetic atypical retinoids.

This study aims at investigating the antitumor activity of ST1926 in RMS. We found that, *in vitro*, ST1926 inhibited RMS cell viability in all tested alveolar rhabdomyosarcoma (ARMS) and embryonal rhabdomyosarcoma (ERMS) cell lines, at an effective Cm as low as 0.5μM. Further analysis showed that ST1926 induced an early DNA damage response, with ATM and H2AX phosphorylation within 24 hours of exposure to drug, along with G1-arrest in all cell lines. After 48 hours of exposure, an S-phase and/or G2/M arrest was observed. On a molecular level, ST1926 resulted in decrease in protein levels of CDK1, which is a kinase essential for G2/M progression. In addition, ST1926 resulted in a decrease in protein levels of PAX3-FOXO fusion protein in ARMS cell lines, and promoted differentiation of ERMS cell lines. We conclude that ST1926 is effective in inhibiting RMS cell proliferation *in vitro*, by inducing a cell cycle block at G1 and S phases of the cell cycle. Molecular mechanisms include activation of a DNA damage response, and possibly alterations in CDK1, and the PAX3-FOXO1 oncogenic fusion protein, both of which are being further investigated. In addition, we are currently exploring *in vivo* effects of the drug in preclinical RMS mouse models.

Keywords: Rhabdomyosarcoma, ST1926, cell cycle, DNA damage

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The synthetic retinoid ST1926 as a novel therapeutic agent in rhabdomyosarcoma

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This study aims at investigating the antitumor activity of ST1926 in RMS. We found that, *in vitro*, ST1926 inhibited RMS cell viability in all tested alveolar rhabdomyosarcoma (ARMS) and embryonal rhabdomyosarcoma (ERMS) cell lines, at an effective Cm as low as 0.5μM. Further analysis showed that ST1926 induced an early DNA damage response, with ATM and H2AX phosphorylation within 24 hours of exposure to drug, along with G1-arrest in all cell lines. After 48 hours of exposure, an S-phase and/or G2/M arrest was observed. On a molecular level, ST1926 resulted in decrease in protein levels of CDK1, which is a kinase essential for G2/M progression. In addition, ST1926 resulted in a decrease in protein levels of PAX3-FOXO fusion protein in ARMS cell lines, and promoted differentiation of ERMS cell lines. We conclude that ST1926 is effective in inhibiting RMS cell proliferation *in vitro*, by inducing a cell cycle block at G1 and S phases of the cell cycle. Molecular mechanisms include activation of a DNA damage response, and possibly alterations in CDK1, and the PAX3-FOXO1 oncogenic fusion protein, both of which are being further investigated. In addition, we are currently exploring *in vivo* effects of the drug in preclinical RMS mouse models.

Keywords: Rhabdomyosarcoma, ST1926, cell cycle, DNA damage

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ABSTRACTS

Clinical

Anticoagulation for patients with cancer and central venous catheters: a systematic review and meta-analysis

Authors and their affiliations: Elie Ramly, Lara Kahale, Elie Akl, American University of Beirut, Clinical Research Unit

E-mail address of corresponding author: ea32@aub.edu.lb

Keywords: Anticoagulation, cancer, central vein catheter

Descriptive statement using simple terms to be accessible to audience from diverse scientific backgrounds: This systematic review evaluates the efficacy and safety of blood thinning agents in patients with cancer and central venous catheters

Indicate your position (student, postdoctoral fellow, etc.): postdoctoral research fellow, Department of Surgery.

Funding source: National Institute for Health Research, UK

ABSTRACT

Background

Central venous catheter (CVC) placement increases the risk of thrombosis in cancer patients. Thrombosis often necessitates the removal of the CVC, resulting in treatment delays and thrombosis related morbidity and mortality.

Objectives

To evaluate the efficacy and safety of anticoagulation in cancer patients with a CVC.

Search Methods

A comprehensive search was performed for studies of anticoagulation in cancer patients including a February 2013 electronic search of: the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, EMBASE and ISI Web of Science. We hand-searched conference proceedings, checked references of included studies, used the "related article" feature within PubMed, and checked clinical trials.gov for ongoing studies.

Main results

Of 9,560 identified citations, we included 12 RCTs (19 reports) enrolling 4,089 patients and assessing either prophylactic dose heparin or low dose VKAs. Parenteral anticoagulation was not associated with a statistically significant effect on death (RR 0.85; 95% CI 0.55 to 1.31) or major bleeding (RR 0.68; 95% CI 0.1 to 4.78) but there was high quality evidence of reduction in VTE (RR 0.54; 95% CI 0.35 to 0.85).

Low dose VKAs were not associated with a statistically significant effect on death (RR 0.97; 95% CI 0.82 to 1.15) or major bleeding (RR 6.93; 95% CI 0.86 to 56.08), but there was moderate quality evidence of reduction in VTE (RR 0.51; 95% CI 0.29 to 0.89).

Conclusions

In this systematic review, our updated meta-analysis of data from twelve trials and 4,089 participants revealed high quality and moderate quality evidence of reduction in VTE with the use of LMWH and VKA, respectively. There was no statistically significant effect on other outcomes of interest for heparin or VKA. However, the findings did not rule out clinically important benefits and harms. Patients with cancer with CVCs considering anticoagulation should balance the possible benefit of reduced thromboembolic complications with the possible harms and burden of anticoagulants. The main limitation of this systematic review is the inclusion of different types and stages of cancer.

Artificial Intelligence modeling using Clinical features, Knowledge and Attitude Predicts Salt Reduction Behavior

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Keywords: Artificial Neural Network, Salt Intake Reduction, Knowledge, Attitude, Behavior

Descriptive statement: The aim of this project is to determine the key knowledge and attitude questions that guide behavior using an artificial intelligence prediction model. Knowing this will guide health campaigns and interventions to raise awareness on adverse effects of excessive salt intake

Indicate your position (student, postdoctoral fellow, etc.): medical student

Funding source: None

Artificial Intelligence modeling using Clinical features, Knowledge and Attitude Predicts Salt Reduction Behavior.

Background: High dietary salt intake is accountable for up to 30% of the prevalence of hypertension. Hypertension in turn is a significant risk factor for hazardous cardiovascular events. The World Health Organization aims for salt intake of less than 5g/day/ person by 2025. This entails individual efforts to change behavior along with policy changes. Predicting behaviors regarding salt intake habits is vital to guide and focus interventions and increase their effectiveness. We **aim** to develop an Artificial Intelligence (AI) statistical based tool that predicts behavior from key knowledge and attitude questions along with clinical data in a high cardiovascular risk population. **Methods:** An AI model was used to analyze collected knowledge, attitude and behavior (KAB) data on 115 high risk patients (Coronary Care unit) in AUBMC (mean age: 60.63 SD 15.39 years). The outcome to be predicted was to classify a person into one of three groups of behavioral tendency: **Group A** with favorable behavior, **Group B** with less favorable behavior, and **Group C** with unfavorable behavior for reducing salt intake. To further refine our model we included the clinical features found to be most important: diastolic and systolic blood pressure, pulse, smoking status and history of hypertension. The initial questionnaire included 34 questions on knowledge, attitude and behaviors related to salt intake. The developed reduced model included only 8 questions found to provide the highest accuracy. The accuracy was calculated using the bootstrapping technique with 100 iterations. The reported accuracy is the average correct prediction over all iterations. **Results:** AI based model achieved accuracy of 66% CI (63%-69%); on the validation

cohort based on the total sample from which 90 were used as a derivation cohort and 25 as validation cohort. The statistical model has been implemented in an online calculator that allows the physician to estimate the patient's behavior from a few questions as illustrated below. **Conclusion:** Using Artificial Intelligence modeling that incorporates a reduced number of knowledge and attitude questions about Salt intake- in addition to baseline clinical information- we can predict favorable salt reduction behaviors with 66% accuracy. This tool can be used in clinics to guide therapeutic salt reduction interventions in high Cardiovascular risk individuals with minimized effort and time from the healthcare (physicians, nurses or dietitians) providers.

Behavior Prediction

Knowledge Questions

Which of these best describes what you think is the effect of the salt/sodium in your diet?

☐ Improves your health

☐ Has no effect on health

☐ Worsens your health

☐ Don't know

Do you think these health problems can be caused or aggravated by salty foods? (Check the box if YES)

☐ Stroke

☐ Osteoporosis

☐ Fluid Retention

What is the maximum daily amount of salt recommended for adults?

☐ 3 grams (1/2teaspoonful)

☐ 6 grams (1 teaspoonful)

☐ 9 grams (1 1/2teaspoons)

☐ 12 grams (2 teaspoons)

☐ 15 grams (2 1/2teaspoons)

☐ Don't know

Which of the following statements best describes the relationship between salt and sodium?

☐ They are exactly the same

☐ Salt contains sodium

☐ Sodium contains salt

☐ Don't know

For each please indicate whether you consider these foods to be: high, medium or low in terms of salt/sodium content.

High Medium Low Dont Know Bread

○ ○ ○ ○

High Medium Low Dont Know Ketchup

○ ○ ○ ○

Clinical Information

SBP

DBP

Pulse

Smoking Status ☐

Hypertension History ☐

Find Behavior Class

A

Association between low vitamin D levels and asthma in children: a systematic review of cohort studies

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Key words

Asthma, wheezing, childhood, pediatric, vitamin D, bronchial hyper responsiveness, lung function tests, systematic review, cohort

Descriptive statement: Available epidemiological evidence suggests a potential association between low serum levels of vitamin D and the diagnosis of asthma in children.

Abstract

Background: There is conflicting evidence about the association between low vitamin D levels in children and later development of asthma. The objective of this study was to systematically review the evidence for an epidemiological association between low serum levels of vitamin D and the diagnosis of asthma in children.

Methods: We used the Cochrane methodology for conducting systematic reviews. The search strategy included an electronic search of MEDLINE and EMBASE in February 2013. Two reviewers completed in duplicate and independently study selection, data abstraction, and assessment of risk of bias.

Results: Of 1081 identified citations, three cohort studies met eligibility criteria. Two of these studies found that low serum vitamin D level is associated with an increased risk of developing asthma late in childhood. The third study found no association either Vitamin D2 or vitamin D3 levels. All three studies suffer from major methodological shortcomings that limit our confidence in their results.

Conclusions: Available epidemiological evidence suggests a potential association between low serum levels of vitamin D and the diagnosis of asthma in children. High quality studies are needed to reliably answer the question of interest

Funding: None

Barriers in Mammography screening; where we went wrong.

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Keywords: Breast Cancer, Mammography screening, Campaigning.

