Cardiologist/Cardiovascular Disease

CONTROL ID: 1315075

INHIBITION OF PRE-B CELL COLONY-ENHANCING FACTOR (PBEF) PREVENTS AND REVERSES MONOCROTALINE-INDUCED PULMONARY HYPERTENSION


CURRENT CATEGORY: Cardiology/Cardiovascular Disease

ABSTRACT BODY: Rationale: We have previously shown that PBEF, a cytokine with extracellular proinflammatory cytokine-like activity and intracellular enzymatic activity as a phospholipase D2, which regulates intracellular NAD levels and cellular redox state, regulates histone deacetylation and has anti-apoptotic activity, is significantly upregulated in patients with pulmonary arterial hypertension. We sought to determine whether inhibition of the cytokine and enzymatic activities of PBEF could prevent and reverse monocrotaline (MCT)-induced pulmonary hypertension (PH) in rats. In addition, we hypothesized that PBEF could affect calcium signaling pathways, which play a role in cell proliferation and vascular constriction.

Methods: Male Sprague-Dawley rats (n=6 per group) received one dose of monocrotaline (MCT) (60mg/kg, IP). In the prevention experiments, rats were simultaneously administered FK866 (an inhibitor of PBEF enzymatic activity) (2.5 mg/kg, IP, twice daily for 2 weeks) or recombinant goat anti-PBEF antibodies (20mg/kg, IP, daily for 2 weeks). In the reversal experiments, the same doses frequency and duration of FK866 or PBEF antibodies were given to rats two weeks after MCT. Right ventricular systolic pressure (RVSP) was determined with a pressure transducer catheter. The right ventricle: left ventricle + septum (RV/LV+S) ratio was calculated. In a cell culture model, human pulmonary arterial smooth muscle cells (PASMC) were stimulated with recombinant PBEF (25ng/ml) for 6 hrs and 48 hrs. [Ca2+]cyt was measured in a fluorescence microscope and cytoplasmic acid (CPA, a specific Ca2+-ATPase inhibitor) was used to induce store-operated calcium entry (SOCE).

Results: Administration of FK866 or PBEF antibody prevented the development of PH (RVSP [mmHg] 21.04 ± 1.77 vs. 39.35 ± 3.24 [MCT] vs. 23.20 ± 0.95 [MCT+FK866] vs 20.70 ± 1.43 [MCT+PBEF Abs], p<0.05) and right ventricular hypertrophy (RVH) (RV/LV+S 0.31 ± 0.04 vs. 0.40 ± 0.05, p<0.05). In the reversal experiments, administration of FK866 or PBEF antibody reversed established PH (RVSP [mmHg] 19.77 ± 0.80 [control] vs. 34.45 ± 3.49 [MCT+FK866] vs 33.86 ± 5.00 [MCT+PBEF Abs], p<0.05) and RVH (0.25 ± 0.001 vs. 0.60 ± 0.019 or 0.43 ± 0.022 vs. 0.40 ± 0.022, p<0.01). In normal pulmonary artery smooth muscle cells (PASMC), short (6 hrs) and long (48 hrs) treatment with recombinant PBEF enhanced PASMC proliferation. Inhibition of PBEF prevented and reversed MCT-induced PH. PBEF may play a role in vascular remodeling via regulation of calcium signaling pathways. These data suggest that PBEF inhibition could be a potential therapeutic target for PH.

CONTROL ID: 1315130

SPHINGOSINE KINASE 1 DEFICIENCY PROTECTS RODENTS FROM CHRONIC HYPOXIA-MEDIATED PULMONARY HYPERTENSION


CURRENT CATEGORY: Cardiology/Cardiovascular Disease

ABSTRACT BODY: Rationale: Sphingosine kinases (SphKs) play a role in cell proliferation and vascular constriction. Inhibition of PBEF prevents and reverses MCT-induced PH. Under normoxia RVSP and RV/LV+S did not differ between SphK1-deficient mice (SphK1KO) and C57Bl6 wild-type littermates (WT). We investigated whether inhibition of PBEF could prevent and reverse monocrotaline (MCT)-induced pulmonary hypertension (PH) in rats. In addition, we hypothesized that PBEF could affect calcium signaling pathways, which play a role in cell proliferation and vascular constriction.

Methods: Male Sprague-Dawley rats (n=6 per group) received one dose of monocrotaline (MCT) (60mg/kg, IP). In the prevention experiments, rats were simultaneously administered FK866 (an inhibitor of PBEF enzymatic activity) (2.5 mg/kg, IP, twice daily for 2 weeks) or recombinant goat anti-PBEF antibodies (20mg/kg, IP, daily for 2 weeks). In the reversal experiments, the same doses frequency and duration of FK866 or PBEF antibodies were given to rats two weeks after MCT. Right ventricular systolic pressure (RVSP) was determined with a pressure transducer catheter. The right ventricle: left ventricle + septum (RV/LV+S) ratio was calculated. In a cell culture model, human pulmonary arterial smooth muscle cells (PASMC) were stimulated with recombinant PBEF (25ng/ml) for 6 hrs and 48 hrs. [Ca2+]cyt was measured in a fluorescence microscope and cytoplasmic acid (CPA, a specific Ca2+-ATPase inhibitor) was used to induce store-operated calcium entry (SOCE).

Results: Administration of FK866 or PBEF antibody prevented the development of PH (RVSP [mmHg] 21.04 ± 1.77 vs. 39.35 ± 3.24 [MCT] vs. 23.20 ± 0.95 [MCT+FK866] vs 20.70 ± 1.43 [MCT+PBEF Abs], p<0.05) and right ventricular hypertrophy (RVH) (RV/LV+S 0.31 ± 0.04 vs. 0.40 ± 0.05, p<0.05). In the reversal experiments, administration of FK866 or PBEF antibody reversed established PH (RVSP [mmHg] 19.77 ± 0.80 [control] vs. 34.45 ± 3.49 [MCT+FK866] vs 33.86 ± 5.00 [MCT+PBEF Abs], p<0.05) and RVH (0.25 ± 0.001 vs. 0.60 ± 0.019 or 0.43 ± 0.022 vs. 0.40 ± 0.022, p<0.01). In normal pulmonary artery smooth muscle cells (PASMC), short (6 hrs) and long (48 hrs) treatment with recombinant PBEF enhanced PASMC proliferation. Inhibition of PBEF prevented and reversed MCT-induced PH. PBEF may play a role in vascular remodeling via regulation of calcium signaling pathways. These data suggest that PBEF inhibition could be a potential therapeutic target for PH.

CONTROL ID: 1310574

VARIANTS IN THE 3' UNTRANSLATED REGION OF THE KCNQ1-ENCODED KV.7.1 POTASSIUM CHANNEL MODIFY DISEASE SEVERITY IN PATIENTS WITH TYPE 1 LONG QT SYNDROME IN AN ALLELE-SPECIFIC MANNER

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CURRENT CATEGORY: Cardiology/Cardiovascular Disease

ABSTRACT BODY: Introduction: Heterozygous mutations in KCNQ1 cause type 1 long QT syndrome (LQT1), a disease characterized by a prolonged heart rate-corrected QT interval (QTc) on ECG and an increased risk of life-threatening arrhythmias. It is unknown why disease penetrance and expressivity is so variable between individuals hosting identical LQT1-causative mutations. Here, we hypothesize that the variable penetrance and expressivity observed in LQT1 can be explained by single nucleotide polymorphisms (SNPs) in the 3'untranslated region (3'UTR) of KCNQ1, which create de novo target sites for cardiac microRNAs (miRNAs) resulting in the microRNA-mediated suppression of KV.7.1 channel expression.

Methods: 168 LQT1 cases (96 females, mean age 36 ± 22 yrs, mean QTc 449 ± 30 msec, range QTc 354 to 592 msec) were genotyped for SNPs in the 3'UTR of KCNQ1 using direct DNA sequencing. The miRanda algorithm was used to predict in silico whether miRNA target sites were created by the 3'UTR SNPs detected in LQT1 cases. Next, miRNA overexpression, anti-miR knock-down, and standard dual-reporter luciferase assays were performed in COS-7 cells and neonatal rat cardiacmyocytes to investigate the effects KCNQ1 3'UTR SNPs on allelic expression in vitro. Finally, allele-specific qPCR and western blotting were used to assess KCNQ1 miRNA and KV.7.1 protein levels in human myocardial necropsy samples.

Results: In total, six SNPs were found in the KCNQ1 3'UTR. The minor variants of SNP rs2319184 (A) and SNP rs8234 (G) were predicted to create
Fifty-seven of these patients (25%) tested positive for cocaine by urine drug screen. The presence or absence of occlusive coronary disease was determined by cardiac catheterization or non-invasive stress testing. Forty-three patients (75.4%) were determined to have occlusive coronary disease. Comparisons between patients with and without occlusive coronary disease were made based on information at admission including demographics, co-morbid conditions, medications, family medical history and baseline laboratory data. Acute coronary syndrome patients with occlusive coronary artery disease were more likely to have a chest pain description of typical angina (31 vs 12, P < 0.05), EKG finding of T-wave inversion >=0.3mV (14 vs. 9, P=0.014), and troponin elevation (p=0.02). No other baseline characteristics were helpful in predicting presence of occlusive CAD.

Conclusions: In patients suspected to have cocaine associated acute coronary syndrome, presence of typical angina, T wave inversion on EKG and positive cardiac enzymes help identify patients who are more likely to have occlusive CAD. Presence of these features should help identify patients who deserve further evaluation and promote proper utilization of resources.

CONTROL ID: 1314007
NOX1 NADPH OXIDASE IS NECESSARY FOR LATE, BUT NOT EARLY, ISCHEMIC PRECONDITIONING AGAINST MYOCARDIAL INFARCTION IN MICE
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CURRENT CATEGORY: Cardiology/Cardiovascular Disease
ABSTRACT BODY: Ischemic preconditioning (IPC) is an adaptive mechanism providing cardioprotection against subsequent ischemia. Although it is widely accepted that reactive oxygen species (ROS) trigger ischemic preconditioning, the source of ROS and mechanisms of cardioprotection during the early phase (1-2 hours) and late phase (12-24 hours) of IPC is incompletely understood. This study was designed to investigate the role of the Nox1 NADPH oxidase in early and late IPC. WT and Nox1 deficient (ko) mice underwent three cycles of 4-min of coronary artery occlusion and 4-min reperfusion. Mice in the early IPC group were then immediately subject to 30-min of coronary artery occlusion, whereas mice in the late IPC group recovered for 24 hours prior to being subjected to 30-min coronary occlusion. Mice subject to 30-min coronary occlusion without IPC served as controls. Twenty-four hour later, the WT and Nox1 ko mice in the early IPC group showed similar protection in myocardial infarct (MI) size, as measured by triphenyltetrazolium (TTC) staining (control 33% vs WT early IPC 17% vs Nox1 ko early IPC 13%). In contrast, although WT mice in the late IPC group were also protected from MI, the Nox1 ko mice in the IPC group had similar infarct size as controls (controls 33% vs WT late IPC 18% vs Nox1 ko late IPC 27%). Echocardiography confirmed these results. In addition, although Nox1 is not typically detected in the myocardium, we observed that the expression of Nox1 increased after IPC. The mechanism of cardioprotection by late IPC has been reported to involve tumor necrosis factor (TNF)-alpha-dependent activation of nuclear factor kappa B (NF-kB). We found that late IPC increased TNF-α levels and NF-kB activation in myocardium of WT but not Nox1 ko mice. Finally, siRNA knockdown of Nox1 in cultured neonatal cardiomyocytes abolished the protective effect of IPC. Hypoxia-induced apoptosis. In summary, expression of the Nox1 NADPH oxidase is increased in response to IPC and necessary for inducing TNF-α and NF-kB mediated late cardioprotection.
eventual loss of sinus rhythm as detected on screening ECGs performed at 12-16 weeks post birth. Surface ECG was suggestive of atrial fibrillation and no atrial contractions were evident by pulse wave Doppler. A computational analysis of target messenger RNAs using the target prediction algorithms, PicTar, Microcosm, and TargetScan, revealed several genes with known functions in cardiac conduction and arrhythmogenesis including, connexin-43, desmocollin-2, and neuregulin. Western blot analysis of adult hearts confirmed significantly reduced protein levels of these genes. Immunofluorescence staining revealed a near complete loss of connexin-43 in both the atrial and ventricular tissues compared to non-transgenic matched control hearts.

CONCLUSIONS: Taken together, miR-130a is an important regulator of normal cardiac conduction via regulation of genes such as connexin 43, desmocollin-2 and neuregulin. Dysregulation of miR-130a may be important in the development of a variety of cardiac arrhythmias.

CONTROL ID: 1344875
OXIDIZED CA2+ AND CALMODULIN-DEPENDENT PROTEIN KINASE II CAUSES SINUS NODE DYSFUNCTION AND INCREASED ACUTE POST-MYOCARDIAL INFARCTION MORTALITY IN DIABETIC MICE
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CURRENT CATEGORY: Cardiology/Cardiovascular Disease
ABSTRACT BODY: Diabetes is a risk factor for sudden death after myocardial infarction (MI) and multiple studies have demonstrated that diabetic patients are twice as likely to die from MI compared to non-diabetic patients. However, mechanisms underlying the increased mortality in diabetic patients are unknown. We found that streptozotocin-treated diabetic mice were twice as likely to die after MI surgery as vehicle-treated control mice, mimicking the increased risk after MI seen in diabetic compared to non-diabetic patients. These mice exhibit profound sinus bradycardia and chronotropic incompetence after MI despite of comparable contractile function to non-diabetic mice and absence of cardiac wall rupture or tachyarrhythmia. Diabetic hearts ex vivo also show sinus node dysfunction (SND) with decreased heart rate, prolonged sinus node recovery time and blunted response to isoproterenol.

Oxidation of the Ca2+ and calmodulin-dependent protein kinase II (CaMKII) in sinoatrial node (SAN) cells was shown to cause SND by increasing apoptosis and fibrosis of SAN in Angiotensin-infused mice. Increased ROS is consistently reported after MI and in the hearts of diabetic patients and in animal models of diabetes. We found that ox-CaMKII was significantly increased in right atrial tissues from diabetic patients and mice. We created a knock-in model of diabetes-resistant CaMKIIβ (CaMKIIβMM-Val281/282), in which Met residues are replaced by Val residues in the dominant CaMKII isoform in heart. Diabetic, streptozotocin-treated CaMKIIβMM-Val281/282 mice, and mice with transgenic myocardial and SAN cell expression of a synthetic CaMKII inhibitory peptide (AC-3-I) were protected from increased mortality after MI and SND. The increase in SAN ox-CaMKII expression in diabetes is likely a result of increased mitochondrial ROS triggered by hyperglycemia, as MitoTEMPOL treated diabetic mice showed similarly improved survival after MI and SND, whereas p47phox lacking a critical component of NADPH oxidase and infused with streptozotocin were not protected from mortality after MI. Our findings are consistent with a pathway where hyperglycemia and MI triggers increased mitochondrial ROS, leading to excessive ox-CaMKII enhanced mortality due to SND.

CONTROL ID: 1315198
A NEW COMPUTER BASED METHOD USING Bipolar ELECTROGRAM CHANGES TO DETECT MYOCARDIAL ISCHEMIA
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CURRENT CATEGORY: Cardiology/Cardiovascular Disease
ABSTRACT BODY: Ischemia is the most common cause of ventricular tachycardia and fibrillation (VT/VF). In a model of VT/VF in the dog we have investigated whether bipolar electrograms (BE) changes of reduction of dV/dt or prolongation of BE duration could correctly predict ischemia measured by standard voltage drop (45-55%) and VT/VF origins of epicardial entry and endocardial foci.

13 open chested dogs had 60 BE, filtered 3-1300Hz, with recordings before and 20 and 60 minutes after coronary artery occlusion (CAO) with 3-D mapping identifying reentry or foci of VT/VF. Automated measurements were performed by custom programming using MatLab 7.11. First, the maximum dV/dt was selected within each BE QRS, guided by the surface ECG. Next, BE duration was calculated by choosing BE onset and offset at the isoelectric line in front of and behind the max dV/dt with both returning to within 25% of the average slope magnitude. The maximum BE voltage drop for the entire QRS was calculated for the BE duration in control and after CAO with BE voltage reduction as gold standard from multiple prior studies. All measurements made by the algorithm were confirmed by hand.

With VT/VF total sites of origin (172) were correctly delineated as ischemic by voltage reduction in 99 and by max dV/dt in 105, but only 69% (p=0.05) by duration. Using either voltage or max dV/dt reduction, reentry origins were correctly identified as ischemic in >80%, but focal origins were ischemic in only 50%. For data recorded at 60 min post occlusion in all experiments, of 373 BE showing ischemia by voltage reduction, only 47% were also designated ischemic by 45-55% prolongation, but 95% were also designated by 45-55% reduction of max dV/dt during atrial pacing. Thus while specificity of the latter two were equal at .82, sensitivity of dV/dt was .94 while duration was .64. Similar performance results were observed in BE at 20 min post occlusion. During ventricular pacing voltage reduction was equally effective as was max dV/dt but BE delay was not.

These results show that 45-55% reduction of max dV/dt correctly indicated ischemic sites after coronary occlusion, occasionally even when voltage reduction did not. Since focal sites were identified as ischemic by voltage drop and dV/dt reduction criteria in only 50% of cases, the results suggest induction may arise from other mechanisms. This automated measurement may be able to correctly identify ischemia in BE such that ischemic VT/VF could be correctly inferred, suggesting therapy to improve heart perfusion in patients with cardiac devices.

CONTROL ID: 1235618
SERUM 2-METHOXYEstradiol, AN ESTROGEN METABOLITE, IS POSITIVELY ASSOCIATED WITH SERUM HDL IN A POPULATION-BASED SAMPLE
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CURRENT CATEGORY: Cardiology/Cardiovascular Disease
ABSTRACT BODY: Purpose: Serum high-density lipoprotein (HDL) is inversely associated with coronary artery disease, ischemic stroke, and atherosclerosis in men and women. Postmenopausal women have lower HDL than men and HDL has been shown to inhibit HMG-CoA reductase in vitro. Because such inhibition can lead to increased serum HDL, we hypothesized that serum 2-MeOE2 would be positively associated with serum HDL.

Methods: Data for this study were gathered in the fifth year of the Chicago Health, Aging, and Social Relations Study (CHASRS), which is a population-based longitudinal study designed to examine the relationships between psychosocial characteristics and health outcomes among middle-aged and older adults. Participants completed surveys regarding demographic characteristics and use of antihyperlipidemic agents, including HMG-CoA reductase inhibitors (statins), niacin, bile acid sequestrants, and cholesterol absorption inhibitors. In addition, serum was analyzed for estradiol and 14 estradiol metabolites (EM) using mass spectrometry. EM concentrations exhibited a positively skewed distribution and were therefore subjected to natural log (ln) transformation.

Results: 51 men and 51 postmenopausal women participated in this phase of the study. Four women were excluded because they were taking hormone replacement therapy. Preliminary analysis revealed no correlation between 2-MeOE2 and serum HDL in men so the current analysis includes only women (N = 40) with no missing demographic, medication, EM, or lipid profile data. Mean age was 57 years; 49% were white, 23% were African-American, and 28% were Latina. The mean body mass index (BMI) was 32 and 33% of the women were taking antihyperlipidemic medications. The mean lipid values (mg/dL) were: total cholesterol (205.6), HDL (56.2), LDL (117.8), and triglycerides (163.0). Correlational analysis revealed a sizeable

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positive relationship between serum in 2-MeOE2 and serum HDL (r = 0.31; p = 0.05). Neither serum estradiol nor any other EM was significantly associated with serum HDL. Multivariate regression analysis showed that in 2-MeOE2 retained a positive association (β = -0.26, p = 0.043) with serum HDL when age, BMI, race/ethnicity and antihypertensive medications were held constant. In the same model, antihypertensive medications were also positively associated (β = 0.307, p = 0.047) with serum HDL.

Conclusions: Consistent with our hypothesis, serum 2-MeOE2 was positively associated with serum HDL in women. However, this association was not present in men. Because this was a cross-sectional study, prospective analyses are needed to determine if increased 2-MeOE2 leads to increased serum HDL. A better understanding of this relationship could lead to new strategies for raising serum HDL and reducing the risk of cardiovascular disease, especially among postmenopausal women.

CONTROL ID: 1344908
CAMKII IN THE TRANSITION FROM PRESSURE-OVERLOAD CARDIAC HYPERTROPHY TOWARDS HEART FAILURE IN THE MOUSE
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CURRENT CATEGORY: Cardiology/Cardiovascular Disease
ABSTRACT BODY: Background: The Ca2+-/Calmodulin dependent protein kinase II (CaMKII) is involved in many cardiac pathologies such as myocardial infarction, heart failure and arrhythmogenesis. Expression and activity of CaMKII are increased in cardiac hypertrophy as a cause of short-term pressure-overload and in heart failure (HF) where decreased myocyte contractility and disturbed intracellular Ca2+ handling are at least in part due to overactive CaMKII. Chronic pressure-overload results in HF, but little is known about the transition from cardiac hypertrophy to HF.
Aim: The aim of our approach was to study the role of CaMKII in the transition from pressure-overload cardiac hypertrophy to heart failure.
Methods: Wildtype female C57BL/6 mice between 4 and 5 months of age were subjected to transaortic constriction surgeries (TAC) by a minimally invasive approach using a 27-gauge-needle for constriction. Continuous investigations were conducted 7, 21 and 35 days after surgery (a.s.) (for protein analyses 7, 29, 45 days a.s.). We measured cardiac hypertrophy, morphological characteristics by echocardiography, protein expression, and myocyte function and Ca2+ handling using epifluorescence microscopy.
Results: NK2a revealed 7 days a.s. increased heart-weight/body-weight (HW/BW) ratios (in mg/g for TAC vs. sham: 10.4±0.4 vs. 7.9±0.2; N=31 and 20; P<0.01). Cardiac hypertrophy achieved a maximum 21 days a.s. (HW/BW: 12.0±0.7 vs. 8.0±0.2; N=27 vs. 23; P<0.01). Likewise, significant myocyte growth occurred after TAC which in the later course was dominated by significantly increased cell length. Echocardiography revealed concentric hypertrophy in TAC 7 days a.s. with a significant thickening of the interventricular septum (in mm: 1.05±0.03 vs. 0.94±0.02, N=5 and 4, P<0.05), and unchanged left ventricular dimensions and preserved fractional shortening. In TAC in the later course (39 days p.a.) dilatation of the left ventricle occurred (LVEDD in mm: 2.16±0.09 vs. 1.32±0.05; N=4 and 3; P<0.01), while fractional shortening was reduced (in %: 41.1±3.2 vs. 54.1±0.3; N=4 and 3; P<0.05) compared to sham. CaMKII expression was increased by 44 % in TAC both 7 days (N=4 vs. 5; P<0.05) and 21 days a.s. by 18%, but 35 days a.s. normalization of CaMKII expression occurred in TAC. SERCA2A expression gradually decreased in TAC, and 45 days a.s. it was significantly reduced by 42%, while expression of Phospholamban (PLB) steadily increased. At basal stimulation (1Hz) myocytes from TAC isolated 7 days a.s. revealed an increased cell shortening (in %: 4.94±0.3 vs. 4.01±0.4; N=44 and 36; P<0.05) and Ca2+ transients (F/F0: 2.53±0.1 vs. 2.24±0.1; N=44 and 36, P<0.05). In the course both shortening and Ca2+ transients decreased to control levels. Concerning relaxation a gradual deceleration occurred in the course with a significantly slowed relaxation in TAC vs. sham 35 d a.s. (RT 80% in ms: 150±10 vs. 100±10; N=33 and 45; P<0.01), but unaltered function of the NCX.
Conclusions: Our model reflects a course from a pressure-overload cardiac hypertrophy with preserved cardiac function and myocyte contractility to a dilatation with decreased cardiac function and impaired Ca2+ cycling. Here, CaMKII expression is increased in early cardiac hypertrophy which is associated with increased cell shortening and Ca2+ transients in early stage of hypertrophy. Interestingly, a normalization of both occurs in the further course suggesting that CaMKII and its function undergo dynamic alterations in the transition from pressure-overload hypertrophy to HF.

CONTROL ID: 1311702
POSTURAL ORTHOSTATIC TACHYCARDIA SYNDROME: PATTERNS OF BLOOD PRESSURE & HEART RATE RESPONSE TO TILT TEST IN RELATION TO ANXIETY SYMPTOMS
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CURRENT CATEGORY: Cardiology/Cardiovascular Disease
ABSTRACT BODY: Postural Orthostatic Tachycardia Syndrome (POTS) is defined as a Head-Upright Tilt Test (HUT), severity of anxiety symptoms may correlate directly with incidences of hemodynamic changes (especially POTS), & inversely with the time to those episodes, while undergoing testing.
Patient Population: 199 pts (57 males, 142 females; mean age of 39.61±14.1 years old with recorded Beck Anxiety Indexes (a metric of anxiety severity that looks at 21 symptoms of anxiety, each graded on a four-point likert scale) (BAI) underwent HUT in a single center. All pts were included in the study & were subdivided by symptom & total HUT time.
Methods: Beck Anxiety Indexes: Surveys were administered to pts prior to undergoing HUT to gauge anxiety levels prior toHUT. In patients (pts) undergoing HUT, severity of anxiety symptoms as determined for comparrison against those of the rest of the cohort.
Tilt Protocol: Patient are: supine, 30° tilt (2min), 45° tilt (2min), & 70° tilt (45min). 12L EKG recordings were taken via a Dash 5000 system at specific intervals. HUT was stopped early if pt developed VVR or other severe syncopal symptoms. No medications were administered in HUT.
Definitions of Tilt Diagnoses:
- Postural Orthostatic Hypotension (POH) – a drop of 20 mmHg systolic (SOH20) or 10mmHg diastolic (DOH10) blood pressure during the HUT.
- Postural Orthostatic Tachycardia (POTS) – increase in heart rate of at least 30 bpm above baseline or above 120 bpm upon HUT.
- Classical POTS – Meets all criteria for POTS (See Above) and occurs within first 10 minutes at 70 degree of HUT.
- Vasodilator Response (VDR) – Sudden drop of SBP and/or DBP without a concomitant drop in HR.
- Vasovagal Response (VVR) – Sudden drop of HR, Systolic Blood Pressure (SBP), and Diastolic Blood Pressure (DBP) with symptoms (such as nausea and dizziness).
Statistics: A series of t-tests were used to test for an association between severity of anxiety symptoms & time to hemodynamic changes (and total time of tilt tolerated by the patient). Mean anxiety score was calculated for each hemodynamic change & compared to the mean score of the entire cohort & all pts not presenting with the aforementioned symptoms. Statistical analysis was performed using JMP software (version 9), p<0.05 was significant.
Results: See table at end
There was no statistically significant relationship between times to any hemodynamic change & severity of anxiety symptoms. The mean BAI score for pts included in this population was determined to be 14.67, which would be considered a mild level of anxiety symptoms in a standard population. Pts reporting with individual hemodynamic changes did not have statistically significant differences in BAI score than the rest of the cohort.
Conclusions: Our study showed that there is no correlation between time to POTS & the severity of anxiety symptoms. The mean of BAI scores for pts presenting with each of the symptoms failed tests for statistical significance.
Future Considerations: Pts with previous diagnosis of anxiety symptoms should not be treated differently when undergoing HUT. Mean BAI scores for pts presenting with each of the symptoms failed tests for statistical significance. Future analysis should examine the impact that anxiety reducing medications, & other treatments, may have on pts' responses to HUT, & answers to questions on the BAI.