Funding: None

Abstract

Introduction: Screening mammography is an established intervention that leads to early breast cancer detection and reduced mortality. The Lebanese Ministry of Health has initiated yearly awareness campaigns and provided free mammography in multiple centers around the country. Despite aggressive campaigning, the rate of adherence to screening mammography in Lebanon remains low. The current study aims at assessing the barriers that prevent women from having such procedure

Methods: The study is a cross sectional survey aiming to assess knowledge about breast cancer screening and screening behaviors in the Lebanese population. The primary outcome of the study was to assess the reasons preventing women from performing screening mammography based on four categories of questions: lack of knowledge about breast cancer, lack of access to screening facilities, failure of primary care physician to encourage screening behavior, other reasons. The study took place in two major areas of Lebanon mainly Beirut and South Lebanon.

Results: Our Results show that the major barriers to seek screening with statistically significant P-values were in order of prevalence: Lack of knowledge about breast cancer, followed by social reasons and lack of access.

Conclusion: Given the prevalence of breast cancer in our population it is important to understand the pitfalls that we experience in promoting awareness. Our study is the first study to reach out to the community to assess perceived barriers against screening and provide solutions for such barriers.

Characteristics, cognitive performance and quality of life of the Multiple Sclerosis patients at AUBMC

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Keywords: Cognition, Quality of life and Multiple Sclerosis

Positions:

Hala Darwish: Assistant Professor

Raed Ismail and Ralph Maroun: Research Fellows

Nuhad Abou Zeid: Assistant Professor

Bassem Yamout: Professor

Samia Khoury: Professor

Funding: AUBMC MS center funds

Background. MS is an autoimmune disease of unknown etiology in which myelin is damaged. The immune system is activated and attacks myelin, and inflammation occurs around blood vessels in the brain and spinal cord. Although several immunologic abnormalities in the peripheral blood of MS patients have been described, there are no blood tests that can be used as biomarkers of disease activity, disease progression, or response to treatment.

The aim of this study is to describe the characteristics of 116 MS patients who presented to the AUBMC multiple sclerosis center over a period of two years.

Design and Methods

The study stems from a prospective 10 years study that consists of establishing a clinical and biobank of urine, serum plasma and frozen cells of MS patients who present to the AUBMC MS center.

Results: Descriptive statistics of 116 MS patients who presented to the MS center over the past two years period will be analyzed. Bivariate correlation of the patients' scores between cognitive performance using the Symbol Digit Modalities Test (SDMT) and the quality of life using the Multiple Sclerosis Quality of life test (MuSQOL) and their clinical findings will be measured. It is expected to find a significant positive correlation between the subjects' cognitive performance and years of education. It is also expected to find a statistically significant positive correlation between the quality of life and speed of walking and a negative correlation between their MuSQOL score, disease duration and their Expanded Disability Status Scale (EDSS) score. Similarly, a negative correlation is expected between their SDMT performance and disease duration, EDSS and smoking.

Conclusion: Results from this study will guide the clinical management and treatment of the MS patients who present to the AUBMC MS center.

Effect of Vitamin D replacement on immune function and cognition in Multiple Sclerosis (MS) patients

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Keywords: Vitamin D, Multiple Sclerosis, Cognition

Positions:

Hala Darwish: Assistant Professor

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Nuhad Abou Zeid: Assistant Professor

Bassem Yamout: Professor

Samia Khoury: Professor

Funding: MPP and CNRS-L

Background: Multiple Sclerosis (MS) is a chronic inflammatory disease of the central nervous system that is linked to genetic and environmental factors such as vitamin D status. Moreover, recent population-based studies linked low serum vitamin D levels to cognitive dysfunction in older adults. New evidence suggests that vitamin D has an immunomodulatory effect in multiple sclerosis. This is a prospective study to evaluate the effect of vitamin D supplementation in MS patients with Vitamin D deficiency (serum level <25µg/ml) to those with normal Vitamin D (serum level >35 µg/ml) on immunological and neuropsychological (cognitive) measures.

Methods: 86 patients diagnosed with relapsing remitting MS or clinically isolated syndrome aged 18 years and older treated with interferon-beta and without signs of active inflammation or cognitive impairment were recruited. Demographic and health behavior information were collected, patients were also screened for depression and anxiety using the Arabic- Hopkins Symptoms Checklist (HSCL-25), cognitive performance was measured using the Arabic-Montreal Cognitive Assessment (MoCA) and Stroop Test, Symbol digit Modalities Test (SDMT) and the Brief Visual Memory Test (BVMt). Blood was collected to examine their vitamin D, calcium and immune profile. Subjects were evaluated at baseline and 3 months after vitamin D supplementation (10,000 IU daily for 3 months or 50,000 IU weekly for 3 months).

Results: Preliminary descriptive behavioral data analysis of the first 63 patients will be performed. Data on mean age, vitamin D level, depression and anxiety scores, and cognitive performance levels will be calculated. Bivariate correlation analysis between the cognitive tests (Montreal cognitive assessment, Stroop and brief visuospatial memory test) scores and between vitamin D and years of education, age and cognitive performance will be performed. Paired t-test to compare the mean vitamin D level and cognitive performance scores before and 3 months after vitamin D supplementation will be analyzed. Independent t- test between the groups with normal vitamin D and deficient vitamin D level will be compared before and after 3 months of vitamin D supplementation.

Conclusion: We expect this preliminary analysis to show a significant proportion of subjects with depression, vitamin D deficiency and cognitive impairment. Also, we expect to find statistically significant negative correlations between the cognitive tests and age and a positive correlation between cognitive performance and years of education in addition to a significant improvement in vitamin D level before and after vitamin D supplementation. Given short study duration of 3 months, we don't expect to see statistically significant difference on cognitive performance between and within the groups.

Identifying participants with missing data in trials included in systematic reviews: challenges and potential solutions

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Funding: Cochrane Collaboration

Keywords:

Missing participant data, premature end of follow up, bias, randomized control trials, systematic reviews, discontinuation of treatment

Statement:

Identifying participants with missing data in trials included in systematic reviews may be very challenging. Given the importance of identifying these participants for the assessment of risk of bias, we have developed suggestions for authors of systematic reviews on how to deal with this challenge.

Abstract:

Background:

Missing data for the outcomes of study is a prevalent problem in clinical trials and is recognized as a potential source of bias. For authors of systematic review of trials to assess the risk of bias associated with missing data, they need to identify which participants actually have missing data. We will discuss the challenges with identifying participants with missing data in trials included in systematic reviews, and make suggestions for how to deal with those challenges.

Methods:

We identified the challenges during the process of updating a series of six Cochrane systematic reviews on the topic of anticoagulation in patients with cancer. The data abstractors noted the challenges and sought potential ways of dealing with them. They discussed those challenges and potential solutions with other clinical epidemiologists and refined them in an iterative manner.

Results:

We distinguish between the concept of 'premature end of follow-up', which is specific to a participant, and the concept of 'missing participant data', which is specific to an outcome. We identified three challenges along with suggestions to dealing with them:

Challenge 1: missing participant data reported by participant and not by outcome; suggestion is to count these participants as having missing data for all outcomes

Challenge 2: lack of clarify whether certain participants (e.g., who discontinued treatment) were actually followed-up; we provide an algorithm on how to deal with this challenge

Challenge 3: lack of clarify how trialists dealt with participants with missing data (e.g., whether any and which assumptions were made); in the absence of clarifying details, consider that the trialists included those participants in the denominator and not the numerator (assumption of no event)

Conclusion:

We suggest ways for dealing with few challenges of with identifying participants with missing data in systematic reviews. We hope that highlighting these challenges will help in improving the quality of reporting of trials.

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Incidence of Alpha Globin Gene Defect in the Lebanese Population: A Pilot Study

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Funding Source: Lebanese National Council for Scientific Research (CNRS)

Abstract

Background

Inherited hemoglobin disorders are the most common monogenic defects described worldwide. It is well established that Mediterranean and Arab populations are at high risk for thalassemia in general, and for α -thalassemia in particular. Prenatal as well as premarital screening programs in countries with high prevalence have already been founded. In this study, we aim at assessing the incidence of alpha thalassemia deleterious alleles in the Lebanese population.

Methods

Following informed parental consent, DNA was extracted from 200 newborns dried blood cards remaining from routine neonatal screening at the American University of Beirut Medical Center. DNA samples were screened for the 21 most common α -globin deletions and point mutations reported worldwide, through multiplex Polymerase Chain Reaction (PCR) and Reverse-Hybridization technique.

Results

The carrier rate of α -thalassemia in our sample population was 8% which is higher than that reported from Jordan (2-4%). This finding is comparable to Mediterranean countries (Israel: 5-9%, Greece: 7%, Adana-Turkey: 7.5%) but lower than that reported in other Arab countries (UAE: 49%; Oman: 48.5%; Saudi Arabia: 50%). Two mutations were detected: the -3,7del single gene deletion (75%) and the non-gene deletion $\alpha 2$ IVS1 [-5nt] (25%). These mutations are common worldwide. Interestingly, the - α 4.2 and MED mutations, particularly common in Arab and Middle Eastern populations, were absent in our survey

Conclusion

This study is the first dedicated to investigate α -thalassemia genotype incidence in Lebanon. Data obtained demonstrates a high carrier rate in a relatively, highly consanguineous population. These results may impact premarital and newborn screening policies in our country.

Keywords: hemoglobin disorders; alpha thalassemia; newborn screening.

Interventions targeting physicians' interaction with pharmaceutical companies: A systematic review

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Keywords: Pharma, Drug Industry, Physician, conflict of interest.

Descriptive statement: There is a potential positive impact of policies aiming to reduce interaction between physicians and the pharmaceutical companies on physicians' prescription behavior.

ABSTRACT

Background

Pharmaceutical company representatives likely influence the prescribing habits and professional behavior of physicians. The objective of this study was to systematically review the effects of interventions targeting physicians' interactions with pharmaceutical companies.

Methods

We used the Cochrane approach to systematic review. The search strategy included an electronic search of MEDLINE and EMBASE. Two reviewers completed in duplicate and independently study selection, data abstraction, and assessment of risk of bias. We assessed the quality of evidence by outcome using the GRADE methodology.

Results

Of 10,189 identified citations, one randomized clinical trial and three observational studies met the eligibility criteria. The RCT provided moderate quality evidence of no effect of a "collaborative approach" between the pharmaceutical industry and the health authority. The three observational studies provided low quality evidence suggesting a positive effect of policies aiming to reduce interaction between physicians and the pharmaceutical companies (in the form of free samples,

promotional material, and meeting with pharmaceutical company representatives) on prescription behavior.

Conclusion

Available evidence suggests a potential impact of policies aiming to reduce interaction between physicians and the pharmaceutical companies on physicians' prescription behavior.

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Intravitreal adalimumab for the management of active non-infectious uveitis: a pilot study

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Purpose: Evaluate efficacy & safety of intravitreal adalimumab (IVA) for treatment of active noninfectious uveitis.