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The relationship between psychiatric disorders and mortality in patients with POTS; in particular, a history of orthostatic intolerance, has been known for some time. However, the specific mechanisms underlying this relationship are incompletely understood.

**Background:**
Psychiatric disorders and orthostatic intolerance can coexist, with orthostatic intolerance being common among patients with POTS; in particular, a history of orthostatic intolerance has been associated with increased mortality. The cause of syncopal episodes may be related to coexistent medical conditions which affect the nervous system or could cause inadequate metabolic activity. The cause of syncopal episodes may be related to coexistent medical conditions which affect the nervous system or could cause inadequate metabolic activity.

**Methods:**
We performed a retrospective review of patients admitted to the Cincinnati VA Medical Center, Cincinnati, OH, from 8/1/1996 to 8/31/2010 for ICD implantation. Our study endpoint was death or ICD discharge for ventricular tachycardia or supraventricular tachycardia. We hypothesized that patients with a psychiatric diagnosis (PTSD, anxiety, or depression) prior to implantation of an ICD would have an increased all-cause mortality.

**Results:**
Eighty-three (21.6%) patients had at least one psychiatric diagnosis prior to implantation of an ICD (28.7% with PTSD, 19.4% with anxiety, and 56.14% with depression). No significant difference in mortality was observed between patients with or without a psychiatric diagnosis (14.17% with prior diagnosis vs. 18.90% patients with no prior diagnosis, p=0.79). Likewise, no difference was seen in the proportion reaching the study endpoint for mortality and ICD discharges; 20 (25.3%) with death or defibrillator discharge in patients with a psychiatric diagnosis, and 87 (28.5%) with death or defibrillator discharge in patients without prior diagnosis (p=0.57). Taking psychoactive medications was associated with improved survival. 331 patients were on such medications, 53 were not. Of these patients on medications, 68 (20.5%) died, whereas of the 53 patients who were not taking medications, 39 (73.6%) died. This translates into a relative risk for death of 3.6 for patients not taking psychoactive medications.

**Conclusions:**
Our data show no relationship between prior diagnosis of PTSD, anxiety, or depression and mortality in patients who subsequently undergo ICD implantation. This effect, however, may be due to “pre-treatment” of patients who are at higher risk with psychoactive medications, which may be protective. This investigation highlights the need to carefully assess psychiatric disease and treatment in patients who have or may require an ICD.
CONTROL ID: 1258833
ROLES OF CARDIOTONIC STEROIDS IN PREECLAMPSIA: A TRANSLATIONAL APPROACH WITH IN VIVO, IN VITRO, AND PATIENT STUDIES
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CURRENT CATEGORY: Cardiology/Cardiovascular Disease
ABSTRACT BODY: Introduction: Reentry is the most common mechanism of arrhythmia in cardiomyopathy and is favored by slow conduction. Cardiomyopathy is associated with mitochondrial oxidative stress. We explored the relationship between mitochondrial oxidative stress and slow conduction in a manganese SOD (MnSOD) knockout mouse that produces excess mitochondrial reactive oxygen species (ROS).
Method: Previously, we have shown that c-Src kinase mediates the effects of MnSOD deficiency on conduction. Therefore, MnSOD knockouts (MnSOD−/−) mice (4-6 weeks old) with and without treatment with the c-Src inhibitor, PP1 (1.5 mg/kg IP three times/week x 2 weeks). Western blotting for phospho [Tyr416] Src and Cx43, immunohistochemistry staining for Cx43, Masson’s Trichrome staining for detection of collagen deposition, electron microscopy, mitoSOX fluorimetry for mitochondrial ROS measurement, mito-green tracker for quantification of mitochondria, patch clamping for cardiac sodium current measurement, fluorescent dye diffusion for functional assessment of Cx43 and gap junction conduction, in vivo epicardial mapping for VT inducibility, and Flex-MEA mapping for conduction velocity measurements were performed.
Results: Sustained VT was induced in 85% of MnSOD−/− mice compared to none in the control group (P<0.05). Most of the induced VT episodes (83%) were monomorphic which suggests reentry as the predominant mechanism of those arrhythmias. Ventricular conduction velocity measured by Flex-MEA at 20 ms PCL was decreased in MnSOD−/− mice compared to control mice (0.51 m/s and 0.99 m/s, respectively, P<0.05). Dye diffusion showed decreased in longitudinal spread to approximately 65% of the control (P<0.05). MnSOD−/− mice showed 66% increase in level of mitochondrial ROS compared to the control mice (P<0.05) and electron microscopy showed evidence of mitochondrial damage and early degeneration in MnSOD−/− mice. Total Cx43 and cardiac sodium current were significantly decreased in the MnSOD−/− compared to the control mice (27% and 60% of the control, respectively, P<0.05). There was no difference in the collagen deposition level between MnSOD−/− and control mice (3.1±1 vs. 4.5±1.5, P=NS) at the age they were studied. MnSOD−/− mouse hearts revealed a 2.1 fold increase in phospho [Tyr416] Src protein level compared to the control (P<0.05). Treating MnSOD−/− mice with PP1 normalized cardiac sodium current and dye diffusion and increased Cx43 at the gap junctions to the 73% of the control (all P<0.05) resulting in a significant reduction in VT inducibility to 20%.
Conclusion: Cx43 remodeling and decreased cardiac sodium current are major and early substrates for ventricular arrhythmia in mitochondrial oxidative stress. Inhibition of c-Src prevents the effect of mitochondrial oxidative stress on Cx43, cardiac sodium current, and VT inducibility. Thus c-Src inhibition may be a novel and valuable antiarrhythmic therapy.

CONTROL ID: 1344918
SUBSTANCES FOR VENTRICULAR ARRHYTHMIA IN MITOCHONDRIAL OXIDATIVE STRESS: C-SRC INHIBITION PREVENTS REDUCTION IN CARDIAC SODIUM CURRENT, AND CONNEXIN 43
CURRENT CATEGORY: Cardiology/Cardiovascular Disease
ABSTRACT BODY: Introduction: The goal of this translational study is to understand the role of Cx43 remodeling and decreased cardiac sodium current are major and early substrates for ventricular arrhythmia in mitochondrial oxidative stress. Inhibition of c-Src prevents the effect of mitochondrial oxidative stress on Cx43, cardiac sodium current, and VT inducibility. Thus c-Src inhibition may be a novel and valuable antiarrhythmic therapy.

ABSTRACT BODY: Background: The association disease with the greatest prevalence was hypertension (n=119; 26%). In terms of gender, females were significantly more likely to have POTS (p=0.024; 11.5%) and migraines (p=0.001; 38.7%) than males (4.3% and 20.9% respectively). Males were significantly more likely to have hypertension (p=0.001; 36.7%) and CAD (p=0.001; 12.2%) than females (21.7% and 1.9% respectively). For each year 10 age increase, the odds of having CAD increased by 96%, hypertension by 87%, and diabetes by 58%. For each 10 year age increase, the odds of having POTS decreased by 34% and migraines by 18%.

Conclusions: Among OS patients, those with hypertension, CAD, diabetes, or GI problems tended to be older than those without these conditions; those with POTS or migraines were significantly younger than others without these conditions. Orthostatic syndromes are common; their interaction with co-existent conditions appear to be related to age.

Keywords: Syncope, Epidemiology, Orthostatic Intolerance, Hypertension, Cx43 remodeling and decreased cardiac sodium current are major and early substrates for ventricular arrhythmia in mitochondrial oxidative stress. Inhibition of c-Src prevents the effect of mitochondrial oxidative stress on Cx43, cardiac sodium current, and VT inducibility. Thus c-Src inhibition may be a novel and valuable antiarrhythmic therapy.

Results: Sustained VT was induced in 85% of MnSOD−/− mice compared to none in the control group (P<0.05). Most of the induced VT episodes (83%) were monomorphic which suggests reentry as the predominant mechanism of those arrhythmias. Ventricular conduction velocity measured by Flex-MEA at 20 ms PCL was decreased in MnSOD−/− mice compared to control mice (0.51 m/s and 0.99 m/s, respectively, P<0.05). Dye diffusion showed decreased in longitudinal spread to approximately 65% of the control (P<0.05). MnSOD−/− mice showed 66% increase in level of mitochondrial ROS compared to the control mice (P<0.05) and electron microscopy showed evidence of mitochondrial damage and early degeneration in MnSOD−/− mice. Total Cx43 and cardiac sodium current were significantly decreased in the MnSOD−/− compared to the control mice (27% and 60% of the control, respectively, P<0.05). There was no difference in the collagen deposition level between MnSOD−/− and control mice (3.1±1 vs. 4.5±1.5, P=NS) at the age they were studied. MnSOD−/− mouse hearts revealed a 2.1 fold increase in phospho [Tyr416] Src protein level compared to the control (P<0.05). Treating MnSOD−/− mice with PP1 normalized cardiac sodium current and dye diffusion and increased Cx43 at the gap junctions to the 73% of the control (all P<0.05) resulting in a significant reduction in VT inducibility to 20%.

Conclusion: Cx43 remodeling and decreased cardiac sodium current are major and early substrates for ventricular arrhythmia in mitochondrial oxidative stress. Inhibition of c-Src prevents the effect of mitochondrial oxidative stress on Cx43, cardiac sodium current, and VT inducibility. Thus c-Src inhibition may be a novel and valuable antiarrhythmic therapy.

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CONTROL ID: 1343835

DETERMINANTS OF APOB, APOA1, AND THE APOB/APOA1 RATIO IN HEALTHY SCHOOLGIRLS, PROSPECTIVELY STUDIED FROM MEAN AGES 10 TO 19 YEARS: THE CINCINNATI NATIONAL GROWTH AND HEALTH STUDY

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Internal Medicine, The Jewish Hospital of Cincinnati, Cincinnati, OH. J. Morrison Pediatrics, Cincinnati’s Children Hospital, Cincinnati, OH. S. Daniels Pediatrics, Denver Childrens Hospital, Denver, CO.

CURRENT CATEGORY: Endocrinology/Metabolism

ABSTRACT BODY: ABSTRACT

Objectives: Prospectively assess determinants of apolipoproteins B and A1 and the ApoB/ApoA1 ratio in 797 healthy black and white schoolgirls from mean ages 10 to 19. Materials/Methods There was prospective 9-year follow-up, with measures of ApoB at mean ages 10, 12, 14, 16 and 19 and ApoA1 at mean ages 12, 14, 16, and 19. Studies of 402 black and 395 white healthy schoolgirls were done in public and private schools, in urban and suburban Cincinnati. Outcome measures included determinants of ApoB, ApoA1, and ApoB/ApoA1.

Results: Black girls had lower ApoB, higher ApoA1, and lower ApoB/ApoA1 ratio. From ages 14-19, BMI and TG were positively associated with ApoB. Menstrual cyclicity ≥ 42 days metabolic syndrome, waist circumference, and triglyceride were independent risk factors while increasing ApoB/ApoA1 ratio, while black race was negatively associated. SHBG at age 14 in black girls was positively correlated with ApoA1 and ApoB/ApoA1 ratio.

Conclusions: The atherogenic ApoB/ApoA1 ratio from ages 14 to 19 is inversely associated with race, and positively associated with hyper-androgenism, menstrual cyclicity ≥ 42 days, waist circumference, triglyceride, and the metabolic syndrome, facilitating an adolescent approach to primary prevention of cardiovascular disease.

Key Words: ApoA1; ApoB; ApoB/ApoA1 ratio; triglyceride; race; sex hormone binding globulin; menstrual cyclicity ≥ 42 days; metabolic syndrome; waist circumference.

CONTROL ID: 1310332

PATIENT CHARACTERISTICS AND PARTICIPATION IN GENETICS STUDIES: A TYPE 2 DIABETES COHORT


CURRENT CATEGORY: Endocrinology/Metabolism

ABSTRACT BODY: Background: Recruitment of large, diverse populations into genetics studies remains challenging. One method to overcome this challenge is leveraging electronic health data and minimizing physical patient contact. We sought to identify characteristics associated with participation in a genetic sub-study of insulin-treated patients with type 2 diabetes, whom were identified via electronic hospital administrative data and asked to participate by providing DNA samples by mail.

Methods: During a phone interview for the parent study, participants (N=410) were asked to take part in the genetic sub-study. We examined the socio-demographic characteristics and clinical variables associated with consent and DNA-kit return (saliva sample) using logistic regression.

Results: The overall rate of consent to participate in the genetic sub-study was 90% (n=410). African-American (AA) race and younger age were associated with refusal to participate (all p<0.05). Of those who consented, 70% returned the DNA-kit (n=288). AA race, younger age, female gender, being employed, more physical activity, and higher hemoglobin A1C (HbA1C) were associated with lower DNA-kit return rate (all p<0.05). Stratifying by race, among AA, younger age, being employed, and having higher HbA1C were associated with lower DNA-kit return; whereas among non-AA, only female gender was associated with lower DNA-kit return (all p<0.05).

Conclusion: Consistent with prior studies, we found underrepresentation of AA in this genetic cohort. To our knowledge, this is the first study to demonstrate an association between HbA1C and participation in genetic research. This may indicate a compliance-related trait which calls for further exploration. Furthermore, our lower DNA-kit return rate despite a high consent rate, suggests that a telephone recruitment/mail-in process may not enhance participation. Direct comparison to in-person donation is warranted.

CONTROL ID: 1354885

USE OF RISEDRONATE FOR PREVENTION OF BONE LOSS AFTER LUNG TRANSPLANTATION

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CURRENT CATEGORY: Endocrinology/Metabolism

ABSTRACT BODY: OBJECTIVE: Osteoporosis is a known sequela of lung transplantation. High dose immunosuppressive therapy and preexisting low bone mass due to longstanding respiratory illness and corticosteroid treatment are factors that lead to rapid decline in bone mass after transplantation. One study on the natural history of osteoporosis after lung transplantation found bone loss to be most significant within the first 6 months. Among lung transplant patients, only one randomized, placebo-controlled prospective study using intravenous pamidronate has shown efficacy in the prevention of transplant induced bone loss with bisphosphonates. Risedronate is a potent anti-resorptive agent that has been shown to be effective in the prevention and treatment of glucocorticoid-induced osteoporosis. The aim of this study was to determine whether risedronate with calcium and vitamin D would lead to reduction in bone loss compared to calcium and vitamin D alone in individuals undergoing lung transplantation.

METHODS: We conducted a small randomized, double-blind, placebo-controlled trial conducted from 2002 to 2011 comparing bone mineral density (BMD) changes one year after lung transplantation. Sixteen patients who underwent lung transplantation (9 men, 7 women) at Loyola University Medical Center were evaluated at months 0, 1, 3, 6, 9, 12 after lung transplantation. Patients were given calcium, vitamin D, and either risedronate 35 mg PO weekly or placebo post-transplant. Bone densitometry was performed at baseline, 6, and 12 months after transplant and the BMD between the risedronate group and the placebo group were analyzed.

RESULTS: Out of 16 lung transplant recipients given calcium, vitamin D, and either risedronate or placebo post-transplant, the lumbar spine BMD was not statistically significant at baseline (p = 0.19), 6 months, (p = 0.28), or 12 months (p = 0.28) between the 2 groups. The total hip BMD was also not statistically significant at baseline (p = 0.21), 6 months (p = 0.29), or 12 months (p = 0.50). Bone turnover markers (osteocalcin and urinary N-telopeptide) were obtained and these showed dramatic lowering in bone formation after transplant.

DISCUSSION: Our findings demonstrate that despite early intervention with calcium, vitamin D supplementation, and bisphosphonate use, a difference in BMD was not observed within the first 12 months after lung transplant when compared with controls. The lack of benefit observed in those patients treated with a bisphosphonate may have been secondary to small sample size, a follow-up duration of only 1 year, or the possibility that one year post-transplant, aggressive calcium and vitamin D provides enough protection that a loss in bone mineral density is not observed.

CONCLUSION: Early intervention with calcium, vitamin D, and bisphosphonate therapy during the first year after lung transplantation failed to show a difference in bone mineral density when compared with controls.

CONTROL ID: 1314670

EXPERIMENTAL SLEEP RESTRICTION REDUCES PHYSICAL ACTIVITY IN ADULTS WITH PARENTAL HISTORY OF TYPE 2 DIABETES

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CURRENT CATEGORY: Endocrinology/Metabolism
OBJECTIVE: To prospectively treat PCOS before and during pregnancy with metformin-enoxaparin and to assess efficacy, safety, and pregnancy outcomes in women with PCOS and familial thrombophilia, compared with metformin treatment alone.

Patients: PCOS patients with or without familial thrombophilia, ≥35 at conception (33% vs 16%), much heavier BMI (32.2 vs 25.6 kg/m²), and found a similar trend toward increased expression in obese human islet compared with islets from non-obese individuals.

Methods: Ancillary endpoints included changes in sedentary, light, and moderate plus-vigorous activity, and their association with changes in mood and vigor. Repeated measures analysis of variance was used to compare the total number of daily activity counts and the time spent in light, moderate, and vigorous-intensity physical activity between the two sleep conditions, with time-in-bed as a within-subject factor and exercise category as a between-subject factor. Partial correlations analyses, controlling for age, BMI, gender, race/ethnicity, and exercise category, were used to investigate whether sleep-loss-related declines in subjective mood and vigor were associated with changes in physical activity between the two sleep conditions.

Results: Daily sleep was reduced by 2.3 h (P<0.001) and total activity counts were 31% lower (P<0.020) during the 5.5-h time-in-bed condition. This was accompanied by a 24% reduction in moderate plus-vigorous vigorous activity time (P<0.005) and more sedentary behavior (+21 min/day; P=0.020). In conclusion, experimental sleep restriction decreased the amount and intensity of physical activity in healthy adults at risk for type 2 diabetes. These findings support the hypothesis that sleep-loss-related reduction in physical activity can contribute to the risk of developing type 2 diabetes in susceptible individuals.

CONTROL ID: 1322144
PREVENTION OF PREGNANCY LOSS, AND PREGNANCY OUTCOMES IN WOMEN WITH POLYCYSTIC OVARY SYNDROME AND FAMILIAL THROMBOPHILIA BY GLUCOPHAGE, AND ENOXAPARIN

C. Glueck, J. Pranikoff, J. Paada, Z. Khan, A. Brar, G. Goyal, P. Wang

Cholesterol Center, Jewish Hospital, Cincinnati, OH; X. Deng, C. A. Abuchaibe, G. Boreil Internal Medicine, Jewish Hospital, Cincinnati OH, Cincinnati, OH.

CURRENT CATEGORY: Endocrinology/Metabolism

ABSTRACT BODY: Objective: To assess efficacy, safety, and pregnancy outcomes of metformin treatment before conception and metformin-Enoxaparin during pregnancy in women with polycystic ovary syndrome (PCOS), thrombophilia, and pregnancy loss.

Controls: Prospectively treat PCOS before and during pregnancy with metformin 2.5 g/day, with monthly follow-up during pregnancy, maintain Enoxaparin throughout pregnancy, compared to 252 healthy women without known PCOS or thrombophilia with 1 live birth.

Outcomes: Outpatient clinical research center, suburban obstetrics practice.

Patients: PCOS by Rotterdam Consensus Criteria, familial thrombophilia, recurrent previous pregnancy loss and/or thrombotic events.

Interventions: Metformin 2.5 g/day, high protein (26%), low carbohydrate (44%), low fat (30%), P/S ratio 2/1, Enoxaparin 60 mg/day through pregnancy.

Results: Forty-six women with PCOS treated with metformin-enoxaparin were older than controls (33 ± 5 vs 29 ± 6 y, P<0.001), more likely to be >age 35 at conception (33% vs 16%), much heavier BMI (32.2 ± 7.7 vs 25.6 ± 5.9 kg/m², P<0.001), and had familial thrombophilia. Forty-three women with PCOS had 130 pregnancies and 100 miscarriages (77%) before metformin-enoxaparin and 56 pregnancies, 10 miscarriages (18%) on metformin-enoxaparin, McNemars S = -7.8, P<0.001. The 18% miscarriage rate on metformin-enoxaparin in women with PCOS did not differ (P=0.1) from 252 controls of whom 161 had 286 previous pregnancies with 223 live births, 51 miscarriages (18%). In 17 PCOS women who had 24 live birth pregnancies pre-metformin-enoxaparin and then 18 live birth pregnancies on metformin-enoxaparin, GD was diagnosed in 8 live birth pregnancies (33%) without metformin vs 3 of 18 live birth pregnancies (17%) on metformin-enoxaparin, McNemars S = 4.5, P=0.03. Pre-eclampsia was present in 1 of 49 pregnancies (2%) on metformin-enoxaparin, not different (P=0.1) from 9 of 252 live births (4%) in 252 controls. Of 50 live births (1 twin pair) in 46 women treated with metformin-enoxaparin, 47 fetuses had birth weight <4000 g (94%) and 3400-4500 g (6%), not different from controls 199 <4000 (85%), 30 4000-4500 (13%), and 6 ≥4500 (3%), p = 0.8. On metformin-enoxaparin, of 49 live births, 2 (4%) were <32 weeks, 5 (10%) 32-37 weeks, and 42 (86%) from 37-42 weeks, not different (P=0.06) from 239 live births in control women, 0 <32 weeks, 22 (9%) from 32-37 weeks, and 217 (91%) from 37 to 42 weeks.

Conclusions: In women with PCOS and familial thrombophilia, compared to pregnancies without treatment, metformin-enoxaparin during pregnancy reduced...
miscarriage from 77% to 18%, reduced GD from 33% to 17%, reduced miscarriage and GD to levels comparable to normal women, was not associated with pre-eclampsia, fetal macrosomia, or premature delivery (all comparable to normal women), and was safe for mother and fetus.

CONTROL ID: 1294469
DYNAMIC EVOLUTION OF THYROID TEST ABNORMALITIES IN MCT8 DEFECT
A. Dumitrescu, X. Liao, R. Weiss, S. Refetoff Medicine, Univ. of Chicago Medical Center, Chicago, IL.
CURRENT CATEGORY: Endocrinology/Metabolism
ABSTRACT BODY: In 2004 we reported mutations in the MCT8 (mono-carboxylate transporter 8) gene as a cause for a form of X-linked syndromic mental retardation. MCT8 is a thyroid hormone (TH) specific cell membrane transporter. Among known inherited disorders of the thyroid axis in humans, this was the first involving TH transport into cells. The syndrome has 2 components: 1) a thyroid defect presenting an unusual pattern of serum thyroid function tests (TFTs): a high level of the active TH, triiodothyronine (T3), and decreased level of the inactive metabolite, reverse T3 (rT3). Serum thyroid hormone (T4) is also low but thyrotropin (TSH) is normal or just slightly elevated; 2) a severe neurodevelopmental delay with quadriplegia, dystonia, poor head control, and mental retardation, manifests in late infancy and childhood. To understand the mechanisms underlying the phenotype we generated a mouse model Mct8 knockout (KO). These mice replicate the characteristic TFT abnormalities observed in humans. Compared to wild-type (WT) litter mates, adult Mct8KO mice have low brain content of T3, whereas liver T3 content is high. Therefore, Mct8 dependent tissues, such as brain, are hypothyroid, while tissues expressing other TH transporters than Mct8, such as liver, are hyperthyroid. Thus, depending on the organ or tissue, Mct8 deficient mice have both TH deficiency and excess.

Routine neonatal screening for hypothyroidism is based on the determination of TSH. It is therefore surprising that MCT8 deficiency has not been identified by this means at birth. To investigate the sequence of events that lead to the established thyroid phenotype and to identify the earliest thyroid abnormality, TFTs were measured in mice at postnatal (P) days P7, 11, 14 and 17 (Table). Compared to WT littermates, Mct8KO mice had significantly lower serum rT3 (ng/dL) concentration already at P7, while T4 (ng/dL) became significantly lower at P11, and T3 and rT3 (ng/dL) concentration became significantly higher only at P17. This demonstrates that Mct8KO mice are not born with the TFTs pattern observed later in life and that there is a sequential appearance of TFTs abnormalities before weaning, with rT3 being the earliest distinguishing marker.

To evaluate the effect of these hormonal changes on a central (brain) and peripheral (liver) tissue, we made use of two sensitive enzyme markers. Deiodinase 1 (D1) activity increases in response to a rising local level of T3, whereas D2 activity is increased as a consequence of tissue TH deprivation. Both deiodinases convert T4 to T3. In Mct8KO vs WT litter mates, liver D1 enzymatic activity was significantly increased at P7, 30.3 ± 2.5 vs 24.5 ± 3.1 pmol/h/mg protein, and brain D2 is increased only at P14, 282.1 ± 70 vs 84.2 ± 3.4 fmol/h/mg protein. This time dependent increase in T3 generation, together with the peak TH production by the thyroid gland around P14, contribute to the dynamic evolution of the TFTs. Our findings demonstrate that there is a sequential appearance of the TFT abnormalities and that early postnatal changes in deiodinase activity are important in defining the characteristic thyroid phenotype of the Mct8 defect.

**TFTs in pups**

<table>
<thead>
<tr>
<th></th>
<th>P7</th>
<th>P11</th>
<th>P14</th>
<th>P17</th>
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<tbody>
<tr>
<td>rT3 in WT</td>
<td>72.8 ± 13.2</td>
<td>108.1 ± 11.5</td>
<td>150.2 ± 13.1</td>
<td>78.6 ± 8.6</td>
</tr>
<tr>
<td>rT3 in Mct8KO</td>
<td>12.1 ± 1***</td>
<td>24.4 ± 4***</td>
<td>15 ± 1.2**</td>
<td>6.2 ± 1.7***</td>
</tr>
<tr>
<td>T4 in WT</td>
<td>2.1 ± 0.2</td>
<td>4.8 ± 0.1</td>
<td>7.8 ± 0.4</td>
<td>5.1 ± 0.2</td>
</tr>
<tr>
<td>T4 in Mct8KO</td>
<td>1.5 ± 0.2</td>
<td>2.8 ± 0.3**</td>
<td>3 ± 0.2***</td>
<td>1.8 ± 0.2**</td>
</tr>
<tr>
<td>T3 in WT</td>
<td>90 ± 4.8</td>
<td>98 ± 3.4</td>
<td>107 ± 2.2</td>
<td>94 ± 4.4</td>
</tr>
<tr>
<td>T3 in Mct8KO</td>
<td>87 ± 4</td>
<td>89 ± 8</td>
<td>121 ± 5.8</td>
<td>118 ± 5.2**</td>
</tr>
</tbody>
</table>

Mct8KO vs WT, P value <0.01 (**), <0.001 (***)

**CONTROL ID: 1311096**
INCREASES IN NITRIC OXIDE BIOAVAILABILITY FAIL TO IMPROVE VASCULAR COMPLIANCE IN ELDERLY PATIENTS WITH TYPE 2 DIABETES
C. Flynn, R. Kalaitzidis, C. Schaffer, L. Fondren, K. Wroblewski, G. Bakris
The University of Chicago, Chicago, IL.
CURRENT CATEGORY: Endocrinology/Metabolism
ABSTRACT BODY: BACKGROUND: Nebivolol is a selective beta-1 antagonist that produces endothelium-dependent vasodilation. Patients with diabetes have reduced endothelial nitric oxide (NO) bioavailability due to excessive superoxide generation. Nebivolol restores NO bioavailability by decreasing superoxide concentration. We tested the hypothesis that nebivolol reduces pulse wave velocity (PWV) to a greater extent than metoprolol succinate in hypertensive type 2 diabetic patients after six months of treatment.