Methods: In a prospective noncomparative interventional case series, eyes with active noninfectious uveitis were injected with IVA at 0, 2 then every 4 weeks for total of 6 months. Change in Visual Acuity (VA), grade of inflammation (anterior chamber (AC) cells, vitreal (vit) haze, leakage on fluorescein angiography (FA) adopted from the angiography scoring for uveitis working group, and central retinal thickness (CRT) were recorded. No systemic or ocular therapies were added to the IVA treatment during the study period.

Results: 7 patients (13eyes) were treated: Behcet panuveitis(4), idiopathic panuveitis(2), and idiopathic intermediate uveitis(1). 6/7 patients (12/13eyes) completed 6 months treatment. 1 patient (1 eye) failed treatment and was taken out of the study. Comparing baseline to the 6month visit: Median VA logMar improved from 0.243 (IQR=0.855) to 0.049 (IQR=0.398) (p=0.003); Median CRT improved from 317 (IQR=199) to 277 (IQR=107.25) (p=0.021); Median FA score improved from 14 (IQR=7.5) to 4 (IQR=4.75) (p=0.002). At 6 months, 12/12 eyes had AC grade and vit haze =0. No side effects occurred. Stratifying the data to include one eye per patient revealed similar results.

Conclusions: IVA was safe in this pilot study; was effective in improving the VA in 7/12 eyes, and decreasing AC cells, vit haze and FA score in 12 eyes while 1 patient (1 eye) failed treatment.

(NCT00855608)

Funding Source: URB grant # CUF114140.734188

Keywords: Uveitis; Non-infectious; Adalimumab (Humira)

Knowledge, attitudes and behaviors related to sodium intake of high risk Lebanese adult patients

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INTRODUCTION: Ischemic heart disease is the leading cause of death worldwide with an estimated toll of 7 million, followed by stroke (6.2 million). The World Health Organization (WHO) has estimated that hypertension is the leading preventable risk factor for death in the world. Among other factors, high dietary salt intake has been directly correlated with elevated blood pressure. Moreover, assessing knowledge, attitudes, and behaviors of high risk patients would direct future interventions to reduce intake of salt and consequently the severity of hypertension.

AIMS: To assess the knowledge, attitudes, and behaviors of high risk patients in the community regarding salt intake to guide future interventions. We also aim to compare the awareness level of our study sample to healthy individuals in the community from a previous study*.

METHODS: 115 Lebanese high risk patients admitted to the Coronary Care Unit were administered a questionnaire developed to assess awareness of salt intake and its associated cardiovascular risk. The questionnaire included questions on sociodemographics, knowledge regarding daily salt intake recommendation, daily salt habits, and knowledge about salt content in food and on associated health hazards. Patients will also be asked about previous dietary consults. Descriptive and correlative statistics were conducted using SPSS 20 for Windows (SPSS Inc, Chicago, IL).

RESULTS: In terms of knowledge, 22.6% of sample knew that bread is high in salt, compared to 67.2% of healthy individuals. Moreover, only 19.1% of patients and 28.9% of healthy individuals knew the correct maximum daily amount of salt recommended (5 grams/day). In terms of attitudes and behaviors, 53.1% of patients never look at labels (or not applicable) compared to 36.0% of healthy individuals. When asked about the main motive to reduce salt intake, 65.8% admitted that it would be a dramatic change in health status compared to 51.7% of healthy individuals.

CONCLUSIONS: Our study shows a lack of knowledge regarding salt major contributors in the Lebanese diet and allowed maximum intake levels relative to the healthy sample. Similarly, favorable attitudes and behaviors were documented in higher percentage among healthy individuals.

* Knowledge, Attitudes and Behaviors Related to Sodium Intake of Lebanese Consumers aged 19-60

Keywords: Hypertension; Knowledge; attitudes; behavior; high salt intake; Lebanese high risk patients

Table 1. Characteristics of the study population (n=115)		n (%)
Gender		
Male		74 (64.3)
Female		41 (35.7)
Age		
19-30		6 (5.2)
31-40		5 (4.3)
41-50		14 (12.2)
51-60		34 (29.6)
61 plus		56 (48.7)
Educational Level		
High school or lower		65 (56.5)
University BS or higher		50 (43.5)
Place of living		
Beirut		70 (60.9)
Other governorates		45 (39.1)
Specialized in a Health related major		
Yes		12 (10.4)
No		103 (89.6)

Table 2. Physical Characteristics of the study population (n=115)	Count	Mean	Standard Deviation
Age	115	60,63	15,39
Crowding index	115	,68	,67
Height	115	166,59	21,69
Weight	115	82,00	17,85
BMI	115	29,37	5,41
Systolic Blood pressure	115	125,61	22,14
Diastolic Blood pressure	115	67,10	14,27
Pulse	115	76,56	20,67

Table 3. Medical Characteristics of the study population (n=115)		Count	Column N %
smoking status	yes	43	37.7%
	no	71	62.3%
Family History - CAD	yes	30	26.3%
	no	84	73.7%
Family History - HTN	yes	28	24.6%
	no	86	75.4%

Family History - DM	yes	29	25.4%
	no	85	74.6%
Medical Hx - HTN	yes	85	74.6%
	no	29	25.4%
Medical Hx - DL	yes	73	64.0%
	no	41	36.0%
Medical Hx - DM	yes	49	43.0%
	no	65	57.0%
Medical Hx - S/P PCI	yes	44	38.6%
	no	70	61.4%
PCI during Current visit	yes	35	30.7%
	no	79	69.3%

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Neurocognitive Changes in SSRI (Selective Serotonin Reuptake Inhibitors) – Treated Adolescents with Depression

Abstract

Depression in adolescents often has debilitating physical and psychological effects, with around 40% of sufferers not showing adequate response to the most well-established treatment modalities currently present. While Selective Serotonin Reuptake Inhibitors (SSRIs) have been shown to be efficacious in the treatment of pediatric depression, the rate of responders to these medications is less than optimal. Recent literature has highlighted the role of neurocognitive markers in pediatric depression. The current study aimed to specifically investigate the changes in neurocognitive functioning in adolescents with depression after SSRI treatment. **Methods:** Twenty four adolescents with Major Depression currently in a depressed episode (MDD) were administered subtests of the CANTAB (Cambridge Neuropsychological Test Automated Battery) prior to SSRI treatment and at 6 weeks into treatment. In addition, 24 Healthy Controls (HC) were administered the same battery at week 0 and week 6. **Results:** MDD had more pre-Extra-Dimensional Shift errors ($p = 0.006$), but less Extra-Dimensional shift errors ($p = 0.02$) than HC, in the subtest measuring attentional set formation, maintenance, shifting, and attention flexibility (Intra/Extradimensional Set Shift) across both testing times. In the visual memory subtest (Delayed Matching to Sample) MDD did worse than HC in all delays for both assessments ($p = 0.004$ for all delays; $p = 0.04$ for 0 ms delay; $p = 0.01$ for 4000ms delay; $p = 0.04$ for 12000 ms delay). Also, MDD had more probability error in both assessment sessions for this task ($p = 0.005$ for probability error given correct; $p = 0.02$ for probability error given error). Finally, MDD showed more impulsivity across sessions on the subtest measuring speed of processing and impulsivity (Rapid Visual Information Processing), with lower B'' scores indicating that they required less visual trace to elicit a response. **Conclusion:** These results suggest that adolescents with depression show improvement in attention shifting after SSRI treatment when rule shifting is required; while they remain sensitive to distractors in a set maintenance task, regardless of treatment. Also, it is suggested that visual memory and impulsivity might not improve as a result of SSRI treatment.

Keywords: depression; adolescent; SSRI; neurocognitive changes; CANTAB; treatment response

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Funding source: Medical Practice Plan

Out of field dose measurement in an anthropomorphic phantom for a localized brain tumor treatment

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Funding: Fogarty International Center of the U.S. National Institutes of Health, award K01TW008409

Purpose: Cancer patients receiving radiation therapy are exposed to radiation doses outside of the treatment field that are not well understood. These out-of-field doses can lead to radiogenic late effects, for example second cancer. The purpose of this project was to determine the amount of dose received in the out-of-field regions for patients receiving radiotherapy for localized brain cancer.

Materials & Methods: We planned a radiotherapy treatment for an anthropomorphic phantom, as if it were a patient in our clinic with a localized brain tumor. The radiotherapy fields were delivered to the phantom after it was loaded with over 200 thermoluminescent dosimeters. This was performed in two phases, a high-dose phase and a low-dose phase. The TLDs were placed in all major organs, bone marrow, and on the skin, as well as surrounding tissues. The in-field dose was calculated by the clinic's treatment planning system and also measured with TLDs.

Results: The analysis of the extracted data highlights the importance of using measurement-based models rather than calculations from commercially-available treatment planning systems when estimating out-of-field dose. The measured data followed a double-Gaussian relationship between absorbed dose and distance from the field edge.

Conclusion: The model developed in this study may be used for estimating out-of-field dose in clinical and research studies for patients receiving localized radiotherapy for brain cancer. This information is useful for follow-up care of long-term survivors, in particular, in estimating the risk of developing second cancers and other late effects.

Paraproteinemic Maculopathy

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Keywords IgM; OCT; serous retinal fluid; Waldenström macroglobulinemia; multiple myeloma; hyperviscosity syndrome; serum viscosity; paraproteinemia; plasmapheresis; rituximab; intravitreal bevacizumab

ABSTRACT

Purpose: Paraproteinemia relates to monoclonal gammopathy producing pathologic antibodies with increased blood viscosity. Serous macular detachment is an uncommon ocular manifestation of paraproteinemia. We ascertain the clinical course of maculopathy in paraproteinemia and propose a mechanism for development and resolution of subretinal fluid based on various therapeutic modalities.

Methods: The records of patients with paraproteinemia with ocular coherence tomography (OCT) documentation of serous macular detachment were reviewed. Data collection included coexisting morbidity, rheology data (immunoglobulin level, hematocrit, and blood viscosity), clinical examination, as well as findings on OCT.

Results: A total of 33 cases were collected with 11 new and 22 previously reported in the literature. Diabetes was present in 7, systemic hypertension in 9, and anemia in 18. Mean initial immunoglobulin level was 6,497mg/dL and mean serum viscosity was 5.5cP. Mean logMar initial visual acuity was 0.55 (20/71) in the right eye and 0.38 (20/48) in the left eye. Final visual acuity was right eye 0.45 (20/56) and left eye 0.50 (20/63). Plasmapheresis was given in 18, chemotherapy in 30, blood transfusion in 2 and transplant of progenitor hematopoietic cells in 2. Oral rituximab was used in 10 patients. Immunoglobulin levels normalized in 8 patients and were unchanged in 1 patient after plasmapheresis and/or chemotherapy. Ocular therapy in 8 patients included vitrectomy in 1, laser photocoagulation in 4, intravitreal bevacizumab in 5, intravitreal triamcilonone in 2, intravitreal dexamethasone implant in 1, intravitreal rituximab in 1, and subtenon corticosteroid in 1. The maculopathy resolved partially or completely in 17 patients and worsened or remained unchanged in 14 patients over a median follow-up of 7 months. The maculopathy was unilateral in 9 cases. Maculopathy occurred at a lower initial immunoglobulin level in diabetics.