METHODS: Subjects with stage 1 hypertension and type 2 diabetes with HbA1c<8.5% on maximum dose RAS blockade were randomized to nebivolol or metoprolol succinate daily. Fifty of the 60 subjects randomized have completed at least 3 post-randomization visits and were included in this analysis. Comparisons were by paired t tests.

RESULTS: No significant difference in baseline demographics were observed, mean age 65, HbA1c 6.6%, 70% females, BMI 34. The primary end-point, change in PWV was not different from baseline or between groups, Table. Results were not confounded by differences in heart rate. Of the metoprolol group 8/25 (32%) were unable to tolerate an optimal dose due to fatigue compared to 2/25 (8%) of the nebivolol group.

<table>
<thead>
<tr>
<th></th>
<th>PWV (m/s)</th>
<th>AUGMENT. INDEX (%)</th>
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</thead>
<tbody>
<tr>
<td>Nebivolol (mean dose=12-17 mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BASELINE</td>
<td>6.6 +/- 1.4</td>
<td>27 +/- 7*</td>
</tr>
<tr>
<td>TREAL END (change@6 months)</td>
<td>-0.5, 95% CI (-0.8,0.2)</td>
<td>-3, 95% CI (-5,0)</td>
</tr>
<tr>
<td>Metoprolol (mean dose=81-20 mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BASELINE</td>
<td>6.7 +/- 0.9</td>
<td>21 +/- 7*</td>
</tr>
<tr>
<td>TREAL END (change @ 6 months)</td>
<td>-0.1, 95% CI (-0.6,0.1)</td>
<td>0, 95% CI (+4.3)</td>
</tr>
</tbody>
</table>

*P<0.05 compared to nebivolol

**CONTROL ID: 1342700**
INSULIN RESISTANCE IN THE PERIPHERAL NERVOUS SYSTEM OF TYPE 2 DIABETIC MICE
C.W. Grote, A.L. Groover, J.M. Ryals, D.E. Wright
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Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS.
CURRENT CATEGORY: Endocrinology/Metabolism
ABSTRACT BODY: Diabetes is the leading cause of peripheral neuropathy worldwide; however the etiology of diabetes-induced neuropathic complications remains unclear. The current research investigating the pathogenesis of diabetic neuropathy (DN) is predominantly focused on neuronal injury associated with the sequelae of hyperglycemia. However, a growing body of literature suggests that the loss of neuronal insulin support may also be a crucial factor contributing to DN. While decreased peripheral nervous system (PNS) insulin signaling is presumably involved in 1 diabetic (insulinopenic) models due to the lack of insulin, loss of insulin signaling in PNS neurons in type 2 (hyperinsulinemic) models has not been explored. The purpose of this study is to determine if insulin-resistant mice display alterations in activation of the insulin signaling cascade in peripheral neurons. Both in vitro and in vivo methods were employed to investigate PNS insulin signaling in insulin-resistant ob/ob mice. Dorsal root ganglia (DRG) cultures from non-diabetic control and diabetic ob/ob mice were treated with insulin and downstream signaling was assessed with Western blots and neurite outgrowth assays. In addition, mice were also directly administered insulin through intrathecal injections and the responsiveness of insulin-signaling pathways was quantified with Western blots in the DRG and sciatic nerve. Our results indicate that insulin-signaling abnormalities documented in other “insulin-sensitive” tissues (i.e. muscle, fat, liver) of ob/ob mice are also present in the PNS. A

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robust increase in insulin-induced downstream signaling (Akt activation) was detected in nondiabetic mice in both the sciatic nerve and DRG; however this response was blunted in both tissues from ob/ob mice. Furthermore, insulin supplementation resulted in a significant increase in nerve outgrowth in DRG cultures from nondiabetic mice, but not in DRG cultures from ob/ob mice. We also explored several pathways of molecular insulin resistance that have been defined in muscle. Our results suggest that upregulated JNK activation, IRS2 serine phosphorylation, and reduced insulin receptor expression could be contributory mechanisms of PNS insulin resistance. In conclusion, these results provide further evidence that alterations in insulin signaling occur in the PNS and may be a key factor in the pathogenesis of diabetic neuropathy.

**CONTROL ID: 1343838**

**NOVEL PATHWAY INVOLVED IN THE DEVELOPMENT OF PAINFUL DIABETIC NEUROPATHY**

N. Katz, J.M. Ryals, D.E. Wright 

**ABSTRACT BODY:** Diabetes affects more than 220 million people worldwide, and up to 50% suffer from peripheral neuropathy. Of those patients with neuropathy, approximately 15-30% experience debilitating pain. Recent evidence suggests the enzyme prostatic acid phosphatase (PAP) plays an important role in regulating nociception; however, the biology of PAP associated with pain sensation in diabetic neuropathy (DN) has not been investigated. PAP reduces pain by cleaving adenosine monophosphate (AMP) to adenosine, a well-known analgesic small molecule that signals through the A1 adenosine receptor (A1R). Our goal is to evaluate the effect of diabetes on this signaling pathway using a murine model of Type 1 diabetes. In this study, male A/J mice were given intraperitoneal streptozocin to induce Type 1 diabetes. Blood glucose levels, weight and behavior were monitored weekly for seven weeks beginning at 8 weeks of age. Diabetic mice had significantly elevated blood glucose levels (p<0.0001) throughout the study, and their weight decreased by almost 19% whereas nondiabetic mice gained weight as expected. Diabetic mice displayed significant mechanical allodynia beginning four weeks post-diabetes induction (p<0.05) that continued throughout the study. At the conclusion of the study, lumbar spinal cord (SC) and dorsal root ganglia (DRG) were harvested and analyzed for PAP expression via immunohistochemistry and Western blot analysis. PAP expression was observed primarily on small-diameter non-peptidergic neurons in the DRG and in nerve terminals in lamina II of the dorsal SC. PAP expression overlapped extensively with the non-peptidergic marker isolectin B4 (IB4). Little to no expression of A1R was observed in the DRG. Western blot analysis revealed that protein expression levels of PAP were significantly decreased in the DRG of diabetic mice compared to their nondiabetic counterparts (p<0.05). However, there was no significant difference in PAP expression in the SC of diabetic mice compared to nondiabetic mice. A1R protein expression in the SC and DRG does not appear to be affected by diabetes (data not shown). Collectively, these studies suggest that diabetes may alter the level of PAP expression in sensory neurons, which may lead to a decrease in adenosine production, contributing to the development of painful diabetic peripheral neuropathy.

**CONTROL ID: 1343851**

**PREVENTION OF PREGNANCY LOSS AND PREGNANCY OUTCOMES IN WOMEN WITH POLYCYSTIC OVARY SYNDROME AND FAMILIAL THROMBOPHILIA BY GLUCOPHAGE AND ENOXAPARIN**


**ABSTRACT BODY:**

**Objective:** Assess efficacy, safety, and pregnancy outcomes of metformin-enoxaparin treatment before conception and metformin-enoxaparin during pregnancy in women with polycystic ovary syndrome (PCOS), thrombophilia, and early pregnancy loss (EPL).

**Setting:** Outpatient clinical research center, suburban obstetrics practice.

**Patients:** PCOS by Rotterdam Consensus Criteria, familial thrombophilia, recurrent previous recurrent EPL, and/or previous thrombotic events.

**Interventions:** Metformin 2.00-2.55 g/day, enoxaparin 60 mg/day through pregnancy, diet high in protein (26%), low in carbohydrate (44%), low in fat (30%), with a polyunsaturated fat-to-saturated fat ratio (PS ratio) of two to one.

**Results:** Forty-six women with PCOS treated with metformin-enoxaparin (59 pregnancies) were older than controls (33 ±5 vs. 29 ±6 yr, p<0.0001), more likely to be >age 35 at conception (33% vs. 16%), and had much heavier body-mass indices (32.2 ±7.7 vs. 25.6 ±5.9 kg/m², p<0.0001). Of the 46 women, 43 had familial thrombophilia, three had >3 eCL without familial thrombophilia. Before taking metformin-enoxaparin, 43 women had 130 pregnancies, 100 EPL (77%), and 23 live births. On metformin-enoxaparin, these 43 women had 56 pregnancies, 10 EPL (18%), and 46 live births (McNemar’s S=78.7, p<0.0001). On metformin-enoxaparin, the full cohort of 46 women had 59 pregnancies, 10 EPL (17%) and 49 (83%) live births. The 17% miscarriage rate on metformin-enoxaparin in these 49 women did not differ (p=0.10) from 252 controls of whom 161 had 286 previous pregnancies with 223 live births, 51 miscarriages (18%). In 17 women who had 24 live birth pregnancies pre-metformin-enoxaparin therapy and then 18 live birth pregnancies on metformin-enoxaparin, gestational diabetes (GD) was diagnosed in eight live birth pregnancies (33%) without metformin versus three of 18 live birth pregnancies (17%) on metformin-enoxaparin, McNemar’s S=4.5, p=0.03. Pre-eclampsia was present in one of determinations (2%) on metformin-enoxaparin, but not different (p=1.00) from 9 of 52 live births (4%) in 252 controls. Of 50 live births (one twin pair) in 46 women treated with metformin-enoxaparin, 47 neonates had birth weights < 4000 grams (94%) and three were between 4000 and 4500 grams (6%), not different from 39 control births. Of 59 live births on metformin-enoxaparin, 45 neonates had birth weights < 4000 grams (19%), and six were between 4000 and 4500 grams (13%), and six were > 4500 grams (3%), (p=0.08). On metformin-enoxaparin, of 49 live births, two (4%) were < 32 weeks, five (10%) between 32 and 37 weeks, and 42 (86%) between 37 and 42 weeks, not different (p=0.062) from 239 live births in control women, none were <32 weeks, 22 (9%) were between 32 and 37 weeks, and 217 (91%) were between 37 and 42 weeks.

**Conclusions:** In women with PCOS and familial thrombophilia, compared to pregnancies without treatment, metformin-enoxaparin during pregnancy reduced miscarriage from 77% to 18%, reduced GD from 53% to 17%, reduced miscarriage and GD to levels comparable to normal women, was not associated with pre-eclampsia, fetal macrosomia, or premature delivery (all comparable to normal women), and was safe for mother and fetus.
which contributes to the beneficial effects of improved systemic insulin sensitivity.

**Methods:** The subjects were recruited from the Center for Surgical Treatment of Obesity at University of Chicago and were approached for participation only after they had been approved as eligible candidates for bariatric surgery. Subcutaneous adipose tissue was obtained by needle biopsy from the same patient two weeks before and two weeks after bariatric surgery. Adipocytes were isolated from the fat biopsy tissue by collagenase digestion immediately following the biopsy procedure. Purified adipocytes were incubated at 37 degrees celsius for 10 minutes with increasing amounts of insulin, and the reaction was terminated by addition of Laemmli buffer and boiling. Samples were stored at -80 degrees celsius until both biopsies from each subject had been performed. Insulin sensitivity was assessed pre- and post-surgery by an insulin-phospho-AKT immunoblotting using the same gel for all samples from each patient. In parallel, mRNA was purified from the patients' samples and analyzed by quantitative RT-PCR for specific proteins in the insulin signaling pathway leading to the phosphorylation of AKT, such as IRbeta (Insulin receptor-beta), IRS-1 (insulin receptor substrate), the subunits of phosphoinositide-3-kinase (PI3K) including p85 regulatory and p110 catalytic regions, and also 3-phosphoinositide-dependent protein kinase 1 (PDK1). Immunoblotting was performed to assess the up- or down-regulation of these proteins before and after bariatric surgery.

**Results:** Our data showed that DS induced an increase in insulin-stimulated AKT phosphorylation in primary human adipocytes from 3 subjects within two weeks after surgery, before any significant long-term weight loss had occurred. No detectable change in the expression of the regulatory or catalytic domains of PEK was observed in parallel with the change in insulin signaling. In contrast, preliminary results indicated there was a marked increase IRS-1 as detected by qRT-PCR and confirmed by immunoblotting.

**Conclusions:** These findings support the hypothesis that DS acutely improves insulin signaling at the cellular level of the adipocyte, resulting in the beneficial effects of improved systemic insulin sensitivity after surgery. Successful completion of this study will provide novel insights into the insulin signaling pathway involved in pancreatic beta cell adaption to insulin resistance. Considering that this receptor is a GPCR, where GPCRs are major drug targets, these studies suggest that this may be a new exciting therapeutic target for diabetes.

**CONTROL ID:** 1344722
**SMRT REGULATES GLUCOCORTICOID ACTION IN ADIPOCYTES**
S. Mantis, M. Landeche, X. Han, R. Cohen Endocrinology, University of Chicago, Chicago, IL.

**CURRENT CATEGORY:** Endocrinology/Metabolism

**ABSTRACT BODY:** Increased glucocorticoid action in adipocytes has been linked to systemic metabolic abnormalities including insulin-resistant diabetes, obesity, dyslipidemia, and hypertension. However, circulating levels of glucocorticoids are not clearly altered in patients with the metabolic syndrome. Glucocorticoid action is regulated by the glucocorticoid receptor (GR), a member of the nuclear hormone receptor (NR) superfamily. NRs recruit coactivators or corepressors either in the absence of ligand or in the presence of antagonists. Although multiple studies have suggested that coactivators are important for GR action, the role of corepressors is much less clear. The two most important nuclear receptor corepressors are the silencing mediator of retinoid and thyroid hormone receptors (SMRT) and the nuclear receptor corepressor (NCoR). To investigate the role of corepressors in GR function in the adipocyte, we created SMRT +/- heterozygous knock-out mice. These mice exhibit normal adiposity on a chow diet, but gain excess weight when placed on a high-fat diet. To investigate the role of SMRT in glucocorticoid action, we isolated adipose tissue from SMRT +/- and WT mice and treated it with and without dexamethasone. RNA was isolated, and we performed quantitative RT-PCR of the dexamethasone-responsive gene GILZ. Interestingly, dexamethasone-mediated GILZ expression was 50% higher in adipose tissue derived from SMRT +/- mice compared to WT controls. To determine if glucocorticoid-responsive changes in gene expression were due to direct recruitment of SMRT by the GR, we performed chromatin immunoprecipitation (ChIP) experiments, taking advantage of the well-characterized glucocorticoid response element (GRE) in the lipin gene. In fact, SMRT was recruited to the GRE, and this recruitment was dramatically up-regulated in the presence of dexamethasone. Thus, GR recruits SMRT in the presence of hormone, and SMRT then modulates glucocorticoid-mediated transcription. These data suggest that SMRT is a key regulator of metabolism in adipocytes, and that alterations in SMRT expression may dictate sensitivity to glucocorticoids in vivo.