Conclusions: Paraproteinemic maculopathy can be unilateral. Decreasing the blood immunoglobulin level is the primary goal in the therapy of paraproteinemic maculopathy and this can be achieved by systemic route. Coexisting diabetes facilitates leakage of immunoglobulins at lower levels than non-diabetics. Despite systemic treatment of paraproteinemia, the long term visual prognosis in maculopathy remains guarded.

Perspective of Lebanese Oncologists on the Symptom Burden Among Adult Cancer Patients

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Keywords: Cancer Patients, Lebanese Oncologists, Symptom Burden

Description:

Background

Palliative oncology is a continuously growing multidisciplinary approach to symptoms among cancer patients. An essential component of palliative care remains the appropriate assessment of symptom burden in cancer patients. On the other hand, better delivery of palliative care services entails the awareness of oncologists about the epidemiology of patients' symptoms especially in our era where the trend is more toward unification of oncology and palliative care. Our study aims at assessing the Lebanese oncologists' point of view concerning the symptom burden among cancer patients of Lebanon and comparing their opinions to the real complaints of patients themselves in order to depict the level of awareness among Lebanese oncologists regarding cancer symptom burden.

Methods

A cross-sectional study was conducted among a representative sample of the Lebanese oncologists. A total of 35 physicians were overall interviewed. After giving oral consent to participate in the study, all study participants were asked to fill out a questionnaire regarding their demographics as well as the symptom profile of their patients. Specifically, they were asked to fill in the percentage of their patients suffering from each one of the physical symptoms listed and they were asked to name the most distressing symptom their patients suffer from.

The symptoms reported by physicians were ranked from the most distressing to the least distressing and the most distressing symptom that is reported by the largest number of physicians was revealed. Those results were compared to the ones obtained from our previous study about symptom profile as reported by patients and the discrepancy between the two profiles evaluated.

Results:

Fatigue was par excellence the symptom most our patients suffered from according to their physicians (64.167%). Also, a good percentage of physicians agree that patients suffer from appetite loss, pain, weight loss, and nausea). Less rated distressing symptoms included dry mouth, constipation, dyspnea, edema, taste change, cough, diarrhea, and skin symptoms. This is probably related to the fact that those symptoms are cancer type specific while the highly rated symptoms are more common to all types of oncologic diseases as well as the different treatment modalities. Among the symptoms that were rarely

reported by physicians to distress their patients, we can mention urinary symptoms, dysphagia, hoarseness, and early satiety. When compared to the patient reports of their own symptoms....

Conclusion:

When it comes to the Lebanese oncologists perspectives regarding the symptom burden profile of their cancer patients, it is non-debatable that overall they are aware of the overall distress their patients are suffering from which is represented by major fatigue and ill feeling. The more detailed awareness is still questionable as the concordance rate of the symptom reports by physicians and patients did not exactly match. We conclude that the issue of symptom burden should be more addressed in the plan of management of cancer patients by oncologists. This will help unveil one important barrier to optimal symptom management.

Prevalence of EBV Seropositivity in Multiple Sclerosis and Normal Individuals in the Lebanese Population

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Keywords: Multiple Sclerosis, Epstein-Barr Virus and Prevalence

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Multiple sclerosis (MS) is a chronic autoimmune disease characterized by a progressive inflammatory demyelination of the central nervous system, leading to neurological symptoms. The cause of MS is unknown but there are several risk factors that have been identified. The risk factors include genetics, smoking, low serum vitamin D, and history of infection with the Epstein-Barr Virus (EBV). EBV is a gamma-herpes virus that infects and remains dormant within B-lymphocytes throughout life. In western countries, around 90% of adult population and more than 99% of patients diagnosed with MS, were found to be infected with EBV. MS patients had increasing titers of antibodies against the EBV nuclear antigen EBNA1 before the onset of neurological symptoms. However, the prevalence of EBV infection in the Lebanese population is unknown. Given the high correlation between MS and EBV infection, and the increasing number of MS cases in Lebanon, we sought to determine the prevalence of EBV infection in the Lebanese population and whether we find an increased rate of EBV sero-positivity in MS patients as reported in western MS patients. In this pilot study we evaluated 216 MS patients and 247 healthy subjects. Frozen serum samples were tested for EBV viral capsid antigen (VCA) IgG antibody by chemiluminescent microparticle immunoassay (CMIA). Out of 130 MS samples tested so far, 129 samples are EBV VCA IgG positive (99.23%), while 23 out of 25 control samples are EBV VCA IgG positive (92%). Moreover, it was noted that control samples that were EBV VCA IgG positive, generally had a lower signal/cut off (S/CO) ratio compared to MS samples. This means that the controls have a lower antibody titer compared to MS patients. Results of the complete pilot study will be available shortly.

Retrospective evaluation of clinical characteristics and outcome of patients diagnosed with Neuroblastoma enrolled in the Children's Cancer Center of Lebanon (CCCL)

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Neuroblastoma (NBL) is the most common extra-cranial malignant tumor of childhood and accounts for around 10% of cancer-related death in childhood.

We present the characteristics of 37 patients treated at the multidisciplinary Children's Cancer Center at the American University of Beirut Medical Center, between April 2002 and December 2013. Median age at presentation was 29 months (range 0 - 168), and median follow-up from time of diagnosis was 14 months (range 0.5 - 97).

At presentation, 26 patients (70%) had stage IV disease (INSS staging system), 4 patients (11%) had stage III disease, 4 patients (11%) had stage II disease, and 3 patients (8%) had stage I disease. N-myc gene amplification in tumor tissue was tested in 24 patients (65%), and was found to be amplified in 6 of the samples. Based on the North American Children's Oncology Group disease risk stratification, 24 patients (65%) had high-risk neuroblastoma, and 23 were treated as per COG A3973 protocol, whereas 1 patient was treated on the European SIOP protocol. Of those 24 patients, 10 (42%) achieved a complete response (CR), whereas 4 (17%) patients achieved CR and then relapsed, 6 (25%) had progressive disease, 3 patients passed away due to infectious cause, and 1 patient was transferred to another hospital. Seventeen of the 24 patients (71%) received radiation therapy; reasons for not receiving radiotherapy in the remaining 7 patients included progressive disease, toxicity, or death of disease. Also, 75% underwent autologous stem cell transplantation as treatment consolidation, the rest were not qualified due to poor response to chemotherapy (n=6).

Seven patients (19%) had intermediate risk neuroblastoma, 4 of whom were treated as per COG A3961 protocol and all attained remission. Six patients (16%) had low risk disease, and all did well. Five were treated with surgical resection, while one patient received two cycles of chemotherapy. Currently, 23 patients (62%) are alive; by risk group, 6 (100%) patients with low-risk disease, 7 (100%) patients with intermediate risk, and 10 (42%) patients with high-risk disease, at median follow-up of 18.5 months (range 0.5 - 97), 30.6 months (range 8 - 60.5), and 12.6 months (range 5 - 79), respectively.

Keywords: neuroblastoma, characteristics, outcome

Role of Baseline Echocardiography prior to initiation of anthracycline-based chemotherapy in breast cancer patients

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Keywords: Breast Cancer, Anthracycline, Baseline Echocardiography

Description:

Background

Anthracycline adjuvant therapy has taken a particular role in the treatment of early stage breast cancer with an associated decrease in rates of both relapse and death. Although its efficacy has been unquestionable, it has been limited by their well-established risk of cardiac dysfunction. . Guidelines have emerged that would limit the maximum lifetime dose of anthracyclines and make a baseline assessment and periodic monitoring of cardiac function part of the routine practice. These precautionary practices are quite costly and cumbersome, and may condemn the patient to an unwarranted modification of his/her regimen. So the need for baseline echocardiography and periodic monitoring of left ventricular function in previously healthy and asymptomatic patients receiving anthracyclines becomes debatable. Our study aimed at assessing the incidence of abnormal baseline echocardiography in asymptomatic women with breast cancer prior to anthracycline therapy and establishing risk criteria associated with abnormal echocardiograms at baseline.

Methods

229 consecutive patients seen at AUBMC who had non-metastatic breast cancer, and had an echocardiography performed before starting anthracycline chemotherapy were studied. Data about demographic characteristics, tumor characteristics, relevant medical history, baseline echocardiography results, and change in clinical decision was collected. Patients with suboptimal ejection fraction on baseline echocardiography were analyzed for the prevalence of dyslipidemia, hypertension, CAD, diabetes, smoking, and morbid obesity among them. Results were compared to those among the overall study group using chi-square test. A p-value of ≤ 0.05 was used as reference for statistical significance.

Results:

98.7% were females with a mean age of 51.6 years. 55% of patients were post-menopausal. Around 70% of patients had stage I or II, and half had a grade 3 tumor. The majority of tumors were ER/PR positive

(72.2% ER positive and 63.9% PR positive) and 23.5% were her-2 overexpressing. All 229 of our patients received a baseline echo prior to initiation of anthracycline therapy. 15 (6.7%) of these patients had already some abnormality in wall motion but only 6 (2.7%) had a suboptimal ejection fraction (EF). Three of those, i.e. 1.3% had a change in chemotherapy regimen based on ejection fraction reading. Of the 6 patients with depressed EF, none had a prior diagnosis of coronary artery disease, only one patient had diabetes, one patient was a smoker, one patient morbidly obese, and one patient with dyslipidemia as compared to 3.5% of the overall study group with coronary artery disease, 21.6% with diabetes, 27.5% with hypertension, 35.8% smokers and 34.8% obese (BMI>30).

Conclusion:

The impact of the baseline echocardiography on the managerial decision of AUBMC oncologists has been minute. A more sensible and perhaps practical approach could limit the baseline echocardiography and periodic Ejection Fraction monitoring thereon after to patients who on breast cancer diagnosis, have symptoms of cardiac disease or are known to have an already depressed ejection fraction. Also, after starting anthracyclines, patients who newly develop cardiac symptoms would be eligible for echocardiography.

My position:

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Funding Source: No funding

Vitamin D levels in patients seeking Bariatric Surgery Consultation, a retrospective chart review

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Funding: Not needed

Abstract:

Introduction: Obesity is one of the most prevalent health problems today ⁽¹⁾. A Body Mass Index (BMI) greater than 35 kg/m² reduces life expectancy by two to four years, while severe obesity (BMI > 40) reduces life expectancy by 10 years⁽²⁾. Low vitamin D is prevalent in the morbidly obese⁽³⁾. Since the emergence of Weight loss surgeries as an effective tool for intentional and sustained weight loss ⁽⁴⁾, many studies have focused on the nutritional status of patients before and after the operation.