**CONTROL ID:** 1336964
**A ROLE OF A NOVEL NUTRIENT-SENSING GPCR, FFAR2, IN GLUCOSE HOMEOSTASIS DURING PREGNANCY**
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**CURRENT CATEGORY:** Endocrinology/Metabolism

**ABSTRACT BODY:** G-protein coupled receptors (GPCRs) compromise a large group of transmembrane receptors involved in a variety of physiological processes, including metabolic homeostasis. One of these GPCRs, free fatty acid receptor 2 (FFAR2), has recently been discovered and is endogenous ligands are short chain fatty acids. Interestingly, this receptor has been observed to be expressed in a number of metabolically important tissues including pancreatic beta cells. Of relevance here, previous studies from our group have demonstrated that the expression of FFAR2 in pancreatic islets is upregulated during the insulin resistant state of pregnancy. Because of this, the aim of this study was to characterize the role of FFAR2 in glucose homeostasis during pregnancy and investigate possible mechanisms by which this may occur using global FFAR2 knockout (KO) models. Comparison of global FFAR2 female KO mice and their littermate wild type (WT) female controls showed similar glucose tolerance prior to pregnancy by both intraperitoneal (IP) and oral glucose tolerance tests (OGTT). However, during pregnancy (at point of maximum beta cell proliferation, day 15 of gestation), impaired glucose tolerance was observed in the pregnant FFAR2 KO mice by both IPGTT and OGTT. Because an expansion of islet mass occurs to compensate for increased insulin needs during pregnancy, we next investigated whether this impairment in glucose tolerance in the KO mice may be due to altered islet expansion. These studies showed that islet mass at day 15 of pregnancy in the female KO mice was decreased and that these mice had an increase in the number of small islets as compared to the female WT mice. Other abnormalities contribute to impaired glucose tolerance in these mice and are being investigated, including insulin secretion and/or insulin resistance. In conclusion, these results suggest that FFAR2 contributes to glucose homeostasis during pregnancy, and that this may occur, at least in part, via the regulation of insulin mass during pregnancy. Data from these studies have identified a novel pathway involved in pancreatic beta cell adaption to insulin resistance. Considering that this receptor is a GPCR, where GPCRs are major drug targets, these studies suggest that this may be a new exciting therapeutic target for diabetes.

**CONTROL ID:** 1344135
**THE COST-EFFECTIVENESS OF GENETIC TESTING FOR MATURITY-ONSET DIABETES OF THE YOUNG**
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**CURRENT CATEGORY:** Endocrinology/Metabolism

**ABSTRACT BODY:** Increased glucocorticoid action in adipocytes has been linked to systemic metabolic abnormalities including insulin-resistant diabetes, obesity, dyslipidemia, and hypertension. However, circulating levels of glucocorticoids are not clearly altered in patients with the metabolic syndrome. Glucocorticoid action is regulated by the glucocorticoid receptor (GR), a member of the nuclear hormone receptor (NR) superfamily. NRs recruit coactivators or corepressors either in the absence of ligand or in the presence of antagonists. Although multiple studies have suggested that coactivators are important for GR action, the role of corepressors is much less clear. The two most important nuclear receptor corepressors are the silencing mediator of retinoid and thyroid hormone receptors (SMRT) and the nuclear receptor corepressor (NCoR). To investigate the role of corepressors in GR function in the adipocyte, we created SMRT +/- heterozygous knock-out mice. These mice exhibit normal adiposity on a chow diet, but gain excess weight when placed on a high-fat diet. To investigate the role of SMRT in glucocorticoid action, we isolated adipose tissue from SMRT +/- and WT mice and treated it with and without dexamethasone. RNA was isolated, and we performed quantitative RT-PCR of the dexamethasone-responsive gene GILZ. Interestingly, dexamethasone-mediated GILZ expression was 50% higher in adipose tissue derived from SMRT +/- mice compared to WT controls. To determine if glucocorticoid-responsive changes in gene expression were due to direct recruitment of SMRT by the GR, we performed chromatin immunoprecipitation (ChIP) experiments, taking advantage of the well-characterized glucocorticoid response element (GRE) in the lipin gene. In fact, SMRT was recruited to the GRE, and this recruitment was dramatically up-regulated in the presence of dexamethasone. Thus, GR recruits SMRT in the presence of hormone, and SMRT then modulates glucocorticoid-mediated transcription. These data suggest that SMRT is a key regulator of metabolism in adipocytes, and that alterations in SMRT expression may dictate sensitivity to glucocorticoids in vivo.
The testing policy yielded an average lifetime gain of 0.02 QAL Ys. In the United States context, routine screening for MODY would transition to SU therapy with a resultant glycemic benefit of 0.8% and that all patients with MODY would discontinue therapy. We estimated a 2% prevalence of MODY in the screened population with genetic testing costs of $2000 per individual screened. Outcomes included costs and quality-adjusted life years (QALYs), based on lifetime risk of complications and treatments, expressed as the incremental cost-effectiveness ratio (ICER, $/QALY).

**Results:** The testing policy yielded an average lifetime gain of 0.02 QALYs. The base case resulted in an ICER of $913,700/QALY. In sensitivity analysis the testing policy became cost-savings if screening was done in a more limited population where the expected prevalence of MODY was ≥88%. Reducing genetic testing costs in the base case to $500 would also make the screening policy cost-savings.

**Conclusions:** In the United States context, routine screening for MODY subtypes in incident cases of T2D is a cost-effective use of personalized genetic medicine, and may be seen as a compelling argument for routine coverage of genetic testing in diabetes.

**CONTROL ID: 1314588**

**TITRATING LOVAZA FROM 4 TO 8 TO 12 GRAMS/DAY IN PATIENTS WITH FAMILIAL HYPERTRIGLYCERIDEMIA WHO HAD TRIGLYCERIDE LEVELS ≥500 MG/DL DESPITE MAXIMAL TRIGLYCERIDE LOWERING THERAPY**

N.A. Khan, K. Riaz, C.J. Glueck, Z. Khan, P. Wang

**CURRENT CATEGORY:** Endocrinology/Metabolism

**ABSTRACT BODY:** In 18 patients with severe familial hypertriglyceridemia (triglycerides ≥500 mg/dl) despite maximal TG lowering therapy, our specific aim was to assess efficacy and safety of sequential stepwise monthly treatment with Lovaza 4, 8, 12, 4, 4, and 4 g/day.

**Design:** When TG remained ≤500 mg/dl despite diet, diabetes control, and fibrin acid therapy, Lovaza was stepped up monthly first at 4 g/day, then if TG remained >500, to 8 g/day, then if TG remained >500, to 12 g/day, then returned to 4 g/day for 3 months.

**Setting:** Outpatient clinical research center.

**Patients:** Familial hypertriglyceridemia, 18 men, 1 woman, all white, mean ± SD age 48 ± 7 years.

**Interventions:** Before Lovaza, fibrin acid, control of type 2 diabetes, reduction of hyperinsulinemia with metformin, NIH Type V diet, then stepwise Lovaza 4, 8, and 12 g/day added to baseline therapy which was maintained unchanged.

**Results:** Of the 19 patients with familial hypertriglyceridemia, 6 reduced TG (group mean) from 1234 to 167 mg/dl on 4 g/day Lovaza, 3 reduced TG from 1287 to 296 mg/dl on 8 g/day, 8 reduced TG from 1067 to 517 mg/dl when lovaza was titrated up to 12 g/day, and 2 patients failed to lower TG when lovaza was titrated up to 12 g/day. Comparisons were made by analysis of variance and mixed model for repeated measures analysis. In most patients, TG fell on Lovaza treatment in a linear fashion as the dose increased. Compared to pre-Lovaza baseline, no abnormal measures developed in laboratory safety tests: glucose, HgA1C, BUN, creatinine, albumin, globulin, bilirubin, AST, ALT, RBC, Hgb, Hct, platelet count. Patients’ weight and diet were stable. The 4, 8, and 12 g/day Lovaza doses were well tolerated.

**Conclusions:** Titration of Lovaza from 4 to 8 to 12 g safely offers an effective way to lower TG beyond conventional therapy.

<table>
<thead>
<tr>
<th>Dosage</th>
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<tr>
<td>0</td>
<td>1206 ± 684</td>
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<td>8</td>
<td>729 ± 534</td>
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<td>778 ± 731</td>
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TG slope decreasing (when dose 0 to 12), p=0.04

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**CONTROL ID: 1319228**

**THE NOVEL ENDOCRINE DISRUPTOR TOLYLFLUANID IMPAIRS ADIPOCYTIC INSULIN SIGNALING THROUGH A REDUCTION IN INSULIN RECEPTOR SUBSTRATE-I LEVELS**

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**CURRENT CATEGORY:** Endocrinology/Metabolism

**ABSTRACT BODY:** The last several decades have witnessed the emergence of an epidemic of metabolic diseases that threatens both individual and societal health. While increasing caloric intake and declining physical activity undoubtedly contribute to the exploding rates of obesity and diabetes, these two factors fail to fully account for the magnitude and rapidity of these disturbing trends. As such, more attention has been directed at complimentary factors that disrupt energy homeostasis. Emerging data suggest that environmental endocrine disrupting chemicals (EDCs) may contribute to the pathophysiology of obesity and diabetes. Tolylfluanid (TF) is a phenyl-sulfonamide fungicide used on fruit crops outside of the United States, including Canada, Europe, and China. It is also used in the shipping industry as a booster biocide in marine paints to improve hydrodynamics. As such, humans are exposed through contaminated food and water; additionally, occupational exposure is significant among some agricultural and shipyard workers. In prior work, we showed that TF augments adipocyte differentiation, likely through stimulation of the glucocorticoid receptor. The effects of this novel EDC on mature adipocyte metabolism, however, remain unknown. Because of the central role of adipose tissue in global energy regulation, the present study tested the hypothesis that TF modulates insulin action in primary rodent and human adipocytes. Alterations in insulin signaling in primary mammalian adipocytes were determined by the phosphorylation of Akt, a critical insulin signaling intermediate.

**Interventions:** Treatment of primary murine adipose tissue in vitro with 100 nM TF for 48 h markedly attenuated acute insulin-stimulated Akt phosphorylation in a strain- and species-independent fashion. Congenital, perinatal, and neonatal fat were all sensitive to TF-induced insulin resistance. A similar TF-induced reduction in insulin-stimulated Akt phosphorylation was observed in primary human subcutaneous adipose tissue. TF-treatment led to a potent and specific reduction in insulin receptor substrate-1 (IRS-1) mRNA and protein levels, a key upstream mediator of insulin’s diverse metabolic effects. In contrast, insulin receptor-β, phosphatidylinositol 3-kinase, and Akt expression were unchanged indicating a specific abrogation of insulin signaling at the level of IRS-1. Additionally, TF-treated adipocytes exhibited altered endocrine function with a reduction in both basal and insulin-stimulated leptin secretion. For the first time, a specific cause-and-effect responsibility for EDC-induced insulin resistance can be assigned, namely a reduction of IRS-1 expression. These findings raise concern about the potential for this fungicide to disrupt metabolism and thereby contribute to the pathogenesis of diabetes. Current work in the laboratory seeks to identify the mechanism by which TF reduces cellular IRS-1 levels while also exploring the potential for other EDCs to attenuate adipocytic insulin action. Collectively, it is hoped that these studies will advance our understanding of the metabolic impact of environmental pollutants in order to develop sound public policy to mitigate their deleterious effects on human health.

**CONTROL ID: 1314992**

**EXOME SEQUENCING IN FAMILIES WITH OSTEOPOROSIS OR IDIOPATHIC HYPERCALCIURIA**

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**CURRENT CATEGORY:** Endocrinology/Metabolism

**ABSTRACT BODY:** Family history plays a major role in the development of osteoporosis (OP) and idiopathic hypercalciuria (IH). Bone mineral density (BMD) is highly heritable, contributing 57 to 92% of peak BMD in premenopausal twin pairs. Familial osteoporosis (FOP) not associated with aging or menopause and without a specific etiology has been described. Although population-based GWAS or candidate gene association studies have defined multiple genes with small contributions to BMD, FOP likely involves different genes with larger effects. IH may also cause low...
BMD and increased fracture risk but differs from OP by pathophysiology and treatment. A few population-based candidate gene association studies have failed to show conclusive associations with IH. Most studies using genetic associations with kidney stones as a marker of hypercalciuria leave out many subjects with IH and low bone density but without stone formation. We hypothesize that the familial forms of IH and FOP result from mutations in the protein coding regions in a limited number of genes. The study aim is to identify genetic variants associated with OP or IH in families using exome sequencing. Study subjects are selected along with their families from the University of Chicago Bone Clinic based on POP or IH and family history of low BMD with or without IH. DNA is isolated for exome sequencing. Subjects are phenotyped using BMD, 24 hour urine Ca excretion, and other pertinent blood and urine testing. Nine subjects from 2 families have been studied. The families have four myocardial infarctions among 7 family members with low BMD and one with kidney stones. Several other family members have had fractures and await BMD testing. Several subjects in the second family have low BMD and hypercalciuria. Complete data on these families, including exome sequencing, is pending.