Aim: This Study looks into the prevalence of Vitamin D insufficiency/deficiency in patients presenting for bariatric surgery consultation.

Study Methods: This Study is a retrospective chart review of 976 patients who presented to the primary investigator's clinic for bariatric consultation from 2005 till 2013. In addition to Vitamin D levels, information about age, gender, BMI, past medical problems and previous bariatric surgeries were retrieved. Information was then subdivided into BMI categories and analyzed.

Results: The majority of patients seeking consultation (87%) had a BMI greater than 35 kg/m². Review showed that there is a female predominance for bariatric surgery consultations (56% vs 44%). Out of the 976 patients, 386 patients had pre-op Vitamin D levels (39.5%). 65% of those patients had levels ≤20 ng/ml (Vitamin D insufficiency) and 51% of those patients had levels ≤12 ng/ml (deficiency). Vitamin D levels appeared to be inversely related to BMI. Patients with BMI of 35-40, 40-50 and >50 Kg/m² had lower serum Vitamin D with levels averaging at 17.7, 17 and 14.4 ng/ml respectively than patients with BMI <35 kg/m² (22.2 ng/ml).

Conclusion: Our review showed that Vitamin D deficiency/Insufficiency is highly prevalent in patients presenting for bariatric surgery consultation and should therefore be an integral part of the screening and work up of patients planning to undergo intervention for weight loss.

Key words: Vitamin D, Bariatric Surgery, Obesity

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Vitamin D supplementation in children with asthma: a systematic review and meta-analysis

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Abstract

Background

Epidemiologic studies suggest an association between vitamin D deficiency and atopic diseases, including asthma. The objective of this study was to systematically review the benefits and harms of vitamin D supplementation in children with asthma.

Methods

We used standard Cochrane systematic review methodology. The search strategy included an electronic search in February 2013 of MEDLINE and EMBASE. Two reviewers completed in duplicate and independently study selection, data abstraction, and assessment of risk of bias. We pooled the results of trials using a random-effects model. We assessed the quality of evidence by outcome using the GRADE methodology.

Results

Four trials with a total of 149 children met eligibility criteria. The trials had major methodological limitations. For the asthma symptoms outcome, a meta-analysis of three trials found a standardized mean difference of 0.36 (95% confidence interval (CI) -0.66; 1.38; very low quality evidence). For the lung function outcome, a meta-analysis of two trials assessing post treatment FEV-1 found a mean difference of 0.54 liters per second (95% CI -5.28; 4.19; low quality evidence). For the vitamin D level outcome, a meta-analysis of three trials found a mean difference of 6.56 ng/ml (95% CI -0.64; 13.77; very low quality evidence).

Conclusions

The available very low to low quality evidence does not confirm or rule out beneficial effects of vitamin D supplementation in children with asthma. Large-scale, well-designed and executed randomized controlled trials are needed to better understand the effectiveness and safety of vitamin D in children with asthma.

Funding: None

White Matter Tract Development in Autistic Toddlers by Diffusion Tensor Imaging and Correlation with Ongoing Therapies

Principle investigator (PI):

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Keywords:

Autistic spectrum disorders: ASD

Diffusion tensor imaging: DTI

Applied behavioral Analysis therapy: ABA therapy

Verbal Behavior Milestones Assessment and Placement Program: VB-MAPP

Abstract:

Autistic spectrum disorders are neurodevelopmental disorders, characterized by impairments in three major domains: socialization, communication, and behavior. The prevalence of ASD is at an all-time high. Improved objective diagnostic tools and raised awareness have contributed to better ascertainment of cases. Up to a few years ago, no neuroimaging markers were specific for the diagnostic work-up of non-syndromic ASD. More recently, several studies have examined the white matter in ASD at both the macrostructural and microstructural levels. They found widespread white matter disruption that might be related to impaired white matter organization and abnormalities in myelination contributing to reduced connectivity in the central nervous system. Yet, conflicting results have been reported in the literature. What is now well-recognized is that a large number of these children, if diagnosed before the age of two and a half, and if they receive early intervention therapies to include speech, occupational, physical and applied behavior analysis, do quite well and become fully integrated into regular schools. We plan to examine 30 autistic children before and one year after institution of therapies and sixty age matched controls with diffusion tensor imaging to determine: a) The existence of early DTI markers for ASD; and b) the impact of early intervention and ABA therapies on outcome and white matter changes by DTI after one year. A potential benefit is the acquisition of an objective imaging measure for tracking the impact of emerging novel therapies in autism.

Funding source:

The proposal was approved for the spring cycle of the MPP research fund for year 2013.

Prepared By:

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Wilm's Tumor Treatment and Outcome at a Multidisciplinary Pediatric Cancer Center in Lebanon

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Abstract

Nephroblastoma (Wilm's tumor, WT) is the most common renal solid tumor in childhood and the fourth most common pediatric tumor. There is a wide difference in the outcome of patients with WT between developing and developed countries.

Objective: Review the clinical parameters and outcomes of patients with WT at the Children Cancer Center of Lebanon and identify prognostic determinants of outcome.

Methods: We have retrospectively reviewed the clinical records of patients with WT treated at our hospital between April 2002 and June 2013.

Results: Our study included 35 children with WT; 13 were males and 22 were females (with a male: female ratio of 0.59). The mean age at onset was 3.9 years with all patients below 16 years of age. There were 8 (22.8%) patients with stage I disease, 4 (11.4%) with stage II disease, 9 (25.7%) with stage III disease, 9 (25.7%) with stage IV disease and 4 (11.4%) with stage V disease or bilateral tumors. One patient passed away before staging or initiation of treatment, and therefore was excluded from further analysis. Treatment consisted of upfront surgery, when possible, followed by stage-specific chemotherapy, and radiation if needed (as per the North American NWTS protocols). At the time of the analysis, 32 (94.1%) of patients were alive and 30 (88.1%) patients were free of disease, at a median follow-up time of 56.9 months from diagnosis (range 5-124). Four patients had relapse of tumor, at a median time of 8.75 months (range 7.1- 12.2). Notably, all four patients who had relapsed had initial metastatic (Stage IV) disease.

Conclusion: The outcome of our patients with WT is similar to that in the developed world. Interestingly is the higher incidence of patients with bilateral malignancy which would need further studies at genetic and molecular levels.

Keywords: Outcome, multidisciplinary, nephroblastoma, treatment.

Fund Source: NA

ABSTRACTS

**Misc: Neuro, Infectious, Cardiovascular,
Metabolic/Diabetes, Vascular, Signaling,
Immunology, Inflammation, Other**

Antioxidant activity of Pomegranate Juice Reduces Lung Injury secondary to Acute and Chronic Cigarette Smoke Exposure in an Animal Model

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Abstract:

Cigarette smoke exposure (CSE) creates an increased oxidative burden in the lungs. Pomegranate (*Punica granatum* L.) Juice (PJ) possesses potent antioxidant activities that are attributed to its polyphenols. The study aims to determine the effects of PJ supplementation on the damaging effects of acute and chronic CSE in an animal model.

Methods: Male C57BL/6J mice were divided to four different groups: control, CSE, CSE + PJ and PJ. CSE groups received daily exposure, 5 days per week, for different time points (3 days, 1 and 3 months). PJ groups received daily 80 μ mol /kg while other groups received placebo. At the end of the experiments, different parameters were studied: a) oxidative stress (OS), c) histological evaluation of the lung by H&E, d) apoptosis using TUNEL assay, and e) expression levels of IL-1 beta, IL-6 and TNF-alpha by RT-PCR method.

Results: Acute exposure (3 days): A significant increase in OS, IL-1 beta, IL-6, TNF-alpha expression was noted in the CSE only group when compared to control. PJ significantly reduced OS and the expression of inflammatory mediators. TUNEL staining demonstrated significant apoptosis in CSE lungs, which was diminished in the CSE + 80 μ mol PJ group

Chronic exposure (3 months): Lung sections demonstrated multifocal alveolar emphysematous changes that were significantly reduced in the CSE + PJ group.

Conclusion: In this animal model, exposure to CSE resulted in lung injury. PJ supplementation attenuated the expression of inflammatory mediators observed in the acute CSE animal model and reduced emphysematous changes noted in the chronic animal model.

Keywords: Pomegranate, lung injury, smoke exposure and antioxidant.

Descriptive statement: the effect of Pomegranate juice in reducing the lung injury secondary to cigarette smoking exposure in mice

Funding source: The Medical Practice Plan and the University Research Board at the American University of Beirut.

Bradykinin and Thromboxane Receptors Positively Cooperate on the ERK1/2 Pathway in VSMCs and Co-internalize in Response to Bradykinin

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Abstract:

Following endothelial injury, the subsequent development of the atherosclerotic lesion is highly dependent on the proliferative and constrictive state of vascular smooth muscle cells (VSMCs). Individual agonist-induced activation of the G-protein coupled receptors, B2R [Bradykinin (BK) type-2 receptor] or TP [thromboxane (TX) receptor], leads to the successive activation of ERK1/2, which results in enhanced VSMCs proliferation. So far, the only known interface between BK and TX is the ability of the former, once bound to B2R, to activate the arachidonic acid/prostaglandin pathway and subsequently lead to increased thromboxane-A2 production in airway SMCs. However, the hypothesis that receptor-receptor interactions between B2R and TP might contribute to their co-regulation has never been addressed. Here, we investigate the crosstalk between TP and B2R both in VSMCs and in cells overexpressing these receptors. Our findings suggest a synergistic potentiation of ERK1/2 when VSMCs were co-stimulated with a minimal effective concentration of BK and a range of increasing concentrations of IBOP (TP stable agonist). ERK1/2 activation could be totally inhibited in VSMCs pretreated with the TP antagonist, SQ29548, prior to co-stimulation with the aforementioned combination of BK and IBOP. However, this could not be secondary to non-specific binding of SQ29548 to B2R as seen in HEK293Ts overexpressing B2R, pointing to the involvement of a possible physical interaction. This notion was reinforced when human B2R and TP α co-internalized in HEK293Ts overexpressing both receptors following their stimulation with BK. Such findings point to the formation of a possible B2R/TP heterodimer that might be modulating the signaling and trafficking properties of its cognate protomers. The likelihood of such hetero-dimerization would provide insights about the cooperative role of TX and BK in the vasculature providing a novel approach for more effective therapies targeted against vascular injury and atherosclerosis.

Keywords: Bradykinin, thromboxane, vascular smooth muscle cells, ERK1/2

Computationally Modeling DNA Mismatch Repair Mechanism (MMR) for Understanding Missed Defects: Phenotype in Congenital Heart Disease (CHD)

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Abstract

Congenital heart defects (CHD) are the most frequent form of major birth defects in newborns affecting close to 1% of newborn babies (8 per 1,000); atrial septal defects (ASD) and ventricular septal defect (VSD) are the most common types of CHD. Such defects often result from DNA damage, caused by either normal metabolic activities or environmental factors such as UV light and radiation, resulting in as many as 1 million individual molecular lesions per cell per day. The vast majority of DNA damage affects the primary structure, often causing errors of DNA synthesis during replication and missed by proofreading; these errors are a major source of mutation. Our problem of interest is to inspect the reasons behind preventing DNA mismatch repair (MMR) mechanism, which is the next level checkpoint after Proofreading, from fixing the erroneous insertion of 14 additional nucleotides into a single DNA strand; mutational case revealed as a phenotype in CHD. Is it failing to excise the defected single-stranded DNA segment and restore it according to the template strand, due to malfunction reaching the repair proteins? Or is it failing to recognize the defect due to the dual insertion of the 14-add-on into both strands, in a complementary deceptive mode?

Unfortunately, rectifying mechanisms such as proofreading and MMR are still not very well understood. This feeds into the high need of modeling the process of MMR after replication in its entirety (from error detection to error correction). Yet, since regulation plays a key role in controlling this mechanism, it is vital for the model to capture parameters in all biochemical pathways (such as binding free energy, protein concentration, mRNA expression level, type of interactions, etc.) giving rise to all associated quantitative measures.

In this work, we introduce a new Petri net model denoted as integrated static-dynamic Petri net (ISDPN) encompassing the dynamic behavior of MMR in homo sapiens after DNA replication, in addition of revealing the static structural properties of the repair network. ISDPN's goal is to integrate information

from various pathways within MMR. The model starts with a hierarchical modeling of the different regulatory, metabolic, and signaling networks as a Petri net, and continues with the analysis of the qualitative properties of the whole network using p-invariants and t-invariants analysis. It then computes a static quantitative measure (binding free energy), focusing next on reconstructing a quantitative mathematical model of the dynamics of MMR pathways, comprising the changes in compounds concentration and interactions, based on experimental data. The static and dynamic results characterize the net structure of MMR and give insights into the complex net behavior with time.

Two types of mutations are applied to the developed model; one with an induced mutation that is corrected by MMR and another with an induced 14-subsequence-insert. Comparisons of resulting dynamic changes between the two applications (repaired and presumably unrepaired), via network alignment techniques, are the used to infer about a possible defected behavior of MMR in the presence of the 14-subsequence-insert. Furthermore, the model can be extended to explain the phenotype of many well-characterized mutants thru varying concentrations and interaction responses.

Key-words: Congenital Heart Disease (CHD), DNA Mismatch Repair (MMR), DNA Replication, Binding Free Energy, Gene Regulatory pathway, Metabolic pathway, Signaling pathway, Concentration, Expression level, Petri-net model.

Control of Adult Neural Stem Cells (NSCs) by the Retinoblastoma Protein, pRb *in vitro*

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Keywords: Rb, adult neurogenesis, neural stem cells, neurosphere assay, differentiation

Introduction: During development, the tumor suppressor gene, pRb, regulates distinct aspects of neurogenesis including neuronal proliferation, differentiation and migration. We recently investigated the role of Rb in adult neurogenesis and found that it specifically regulates the rate of proliferation and differentiation of neural stem cells (NSCs) and progenitors found in the adult subventricular zone (aSVZ). Here, we examined the properties of Rb-null NSCs *in vitro* including their proliferation rate, self-renewal capacity and differentiation potential compared with Rb-wt NSCs. **Methods:** We induced a temporal deletion of Rb in adult NSCs in 6-8 week-old mice using a Nestin-CreERT2-YFP tamoxifen-inducible system and Rbfloxed/floxed mice. 5 days following treatment, we dissected and dissociated the adult SVZ tissue and performed neurosphere assays in culture. Cells were plated at low and high densities in media supplemented with FGF2 and EGF and cultured for 6 days before passaging. **Results: 1)** we assessed the number and the size of primary neurospheres derived from Rb-null NSCs versus Rb-wt NSCs and found ~ 1.5 fold increase in the number of primary neurospheres generated in the absence of Rb as well as a significantly larger size of neurospheres derived from Rb-null NSCs compared with wt-NSCs, **2)** we examined the self-renewal capacity of Rb-null NSCs by performing secondary and tertiary neurosphere assays and found ~ 1.6 fold increase in secondary neurospheres generated at day 7 in the absence of Rb compared with controls and, **3)** we tested the multipotency of Rb-null NSCs/progenitors by conducting differentiation assays and found a potential increase in Tuj1+ cells in culture derived from Rb-null NSCs as compared to controls. **Conclusion:** Rb controls the rates of proliferation and differentiation of NSCs/progenitors *in vitro*, thus loss of Rb leads to enhanced neurogenesis and accelerated differentiation of NSCs as seen previously *in vivo*. These findings have direct implications for the expansion and manipulation of NSCs in regenerative medicine in the future. *Supported by grants from URB and LNCSR.*

Position: Student

Crosstalk Between Bradykinin and Sphingosine-1-Phosphate Receptors in Vascular Smooth Muscle Cells Mediated Through Reactive Oxygen Species.

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Descriptive statement:

Bradykinin promotes vascular remodeling through the crosstalk activation of S1P receptors via ROS generation

Abstract:

Atherosclerosis is a silent chronic inflammatory disease that is a major cause of death worldwide. Due to the injury of endothelial cells aligning the aorta, the blood contents such as white blood cells, cytokines, growth factors and lipids interact directly with the smooth muscle cells of the vessel wall leading to vascular remodeling and lesion formation. Reactive oxygen species (ROS) generation in vascular smooth muscle cells activates signaling pathways, which contribute to vascular remodeling. We hypothesized that kallikrein-kinin system (KKS) especially bradykinin (BK) has a role in vascular remodeling via ROS generation, crosstalk with Sphingosine-1-Phosphate receptors (S1PR)s, and inducing the signaling pathway. When BK acts directly on the smooth muscle cells leading to the synthesis of ROS; ROS in turn activate signaling pathways like mitogen-activated protein kinases (MAPK)s and Phosphatidylinositol-3 kinase (PI3K). This was confirmed by using Nacetylcysteine (NAC), a scavenger of ROS that led to a significant decrease in extracellular signal-regulated kinase (ERK 1/2) and AKT. We also discovered that there is a crosstalk between bradykinin and S1PRs via sphingosine kinase 1 (SphK1) due to ROS generation by BK. On the other hand, when we used NAC, the ROS generation and the downstream signaling pathways of bradykinin 2 receptor (B2R) were inhibited, also CTGF, Fibronectin and (SphK1) gene expression was decreased. Besides, we verified that S1P activates MAPK and PI3K pathways by increasing ERK 1/2 and AKT respectively, which leads to proliferation and migration of smooth muscle cells. S1P also increases CTGF and Fibronectin production, which leads to the production of extracellular matrix (ECM) particularly by S1PR1. Our results suggest that BK induced ROS generation activates ERK 1/2 and AKT to promote vascular remodeling. Moreover, BK initiates a crosstalk between B2R and S1PRs (EDGRs) via activation of SphK1. On the other hand, S1P plays a role in vascular remodeling by activation of MAPK and PI3K pathways and also increases CTGF and Fibronectin production.

Keywords: Atherosclerosis, Vascular Remodeling, Bradykinin, Reactive Oxygen Species.

Funding Source: NIH Grant

Effect of Metabolic Memory on the DNA Methylation Status of Target Genes Involved in Vascular Complications in Type-1 Diabetes

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Descriptive statement: The aim of this project is to study epigenetic regulation of the phenomenon of glycemic metabolic memory which contributes to micro- and macrovascular complications in Diabetes

Background: Epigenetic alterations are known to be implicated in microvascular and macrovascular diabetic complications. Metabolic Memory is the phenomenon in which diabetic individuals that are exposed to high blood glucose, exhibit diabetic complications even after achieving normoglycemia. This memory results partially from mitotically inheritable changes in DNA methylation pattern on key genes involved in inflammation, oxidative stress, Cellular Fibrosis, Renin-angiotensin, Apoptosis, Migration and Proliferation.

Aim: Study epigenetic regulation of the phenomenon of metabolic memory which contributes to micro- and macrovascular complications in Diabetes.

Methods: In vivo studies will be done on 30 Male Sprague Dawley rats divided into Controls, Diabetics and Diabetics treated with Insulin. Type-1 Diabetes will be induced by a single injection of Streptozotocin at day 0 of experiment while control groups will be injected with Saline at day 0. Subcutaneous injection of insulin will be given twice daily for treated diabetic rats group. After end of treatment and exposure, rats will be sacrificed, and protein, RNA and DNA will be extracted from their Aorta (de-endothelialized), heart, liver, pancreas, and kidney cortex. In vitro studies will be done on primary rat aortic smooth muscle cells (RASMCs), divided into Controls, High Glucose (20 mM) and Osmotic pressure controls (Mannitol 20 mM). qRT-PCR will be used to assess the gene expression status for gene involved in Oxidative stress, Inflammation, Fibrosis, and Kinin System and its downstream targets. Bisulfite specific PCR followed by Methylation-Sensitive High-Resolution Melt (HRM) will be used to assess the variability in DNA methylation status of aforementioned processes.

Preliminary Results: qPCR results showed High Glucose treatment of RASMC for 4 weeks induces gene expression of Bradykinin receptor B1 (B1R), and B2 (B2R), Connective Tissue Growth Factor (CTGF), Fibronectin (FN1), and NADPH Oxidase 1(Nox1). However, it reduced Nox 4 gene expression.

Keywords: Type-1 Diabetes, Vascular Complications, Metabolic Memory, DNA Methylation **Funding source:** Qatar Foundation Grant

EGF Receptor Transactivation by Bradykinin receptor and Vascular Remodeling

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Descriptive statement: Bradykinin promotes vascular remodeling through the transactivation of EGF receptors via MMP2 activation

Abstract: Atherosclerosis is the leading cause of death in diabetes, and is a major source of morbidity and mortality. Early atherosclerotic lesions are characterized by endothelial dysfunction, accumulation of inflammatory cells, VSMC proliferation and migration, and extracellular matrix deposition in the vessel wall. Although the association of chronic hyperglycemia and dyslipidemia with diabetic micro-and macrovascular disease is well recognized, the factors and cellular signaling mechanisms that link hyperglycemia and dyslipidemia with atherosclerotic vascular disease are not fully defined. Both bradykinin and EGF receptor have been shown to promote vascular remodeling, but the cross-talk between the two systems has not been explored. Treatment of vascular smooth muscle cells (VSMC) with BK stimulated the mRNA levels of EGFR, Matrix Metalloproteases 2 (MMP2), MMP9 and NADPH Oxidase 4 (NOX4). On the other hand treatment of VSMC with EGF induced the mRNA levels of NOX1 and the expression of connective tissue growth factor (CTGF). Both BK and EGF stimulation resulted in the activation of p42/p44 MAPK and AKT. These findings are the first to demonstrate that BK can stimulate EGF expression and its receptor in VSMC through MMPs. Transactivation of EGF-receptor (EGFR) by G-protein coupled receptors (GPCRs) through MMP is emerging as an important pathway in cell proliferation, which plays a crucial role in the development of atherosclerotic lesion. Insights into the cellular mechanisms and interrelationships between BK and EGF may provide a novel mechanistic pathway through which both factors interact to promote vascular remodeling.