CONTROL ID: 1287322
THROMBOTIC EVENTS AFTER STARTING EXOGENOUS TESTOSTERONE OR ARIMIDEX IN MEN WITH PREVIOUSLY UNDIAGNOSED FAMILIAL THROMBOPHILIA

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CURRENT CATEGORY: Endocrinology/Metabolism

ABSTRACT BODY: Our specific aim was to describe thrombosis (osteonecrosis of the jaw, deep venous thrombosis, and pulmonary embolism) after testosterone or testosterone plus HCG were given because of sexual dysfunction in 3 Caucasian men, and Arimidex to 1 Caucasian man, all with no antecedent thrombosis and previously undiagnosed familial thrombophilia. Case 1: A 55 year-old male, non-smoker, with four myocardial infarctions at age 50 was placed on Arimidex 0.5mg weekly by his cardiologist to lower high estradiol, 50 pg/ml (normal range < 42.6 pg/ml). On Arimidex, estradiol fell to 21 and 18 pg/ml. Six months after starting Arimidex, he developed severe upper and lower jaw pain, and was diagnosed with osteonecrosis of the jaw by X-ray and biopsy. He was found to have heterozygosity for the Factor V Leiden mutation, high ACLA IgG (16 U/ml) and IgM (23 U/ml), homozygosity for the MTHFR C677T mutation, hypofibrinolytic high Lp(a) (33 mg/dl), and homozygous mutation (4G-4G) of the PAI-1 gene. Arimidex was discontinued due to its estrogen-mimetic effect and his jaw symptoms resolved completely. Case 2: A 52 year-old Caucasian man with a twenty-pack-year smoking history and no history of blood clots developed deep venous thrombosis and pulmonary embolism 18 months after starting testosterone (150mg injection every two weeks for the first 15 months, and 150mg once every three weeks thereafter). He was found to have low antithrombin III (<80%, normal >80%). Case 3: A 71 year-old male, after gradually increasing testosterone gel from 50 to 300 mg/day, developed deep venous thrombosis and pulmonary embolism 1 month later. He was found to have high Factor VIII (215%, normal <150%). In these previously healthy men, thrombotic events after starting Arimidex, testosterone, or testosterone plus HCG were found to be associated with previously undiagnosed familial thrombophilia including Factor V Leiden heterozygosity, high Factor VIII, and low antithrombin III. We speculate that when exogenous testosterone is aromatized to estradiol, and estradiol-induced thrombophilia is superimposed on familial thrombophilia, thrombosis is more likely to occur. Men sustaining thrombotic events on Arimidex, on testosterone, or on testosterone plus HCG should be screened for the Factor V Leiden heterozygosity and other familial and acquired thrombophilias. Testosterone, testosterone plus HCG, HCG alone, or Arimidex should not be given to men already known to have familial thrombophilia.

CONTROL ID: 1342603
REGULATION OF ADIPOSE TRIGLYCERIDE LIPOASE (ATGL) AND GOS2 LIPID DROPLET PROTEINS BY FOXO TRANSCRIPTION FACTORS AND INSULIN IN THE LIVER

W. Zhang, I. O-Sullivan, T.G. Untermeyer Medicine, University of Illinois at Chicago and Jesse Brown VAMC, Chicago, IL. S. Bu, D.G. Mashek Nutrition and Food Science, University of Minnesota, Saint Paul, MN. C. Kahn Joslin Diabetes Center, Harvard Medical School, Boston, MA.

CURRENT CATEGORY: Endocrinology/Metabolism

ABSTRACT BODY: FoxO transcription factors are major targets of insulin action. In the liver, FoxO proteins promote gluconeogenesis and contribute to regulation of glycolytic and lipogenic gene expression (JBC: 281: 10105; 2006). Studies in liver organoids show FoxOs promote lipid mobilization in adipose tissue through regulation of adipose triglyceride lipase (ATGL), and recent findings indicate that ATGL is important in regulating lipid turnover in liver. We asked whether FoxO proteins also regulate ATGL expression and its inhibitors, the G0/G1 switch gene protein 2 (G0S2), in liver. Studies in wild type mice show liver ATGL mRNA and protein levels are abundant after an 18 hr fast and markedly suppressed 6 hr after refeeding. Studies in liver-specific knockout mice show FoxO proteins are required for increased expression of ATGL in fasting. Conversely, transgenic mice expressing constitutively active FoxO1 in liver show that suppression of FoxO function is required for reducing ATGL expression and triglyceride lipase activity after refeeding. Gene array and expression studies reveal constitutively active FoxO1 also suppresses hepatic expression of G0S2, an inhibitor of ATGL. In vitro studies confirm that FoxO1 stimulates ATGL and suppresses G0S2 expression in isolated hepatocytes. To determine if FoxO1 proteins mediate effects of insulin on ATGL and G0S2 expression, we examined ATGL and G0S2 expression in liver-specific insulin receptor knockout mice (LIRKO), and LIRKO mice in which FoxO1 was also inactivated in the liver (LIRFKO). ATGL expression was increased 4-fold in LIRKO mice compared to control and this effect was lost in LIRFKO mice. Conversely, G0S2 expression was decreased 80% in LIRKO mice, and disrupting FoxO1 partially reversed this effect. These studies indicate that FoxO proteins play an important role in regulating ATGL and G0S2 expression and thereby mediate the ability of insulin to suppress lipid catabolism and promote lipid storage in the liver.
ABSTRACT BODY: Cocaine-related acute coronary syndrome (PCOS) is among the most common disorders of reproductive-age women, affecting ~7% of this population. It is characterized by hyperandrogenism and ovarian dysfunction. Affected women have a substantially increased risk for type 2 diabetes. Profound peripheral and hepatic insulin resistance as well as pancreatic β-cell dysfunction confer this increased risk. The contribution of decreased insulin-mediated suppression of lipolysis to the pathogenesis of these defects in glucose homeostasis in PCOS has been incompletely assessed. Indeed, there have been no studies of the regulation of lipolysis in vivo in PCOS. Accordingly, we investigated changes in circulating total and individual free fatty acid (FFA) levels in response to insulin in 31 non-Hispanic White women with PCOS and 18 reproductively normal control women of comparable age, weight and ethnicity. Seven PCOS had impaired glucose tolerance; all controls had normal glucose tolerance. Sequential hyperinsulinenemic euglycemic clamp studies were performed at submaximal (20 mU/m2/min) and maximal (400 mU/m2/min) insulin doses in the post-absorptive state after a three-day high carbohydrate diet. Endogenous insulin secretion was suppressed with the simultaneous infusion of octreotide during the clamp. Body composition was assessed by dual photon x-ray absorptiometry (DXA) scan and visceral adipose tissue (VAT) was measured by computed axial tomography. PCOS and control women had comparable lean body, fat and VAT mass. Baseline insulin levels did not differ in PCOS and control women (PCOS 20 ± 11 v control 15 ± 7 μU/mL, P=0.111). Steady-state insulin levels at submaximal (PCOS 40 ± 6 v control 34 ± 6 μU/mL, P=0.007) and maximal (PCOS 201 ± 253 v control 177 ± 148 μU/mL, P=0.002) insulin doses were significantly increased in PCOS. Steady-state total FFA levels did not differ at baseline or at submaximal or maximal insulin doses in PCOS compared to control women. However, percent suppression of total FFA was significantly lower in PCOS compared to control women at submaximal (PCOS 82% ± 12% v control 91% ± 6%, P=0.015) and maximal (PCOS 92% ± 5% v control 95% ± 2%, P=0.042) insulin doses. Levels of elaidic, linoleic, linolenic, myristic, oleic, palmitic, arachidonic and stearic acid, did not differ at baseline in PCOS compared to control women. Further, there was no difference in the suppression of individual FFA levels at submaximal and maximal insulin doses in the two groups.

We conclude that women with PCOS have decreased insulin-mediated suppression of lipolysis in vivo suggesting adipocyte resistance to the antilipolytic effects of insulin. Although baseline total FFA levels did not differ, the decreased suppression of lipolysis may contribute to FFA-mediated insulin resistance in PCOS. There was no evidence for differences in circulating individual FFA levels suggesting that total FFA levels rather than a shift in individual FFA levels to increase those that are more metabolically adverse, e.g. palmitate, plays a role in the pathogenesis of insulin resistance in PCOS.

Epidemiology/Health Care Outcomes/Quality Improvement/Bioinformatics

CONTROL ID: 1241638
CONTINUED COCAINE USE AND RE ADMISSION IN PATIENTS WITH COCAINE-RELATED ACUTE CORONARY SYNDROME

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CURRENT CATEGORY: Epidemiology/Health Care Outcomes/Quality Improvement/Bioinformatics

ABSTRACT BODY: Background: With the advent of crack cocaine in 1973, a new social disorder emerged in the United States. From 13% to as high as 50% of ER admissions are reported in the literature to be related to cocaine use. Cocaine related chest pain is the most common admission diagnosis in cocaine users. With this large burden on some inner city centers, it is unclear which patients should be allocated more extensive resources for aggressive rehabilitation. Methods: Cocaine-related ACS patients were identified from 231 patients meeting inclusion criteria that were entered into an acute coronary syndrome registry at an urban, inner-city acute-care facility. Comparisons were made between patients who re-presented to the emergency department with chest pain within one-year between those with subsequent positive versus negative urine drug screen. Results: At initial enrollment, 44 of these patients (19%) tested positive for cocaine by self-report or confirmed by urine drug screen. 22 of these patients (50%) returned to the same facility within one year. Of these patients, 13 (59%) tested positive for cocaine again and 9 (41%) were UDS negative. Initial and discharge treatments were similar for both groups of patients on earlier visits. Patients who remained positive (+) UDS had significantly lower ejection fractions (44% vs. 58.6%, p=0.04) as determined on previous visit. All three deaths that occurred in this group of patients occurred in those patients with a subsequent (+) urine drug screen for cocaine. The number of readmissions to the same facility trended to be higher in the population of patients to have a subsequent (+) UDS for cocaine (4.9 vs. 1.8, p=0.09).

Conclusions: Cocaine-related acute coronary syndromes represent a significant challenge for the clinician and a significant burden to society. Patients who continue to abuse illicit drugs have high health care utilization rates and represent patients with worse survival. Targeting these patients for early rehabilitation may improve their significantly poor outcome and decrease utilization of health-care services.

CONTROL ID: 1309817
MEDICAL DEVICE APPROVAL SYSTEM: IS PMA MORE EFFECTIVE THAN 510(K)? STUDY OF RECALLED MEDICAL DEVICES


CURRENT CATEGORY: Epidemiology/Health Care Outcomes/Quality Improvement/Bioinformatics

ABSTRACT BODY: Background: The FDA has recognized three classes of medical devices based on their design, complexity, safety, and potential for harm if malfunctioning occurs. The level of control measures to assure safety and effectiveness of medical devices depends on their class. Most of the Class 3 medical devices have to go through premarket approval (PMA) application that requires a clinical trial. Most of the Class 1 and 2 medical devices are eligible for the 510(k) premarket submission, which is to demonstrate that the device is equivalent to a legally marketed device that is not subject to PMA. The recent concern of the Institute of Medicine is that less stringent 510(k) process cannot assure adequate safety of devices. Therefore, they recommend that the 510(k) approval process should be discarded. The goal of this study was to assess the efficacy and safety aspects of the two medical device approval processes (PAM and 510(k)) by analyzing the recalled medical devices. The hypothesis to be tested was that recalled medical devices approved by PMA spend a longer time on the market without recall than devices approved by the 510(k) process, as well as the proportion of recalled devices is lower for PMA than 510(k) approval processes.

Methods: Data on all medical devices recalled in US from January 2005 to December 2010 was collected from FDA.gov website as: name of device, class of recall, class of medical device, date of recall and approval, date of the beginning and the end of manufacturing, classification according to the process which the device was approved (PMA or 510k). The time on the market was calculated by using the manufacturing dates or the time elapsed between approval and recall. The time on the market and rates of recalls in PMA and 510(k) approved groups were analyzed.

Results: A total of 21,985 medical devices were approved during the 6 year period, 3,874 approved by PMA and 18,111 approved by 510(k). The total number of recalled medical devices was 159; 17 approved by PMA, and 142 approved by 510(k). The proportion of medical devices approved by PMA rose from 13% in 2005 to 20% in 2010. The total number of recalled medical devices for the same period of time grew almost two-fold (from 23 in 2005 to 45 in 2010). The most commonly recalled devices were cardiovascular (32%) and general hospital devices (25%), followed by anesthesia (11%), chemical (10%), and gastroenterology/urology (4%). Recalled medical devices spent on the market between 31 and 69 months (Class 1: 34.6±4.6 months, Class 2: 40.6±4.8 months, Class 3: 36.1±34.5 months). There was a non significant trend toward longer marketing time for 510(k) approved devices (PMA: 31±26 months; 510(k): 40±40 months, p>0.467). The proportion of PMA recalls to PMA approvals and the proportion of 510(k) recalls to 510(k) approvals showed no statistically significant difference (PMA: 0.2-0.6% of 3,874, 510(k): 0.58-0.82% of 18,111).

Conclusions: PMA approved medical devices are not on the market longer than 510(k) approved devices before a recall. The proportion of recalls did...
not differ significantly between 510(k) and PMA approved devices. These findings suggest that clinical trials usually lasting for a year as part of the PMA process will not determine the number of recalls or identify problem leading to recalls. It appears that the 510(k) process does not increase the risk of recalls. While the US device approval system has its strengths and weaknesses, it must be thoroughly evaluated before any attempt made to demolish something that served well for so many years, the 510(k) process.