Keywords: Atherosclerosis, Vascular remodeling, EGF, Transactivation, Bradykinin, MMP

Funding Source: NIH Grant

Expression and Regulation of Connexins in Intestinal cells in an IBD model

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Keywords: Inflammation, Epithelial cells, Macrophage, Connexin, GJIC

Inflammatory bowel diseases (IBD) are related to functional impairment of intestinal epithelial cells (IECs) due to infiltration of the sub-mucosa by inflammatory cells. Three major mechanisms could be responsible for the induction of the impairment: (i) soluble mediators secreted by inflammatory cells, (ii) adhesion and signaling molecules expressed on the surface of immune cells or (iii) direct cytoplasmic exchange of mediators between the inflammatory cells and IECs via gap junction (GJ) channels. We studied the effect of pro-inflammatory mediators on connexin expression, and trafficking in human IECs (Caco-2 and HT-29). Further, we explored the nature of the interaction between IECs and macrophages (M ϕ), and identified the connexins (Cxs) involved in this communication. Live Cell Imaging is used to study trafficking of Cxs in IECs and the effects of inflammatory mediators on GJ coupling between IECs and between IECs and macrophages. Fluorescent dye transfer technique is utilized as a measure of homo- and hetero-cellular gap junction intercellular communication (GJIC). Western blotting and real-time PCR assays are performed to identify the different Cxs and to study the regulation of these Cxs under inflammatory conditions. Our data show that IECs and M ϕ express Cx26, Cx30, Cx43 and Cx45; however, Cx32 is only expressed in IECs. Cx 26 and Cx43 expression in IECs is up regulated upon treatment with inflammatory mediators and by adhesion to immune cells. In IECs and in IECs-M ϕ co-cultures, calcein is significantly transferred between cell types, suggesting the presence of functional gap junctional channels. Further, homo-cellular communication between IECs and hetero-cellular communication between IECs and M ϕ is increased under inflammatory conditions. These data show that Cx26 and Cx43 are involved in the gap junctional communication between IECs and M ϕ suggesting a role for gap junctional channels in the regulation of epithelial cell function in inflammatory bowel disease.

Participant: Sara Al-Ghadban (Research Assistant)

Kinin signaling in kidney glomerular cells: Role in Diabetic Nephropathy

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Descriptive statement:

Bradykinin enhances Diabetic Nephropathy by increasing connective tissue growth factor (CTGF) expression that is associated with increased expression of oxidative stress enzymes Nox 1 and Nox 4 and decreased expression of nephrin.

Abstract

Diabetic nephropathy (DN) is a major health epidemic and is the main cause of morbidity and mortality in diabetes. It is the most common cause of end-stage renal failure. A pivotal event initiated by DN is glomerular injury, characterized by mesangial matrix deposition and podocyte loss. Microalbuminuria, an early marker of DN, signifies high risk for progressive renal failure and is strongly correlated with glomerular injury. The risk factors and mechanisms involved in the pathogenesis of DN are still not completely defined. Previous data generated from our laboratory demonstrated novel mechanisms and functions of B2 kinin receptors in DN. Diabetic B2R^{-/-} null mice display reduced albumin excretion rate (AER), and reduced glomerular and tubular injury compared to diabetic B2R^{+/+} mice. In the current study we aimed to understand the cellular mechanisms through which activation of B2 kinin receptors contribute to the initiation and progression of DN. Stimulation of rat podocytes and mesangial cells with bradykinin (BK) resulted in increased expression of connective tissue growth factor (CTGF) and this effect is associated with increased expression of oxidative stress enzymes Nox 1 and Nox 4 and decreased expression of the mRNA levels for nephrin. In addition BK resulted in the phosphorylation of p42/p44 MAPK and AKT. These findings provide insights into novel aspects of B2 receptor signal transduction pathways and their functional significance in pathogenesis of DN and identify novel targets for interventional strategies.

Keywords: diabetic nephropathy, podocytes, mesangial cells, oxidative stress, nephrin, connective tissue growth factor.

Funding Source: NIH Grant

Knock-out of the bradyzoite marker *P18* in *Toxoplasma gondii*: insights towards a functional characterization during neurotoxoplasmosis

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Toxoplasma gondii is an apicomplexan protozoan parasite that infects all warm blooded animals including humans. *T. gondii* causes a severely morbid or fatal disease in fetus and immunocompromised patients. During its life cycle, *T. gondii* exhibits three morphologically infectious stages: tachyzoites, bradyzoites, and sporozoites. Tachyzoites are rapidly multiplying and responsible for the acute toxoplasmosis leading to tissue damage. Bradyzoites are slow-growing and responsible for the chronic neurotoxoplasmosis that reactivates in immunocompromised patients. Lastly, sporozoites are the infective forms found in oocysts. The back and forth switch between tachyzoite and bradyzoite stages is a key modulator of the progression of toxoplasmosis between acute and chronic phases. However, this switch remains very poorly understood. Here, we are investigating the role of the bradyzoite marker *p18* for which the gene sequence is annotated on www.toxoDB.org. Specifically, we have generated the $\Delta ku80\Delta p18$ knock-out parasites to elucidate the role of *P18* in host cell invasion and immune subversion. We have used the vector (P2854) containing the selectable marker cassette hypoxanthine-xanthine-guanine-phosphoribosyl-transferase (HXGPRT) and having in its multi-cloning sites, the unique enzymatic restriction sites HIND III, Apa I, Not I and Spe I. We have inserted the 5' flanking region of *p18* between HIND III and Apa I of P2854, whereas the 3' flanking region of *p18* was inserted between Not I and Spe I. After verification of the ligations by double enzymatic digestion and PCR, we could successfully generate the construct (5'*P18*-P2854-3'*P18*) harboring the 3' and 5' flanking regions of *p18*. We have then introduced this construct by electroporation into *Toxoplasma* $\Delta ku80$ strain which favors its integration by double crossing over and homologous recombination. We are currently testing these $\Delta ku80\Delta p18$ knock out parasites for their *in vitro* and *in vivo* capability to switch and reactivate.

Keywords: *Toxoplasma*, bradyzoite, $\Delta ku80\Delta p18$

Proteomic Profiling of Nuclei Isolated from LaminA/C-Deficient Mouse Embryo Fibroblasts by Performing a Differential Phage Display Screen

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Key words: lamins, phage display, muscular dystrophy

Presenter: Hind Zahr, M.Sc., Research Assistant, Department of Biology, AUB

Funding Source: AUB's University Research Board (URB), and Lebanese National Council for Scientific Research (CNRS)

Abstract: Laminopathies comprise a group of genetic disorders including skeletal and cardiac muscular dystrophy. They are caused by mutations in the *LMNA* gene which encodes for the nuclear lamina proteins laminsA/C that anchor other nuclear envelope (NE) proteins to the nuclear membrane. To date, the molecular mechanisms underlying the phenotypic diversity and the tissue-specific impaired function in laminopathies have not been deciphered. We rationalized that the phenotypic and mechanistic differences seen between laminopathies may result from varied expression, localization, and/or variably impaired function of a number of nuclear envelope proteins mediated by their interactions, or lack thereof, with wild-type or mutant lamin A/C resulting in differentially deregulated pathways that are tissue-specific. To address this hypothesis, we used a phage display - based approach to map NE proteomic heterogeneity in the context of laminopathies. We successfully employed a phage display - based technology termed Biopanning and Rapid Analysis of Selective Interactive Ligands (BRASIL) to identify proteomic differences in nuclei isolated from either wild-type mouse embryonic fibroblasts (MEFs) or lamin A/C-deficient MEFs. Multiple successive bio-panning rounds on nuclei derived from these cells allowed us to enrich and select for peptide sequences that exhibit strong and differential interactions. Work is underway utilizing a combination of molecular, bio-informatics, and biochemical methods to identify the natural ligands mimicked by the binding peptides and their corresponding receptors. Immunofluorescence and Western Blot analysis will then be used to assess the expression levels and sub-cellular localization of these ligand-receptor pairs to determine their bio-functional relevance to laminopathies. Studying the effects of lamin A/C alterations on the differential expression, localization, and impaired function of nuclear envelope proteins will offer new clues into the molecular mechanisms responsible for the phenotypic complexity of laminopathies including a better understanding of the causes for debilitating diseases such as dilated cardiomyopathy and Emery-Dreifuss muscular dystrophy.

Role of the retinoblastoma protein, pRb, in the development of the olfactory system

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Keywords: Rb, olfactory epithelium, olfactory bulb, neurogenesis, synaptogenesis

Introduction: The Retinoblastoma, pRb, is a tumor suppressor gene that plays important roles in brain development primarily by controlling cell division at the G1-S phase checkpoint. In addition, loss of Rb causes neuronal differentiation and migration defects in the developing brain. We investigated here the role of Rb in the development of the olfactory system (OS) which is comprised of the olfactory epithelium (OE) and the olfactory bulb (OB). We analyzed the layer organization inside the OS and we studied the development of the olfactory sensory neurons (OSN) and olfactory nerve layer (ONL) in the absence of Rb. To do this, we performed a conditional Rb deletion in the telencephalon and OS using Foxg1-Cre mice and Rb^{floxed/floxed} mice, and, used cresyl-eosin staining, immunohistochemistry and *in situ* hybridization. Then we studied the OS phenotype in Rb-null and control mice between E12.5 and birth. Neurogenesis and synaptogenesis in the OS are regulated by reciprocal interactions between the OE and the OB during development. We assessed both processes and found that loss of Rb leads to: 1) ectopic proliferation and enhanced neurogenesis in the OE and 2) aberrant migration and maturation of OSN associated with ectopic localization in the basal side of the OE 3) increased cell death in both the OB and OE with gradual degeneration of the latter around birth 4) axonal guidance defects affecting the olfactory nerve layer and leading to a gradual loss of connectivity between the OB and OE. Our data demonstrates that Rb is required for normal development of the olfactory system and emphasizes a novel role for this cell cycle protein during morphogenesis and the establishment of appropriate neuronal connections between different brain regions.

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Role of tumor suppressor p53 and Noxa in ceramide-mediated mitochondrial apoptosis

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Abstract

The study of tumor suppressor p53 and its relation to apoptosis has contributed greatly to the science of cancer as well as to cancer therapy. One of the mechanisms by which p53 induces apoptosis is through the pro-apoptotic sphingolipid, ceramide. The biochemical molecules that link p53 to ceramide in the apoptotic signaling pathway are still unclear. In exploring several potential mediators, the BH3-only pro-apoptotic protein, Noxa, protrude among other molecules as a chief player in p53-dependent ceramide accumulation. To further investigate the role of Noxa in the p53 and ceramide-dependent apoptosis, we have stably knocked down Noxa in Molt-4 (Human acute lymphoblastic leukemia cell-line) and HCT-116 (Human colorectal carcinoma cell-line) cells. Upon irradiation and exposure to Okadaic acid, Molt-4 and HCT-116 cells underwent a p53-dependent apoptotic response, respectively. Cells expressing both p53 and Noxa exit significantly the cell cycle into apoptosis unlike cells with silenced Noxa which were affected to a lesser extent. Interestingly, we have found that when Noxa was silenced, ceramide accumulation was abolished in our cell models. Tumor suppressor p53 induce apoptosis through different pathways one of these pathways is mediated through Noxa and ceramide. We concluded that p53 is fundamental for apoptosis but not enough to accumulate ceramide in both Molt-4 and HCT-116 cells. These results suggest that the trio p53, Noxa and ceramide mediate together an apoptotic pathway in response to irradiation and Okadaic acid. Further experiments will be done to elucidate the role of mitochondria in this pathway.

Keywords: p53, Noxa, ceramide, apoptosis

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The Effect of Aspirin and Clopidogrel on Bleeding, Platelets aggregation and Neuronal Damage after Moderate Traumatic Brain Injury

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Keywords: Platelets aggregation, Traumatic Brain Injury, Neuronal damage

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Funding Source: CNRSL

Background: Traumatic brain injury (TBI) is a major cause of mortality and morbidity around the world affecting nearly over 2 million persons with approximately 500,000 hospitalizations annually. In Lebanon, little is known about the incidence and mortality related to TBI; however, a Lebanese insurance company reported that 20% of people that had car accidents, suffered from head injury. Antiplatelet agents such as clopidogrel (Clopi) and Acetyl-Salicylic Acid- ASA (Aspirin) are essential adjuncts to the medical care of elderly patients with cardiovascular disease and have been co-administered. Clinical research studies showed that pre-injury antiplatelet and anticoagulant pharmacotherapy are associated with higher mortality rates especially in the trauma population. Clinical observations from our practice showed that among elderly patients receiving antiplatelet therapy with documented traumatic brain injury, intracranial hemorrhage have been significantly high and associated with alarmingly high mortality rate. **The overall aim** of this study is to investigate the effect of the co-administration of aspirin and clopidogrel on the bleeding mechanisms and systemic changes as well as neuronal injury after experimental rat model of brain injury.

Methods: Platelets were isolated from whole blood withdrawn from the rats' inferior venacava after isoflurane anesthesia 24 hours after intra-peritoneal injection or PO administration of Aspirin or Clopidogrel respectively. Arachidonic acid (AA 0.5 mM) or Adenosine Diphosphate (ADP) induced platelets aggregation (15 microM). Bleeding and systemic changes as well as neuronal injury in rats that

will receive either ASA or Clopidogrel or combination will be compared following a controlled cortical head injury of moderate intensity.

Results: Three different concentrations of ASA were examined; 10, 20 and 30 mg/ kg. The preliminary data showed that ip injection of 10 mg/Kg ASA to rats induced partial inhibition of platelet aggregation induced by AA, while 20 mg/Kg led to total inhibition of aggregation; however, a dose of 30 mg/kg induced bleeding. Therefore, the optimum concentration of 20 mg/kg will be used to prevent hemorrhage. The PO administration of 20 or 30 mg/Kg of clopidogrel led to nose bleeding with total inhibition of platelet aggregation induced by ADP. A dose of 10 and 15 mg/ kg will be tested in the future. Later, co-administration of both drugs will be attempted. Eventually, ASA, clopidogrel and combination of both drugs will be administered to normal and animals to receive head injury; platelets aggregation and extent of neuronal injury that will follow will be investigated.

Conclusion: Our anticipated findings will have an impact on the clinical treatment of older adults receiving combination of both drugs, at least on the level of drug prescription and dosage, by translating the findings into clinical research.

The effect of nitric oxide on the blockade of the norepinephrine transporter, uptake-1 by methylphenidate

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Methylphenidate is a blocker of the norepinephrine and the dopamine transporters, a pharmacological effect which makes it useful for the treatment of the most common behavioral disorder in children: attention deficit hyperactivity disorder (ADHD). This disturbance was defined by the American Psychiatric Association in 2000 as a debilitating disorder diagnosed on the basis of persistent and developmentally inappropriate levels of overactivity, poor behavioral organization, inability to sustain attention and concentration, and impulsivity. Methylphenidate improves this condition by enhancement of the extra-synaptic dopamine and norepinephrine levels in specific regions of the brain such as the striatum and the prefrontal cortex. Moreover, its low abuse potential has rendered it a more favorable therapeutic agent for ADHD therapy. In previous studies we showed that fresh synthesis of nitric oxide (NO) is needed for the transport across the norepinephrine transporter, uptake-1, in postganglionic sympathetic nerve terminals, of norepinephrine, a direct agonist of the adrenergic receptors, and tyramine which is an indirectly acting sympathomimetic amine that enters across uptake-1 to the sympathetic nerve terminals to release norepinephrine from the adrenergic vesicles, and also the blocking effect of cocaine, atomoxetine and imipramine on uptake-1. In this study, we explored the role of fresh synthesis of NO in the blockade of uptake-1 by methylphenidate (1.52 ± 0.4 mg/kg) in Sprague Dawley rats in which mean arterial pressure was measured. Synthesis of NO was blocked by Nw-nitro-L-arginine (L-NNA) and the rise in mean arterial pressure it induces was restored to starting level by an infusion of nitroglycerin, a NO donor. Norepinephrine (0.05, 0.1, 0.2 μ g) showed potentiation of its pressor effect after methylphenidate by 34%-56% and did not change further after methylphenidate, L-NNA and nitroglycerin. The pressor effect of tyramine (0.025, 0.05, 0.1 mg) was reduced by 78%-85% after methylphenidate and restored by 424%-658% after methylphenidate, L-NNA and nitroglycerin. The pressor effect of methoxamine, not a substrate of uptake-1, was not affected by pretreatment with methylphenidate, and methylphenidate, L-NNA and nitroglycerin. It is concluded that the blocking effect of methylphenidate on uptake-1 is confirmed and this blockade is significantly dependent on the fresh synthesis of NO.

Keywords: norepinephrine transporter, methylphenidate, attention deficit hyperactivity disorder, nitric oxide, norepinephrine, tyramine, methoxamine, Nw-nitro-L-arginine (L-NNA), nitroglycerin.

The effect of Vitamin D on the differentiation of Th17 cells

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Keywords: Th17, Vitamin D, Multiple Sclerosis

Funding source: CNRS

Background: Th17 cells play an important role in the pathogenesis of Multiple Sclerosis, a demyelinating neurodegenerative disease of the central nervous system, by the secretion of several pro-inflammatory cytokines. The active form of Vitamin D, 1 α ,25-dihydroxyvitamin D₃, has several anti-inflammatory properties. Studies have shown its negative effect on Th1 and Th17 responses and its positive effect on regulatory T-cells and Th2 cell responses. The aim of this study was to investigate the effect of 1 α ,25-dihydroxyvitamin D₃ on the differentiation of Th17 cells *ex vivo* in multiple sclerosis patients and healthy controls prior to and post vitamin D supplementation, and to characterize the phenotype of Th17 cells and its modulation by vitamin D.

Methods: Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Isopaque density gradient centrifugation from collected blood samples or Leukopacks obtained from healthy donors. Naïve CD4⁺ T helper cells were isolated by microbead negative selection. The cells were then cultured in Th17 polarizing conditions in the presence of the cytokines: rIL-6, rTGF- β , rIL-1 and rIL-23, in the presence or absence of 1 α ,25-Dihydroxyvitamin D₃ at a 10 nM concentration. On the 6th day of culture, Interleukin-17A production was assessed by flow cytometry and ELISA. The acquisition of Th17 cells was done on a fluorescence-activated cell sorter (FACS) analyzer.

Results: Preliminary results obtained from healthy controls show an effect for 1 α ,25-dihydroxyvitamin D₃ on Th17 polarization as measured by the frequency of IL-17⁺ Th cells by flow cytometry. The percentage of polarized Th17 cells decreased from 54.2% to 34.5% when a 10 nM concentration of Vitamin D₃ was added.

Conclusion: 1 α ,25-dihydroxyvitamin D₃ has a direct negative effect on the differentiation of Th17 cells and its addition to CD4⁺ naïve T cells in culture, in the presence of Th17-polarizing conditions decreases their differentiation into Th17 cells. Additional experiments are underway to characterize the phenotype of IL-17 secreting Th cells, and determine their expression of the regulatory transcription factor Foxp3.

The full 2D proteome analysis of SLC35b4 and its knockdown.

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Although type II diabetes (T2D) is the most common endocrine disorder, only 10 percent of this disease heritability has been identified. This derives an urge to further investigate the profile and pathway of genes involved in this disorder. SLC35b4 for instance is a solute receptor that has been recently associated with obesity, insulin resistance and gluconeogenesis by Yazbek et al using various genetic and functional studies. This intrigued further investigations in order to decipher the role of SLC35b4 solute receptor in obesity-induced type II diabetes. Thus the aim of this study was to obtain the whole proteome expression profile of SLC35b4 and to identify its subsequent effectors. This was accomplished by knocking-down SLC35b4 gene in HepG2 cells using siRNA, extracting the proteins from the cells and running 2D gel electrophoresis. Using this approach allows us to compare the whole proteome of the SLC35b4 knock-down to that of the control. Furthermore, MALDI TOF was used allowing the analysis of the pathways involved. This was done by peptide mass fingerprinting and de novo sequencing to identify proteins. Preliminary data indicates the involvement of the MAFA gene in the phenotypic effect of SLC35b4. The mapping of molecular and downstream pathways of the solute receptor will allow the development of a hypothesis on the mechanism of action of this protein and set the experiments necessary to discover new therapeutic targets for hyperglycemia, which may lead to clinical trials and alternate treatment regimens. Considering the spike in obesity-induced type II diabetes incidence in Lebanon and the region, this project is of great importance as it is a possible doorstep to finding new treatment options to lower blood sugar levels and avoid complications of T2D.

Keywords: SLC35b4, obesity-induced type II diabetes (T2D), expression profile.

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