CONTROL ID: 1244673
DO INTERNATIONAL MEDICAL GRADUATES HAVE LOW PATIENT SATISFACTION RATES?
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CURRENT CATEGORY: Epidemiology/Health Care Outcomes/Quality Improvement/Bioinformatics
ABSTRACT BODY: BACKGROUND: With the number of international medical graduates (IMG) increasing and their population being more than a quarter of total physician workforce, their ability to satisfy patients has become a question which has never been studied. A recent study published in Health affairs (2010) showed that foreigners who are international medical graduate have the same patient mortality rate as their US graduate counterparts. The purpose of this study is to examine if there is a difference between the patient satisfaction scores of US medical graduates (USMGs) and IMGs. METHOD: This retrospective study was done in the Southwestern Regional of the Mayo Clinic Health System in Minnesota. We evaluated 2627 randomly selected patient experience surveys treated by 55 different physicians between August 2009 and August 2010. All surveys were collected through an independent contracted firm measuring patient satisfaction. The group of physicians contained 42 USMGs and 13 IMGs. Overall patient satisfaction data of these two groups were compared to evaluate the difference in patient satisfaction. Tests included independent t-test and t-inverse test, with a significance level of 0.05. RESULT: No statistical difference in overall patient satisfaction between the two groups was identified. The results of the t-test lead to conclude with 95% confidence that there is no difference between the patient satisfaction scores of the two groups of physicians. T-inverse test confirms the results of t-test and lead to conclude with 95% confidence that the scores of the two groups of physicians are similar. CONCLUSION: It is concluded that USMGs and IMGs have similar capability to satisfy their patients and also that patients did not rate USMGs and IMGs differently.

CURRENT CATEGORY: Epidemiology/Health Care Outcomes/Quality Improvement/Bioinformatics
CONTROL ID: 1343195
CHICAGO INEQUITIES IN GEOGRAPHICAL ACCESS TO HEALTH CARE
CURRENT CATEGORY: Epidemiology/Health Care Outcomes/Quality Improvement/Bioinformatics
ABSTRACT BODY: Access to health care was once thought of in terms of access to physicians, nurses, hospitals and clinics. However, the concept of health care access has broadened, and social determinants of health and distribution of access have become significant considerations when examining differences in clinical outcomes between populations. Increasingly, pharmacists are providing supportive roles in both health education and medical management, and some studies have shown a benefit of these interactions. The use of Geographic Information Systems (GIS) mapping technology can be used as a tool to help identify areas that are vulnerable to distribution inequities. By using GIS to identify medically underserved areas, community health centers, pharmacy locations, and distance to pharmacies for each census tract in Chicago, we demonstrate that there are significant distribution inequities for access to pharmacies. Thus, with unequal distribution of pharmacies, particularly in areas that are already medically underserved, patients may face a two-fold problem: 1) less access to medicines and 2) less opportunity for access to their pharmacist for health education and medical management. By using GIS as a tool to investigate health inequities, locations in need can be focused on through a collaboration of medical, public health, and public policy professionals. Future research should explore whether inequities in pharmacy access are a significant factor in the clinical outcomes of the populations affected.

Gastroenterology/Clinical Nutrition
CONTROL ID: 1343359
SAFETY OF ENALAPRIL AFTER ABDOMINAL IRRADIATION
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CURRENT CATEGORY: Gastroenterology/Clinical Nutrition
ABSTRACT BODY: Angiotensin-converting-enzyme inhibitors (ACEi) mitigate experimental and clinical radiation injuries to kidneys, lung, and skin. Whole or partial body irradiation may expose the gastrointestinal (GI) tract. ACEi do not mitigate GI radiation injury in rats, but the GI tract is likely to be included in a radiation exposure to kidneys. GI Tolerance of ACEi must be understood. We tested the ACEi enalapril, then enalapril, in a canine model. Five 20 to 25 kg dogs underwent 600 cGy abdominal irradiation. Each dog served as their own non-irradiated control. Gastrointestinal motility was assessed by surgically-placed intestinal and colonic strain gauges. Enalapril, 0.625 mg/kg, intravenously, twice daily, alone did not affect GI motility or animal well-being. Irradiation (rads) caused significant increases in retrograde giant contractions (RGC), giant migrating contractions (GMC), and their respective correlates, vomiting and diarrhea, within the first week after rads. Rads followed by enalapril caused no significant change in RGCs or GMCs but all dogs in this group were clinically worse than irradiated dogs not on drug; they were euthanized within a week after rads. In a second experiment, 6 dogs also underwent 600 cGy abdominal rads, 3 were instrumented with strain gauge transducers and 3 were non-instrumented. Animals were irradiated with a single dose of 600 cGy and started enalapril 0.5 mg/kg, once a day at one week after rads. All dogs had vomiting and diarrhea, which were maximal on the day of radiation. During the second week after rads, irradiated dogs taking enalapril were clinically well, had no vomiting or diarrhea, and their GI motility monitoring was not different from their control recordings before rads. We conclude that after abdominal irradiation, immediate use of ACEi may enhance GI toxicity and cause greater mortality, but starting ACEi at one week after abdominal x-ray exposure has no adverse effects. This is critically important for the use of ACEi to mitigate other organ injuries, such as those to kidneys and lungs, since starting ACEi to mitigate radiation nephropathy or pneumonopathy is not needed until one week or more after irradiation. Starting ACEi within the first week of a total body exposure is not necessary and may be harmful.

CURRENT CATEGORY: Gastroenterology/Clinical Nutrition
CONTROL ID: 1319068
IMIPRAMINE BLOCKS ETHANOL-INDUCED ASMASE ACTIVATION, CERAMIDE GENERATION, AND PP2A ACTIVATION, AND AMELIORATES HEPATIC STEATOSIS IN ETHANOL-FED MICE
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CURRENT CATEGORY: Gastroenterology/Clinical Nutrition
ABSTRACT BODY: Our previous data showed the inhibitory effect of ethanol on AMPK phosphorylation appears to be mediated in part through increased levels of hepatic ceramide and activation of PP2A (protein phosphatase 2A). The effect of ethanol on AMPK phosphorylation was reversed by imipramine, suggesting that the generation of ceramide via ASMase is stimulated by ethanol. In this study, we determined the effects of imipramine on the development of hepatic steatosis, the generation of ceramide, and downstream effects of ceramide on inflammatory, insulin, and apoptotic signaling pathways, in ethanol-fed mice. Methods: The effect of ethanol and imipramine (10 μg/g body weight intraperitoneally) on ceramide levels, as well as inflammatory, insulin, and apoptotic signaling pathways was studied in C57BL/6d mice fed the Lieber-DeCarli diet. Results: Ethanol-fed mice developed the expected steatosis and co-treatment with imipramine for the last 2 weeks of ethanol feeding resulted in improvement in hepatic steatosis. Ethanol feeding for 4 weeks induced impaired glucose tolerance when compared to controls and this was modestly improved with imipramine

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Interleukin 6 (IL-6) is a pro-inflammatory cytokine whose expression has been found to be increased in patients with acute lung injury (ALI), an inflammatory disease often found in conjunction with severe sepsis. Our aim was to identify known single nucleotide polymorphisms (SNPs) in a large cohort of ALI/severe sepsis patients, and analyze the association of those variants with disease. METHODS: Genomic DNA was collected from blood from 602 individuals Johns Hopkins University, The University of Tennessee Health Science Center, and The University of Chicago (n=224 cases: 77 African American (AA), 147 European American (EA)). Fifteen IL-6 SNPs were genotyped using Applied Biosystems SNPlex multiplex system. Data were analyzed using SNPStats, and PLINK.

RESULTS: Four SNPs were found to be protective against ALI/severe sepsis (rs2069824, rs2069837 in EA populations, and rs2069842 in AA populations). Six SNPs were found to increase susceptibility to ALI/severe sepsis in patients. Four SNPs in EA populations, including one variant that has previously been identified with increased plasma IL-6 levels, increased susceptibility in AA populations. Haplotype analysis was performed using PLINK. Significant haplotypes (Bonferroni adjusted p<0.01) include one 9 SNP haplotype, and one 6 SNP haplotype in the EA population that have previously been associated with ALI. The highest omnibus p-value in these groups was equal to 4.78 E-05. Additionally, in the EA population we have also identified a novel 3 SNP haplotype that modifies ALI susceptibility, with significant omnibus p-values less than 0.01. CONCLUSION: We have identified several IL-6 SNPs and haplotypes that modify ALI/severe sepsis susceptibility, including one polymorphism that has previously been linked to increased IL-6 protein levels in plasma.

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CONTROL ID: 1344901
RESEARCH FINDINGS IN A PUBLIC CONSULTATION TO MAP THE FUTURE OF PUBLIC HEALTH GENOMICS
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CURRENT CATEGORY: Genetic & Molecular Medicine
ABSTRACT BODY: Purpose of Study: In the Summer of 2011 the U.S. Centers for Disease Control and Prevention Office of Public Health Genomics (CDC/OPHG) conducted a stakeholder consultation, administered by the University of Michigan Center for Public Health and Community Genomics and Genetic Alliance, to recommend priorities to advance the field of public health genomics from 2012 through 2017. This consultation brought to fruition several prior anticipatory efforts by the Institute of Medicine (IOM 2004) and others. Inter-rater feedback on priority areas and practices were solicited by the CDC/OPHG from June 30, 2011 through August 1, 2011; (2) 9 key informant interviews representing public health practice, academy and the community; (3) 3 informal discussion groups involving diverse communities, public health practitioners, and stakeholders at Genetic Alliance’s annual meeting; and (4) a concluding all-day meeting of ~70 public health genomics leaders. Steps in the analysis: (1) formation and refinement using NVivo9 of an initial list of topic areas and sub-themes from existing literature and prior community dialogues; (2) raw compilation of respondent data into Word tables; (3) Planning Committee review of raw data and data summaries; (4) formation of preliminary recommendations based on IOM-developed public health core functions and Committee-identified major themes; (5) breakout groups review of literature and materials developed, yielding outcome areas and priority action items; and (6) development of final short- and long-term recommendations.

Consensus: Nine major response categories (e.g., cancer and cardiovascular disease intervention, genetics education) were consolidated into 5 core areas – assessment, policy development, assurance, system management, and research. Areas of contention between respondents included: promotion of genetic testing vs. focus on the environment; amount of funding allocated to validating population-based genetic testing; focus on rare, highly penetrant conditions vs. common, chronic illness; appropriate fit of pharmacogenomics into the population framework. Final priority areas included: engaging healthcare organizations in the study of “low hanging fruit” – valid markers (including family history) needing trial testing; increased “T3/T4” investigation of genetic services delivery and outcomes; concerted translation to end users – physicians, public health practitioners, policymakers, and consumers (including guides to access information from sources like NIH’s Genetic Testing Registry); increased utility through cascade and life stage screening; embedding genetics into all aspects of health care, e.g., through national data standards and health IT / EMR priorities. Conclusions: Methodologic – Inter-rater feedback on priority areas and committee consensus building on useful frameworks and recommendations priorities are a necessity. Research teams must deal with practical constraints, e.g., in the number of permissible interviews, time constraints for qualitative analysis, and variety of responses that can be gathered. Summative – The recommendations require participation of a diversity of stakeholders to be realized. Interagency (CDC, NIH, HRSA) coordination and public/private partnering are needed. Engagement of communities will require creative communication vehicles such as social media, continued trust-building, and clear, community-partnered information. Various objectives (e.g., developing key genetics innovation driving questions for clinicians – 2 years / integration into national health information systems – 5 years) occupy differing time scales.

CONTROL ID: 1344398
DNA METHYLATION CONTRIBUTES TO THE POPULATION VARIATION IN EXPRESSION OF THE PHARMGKB VERY IMPORTANT PHARMACOGENES
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CURRENT CATEGORY: Genetic & Molecular Medicine
ABSTRACT BODY: Background: DNA methylation plays an important role in regulating gene transcription, and the genome-wide landscape of DNA methylation has been observed to be altered in some human diseases including cancer. In addition, it is likely that DNA methylation could affect individual response to therapeutic treatments through regulating genes related to drug metabolism. The very important pharmacogenes (VIPs) (http://www.pharmgkb.org) were selected by the PharmGKB owing to their significant effects on drug treatment both at the pharmacokinetic and pharmacodynamic levels. To further explore the baseline variation of DNA methylation and its potential regulatory role on the expression of these genes between human populations, we analyzed genome-wide DNA methylation data on individual CpG methylation sites in a collection of human lymphoblastoid cell lines (LCLs) from the HapMap Project. Methods: DNA methylation was evaluated in 74 unrelated African (YRI - Yoruba people from Ibadan, Nigeria) and 60 European ancestry (CEU - Caucasians from Utah, US) LCLs using the Illumina HumanMethylation450 BeadChip, which interrogates methylation level (represented by average beta value) for CpG methylation sites at single-nucleotide resolution. In total, 715 CpG methylation sites (probes) within 43 VIPs were analyzed between the two populations. Particularly, CpG methylation sites showed hypomethylation (average beta value ≤0.2) or hypermethylation (average beta≥0.8) in both two populations were removed. The Wilcoxon rank sum test was performed to identify differential methylation sites between the YRI and CEU samples. The results showed that DNA methylation may play a critical role in regulating drug response-related genes. Therefore, integration of DNA methylation may provide novel insights into the regulation of current pharmacogenomic loci. Finally, by analyzing these VIPs as an example, we illustrated the possibility of identifying the epigenetic contribution to gene regulation in humans.

CONTROL ID: 1344887
PATIENTS WITH FLT3 NEGATIVE ACUTE MYELOID LEUKEMIA AND NORMAL CYTOGENETIC WHO HAVE THE COMBINATION MUTATIONS (NPM1/CEBPA) HAVE BETTER SURVIVAL THAN PATIENTS WHO HAVE THE COMBINATION MUTATIONS (NPM1/CEBPA+)
S.A. Srour, P. Akl, S. Butt, H. Bhawardj, T. Dunn, S. Pant, J. Holter, M.A. Cherry OUIISC, Oklahoma City, OK.
CURRENT CATEGORY: Genetic & Molecular Medicine
ABSTRACT BODY: Introduction: Acute Myeloid Leukemia (AML) is the most common type of acute leukemia in adults. About 50% of patients with AML have normal karyotype, and are categorized as intermediate-risk group. However, the response to treatment in this group is heterogeneous. There is high interest in characterizing molecular genetic features in the intermediate-risk AML patients (pts) that might rectify their stratification risk. In this group, FLT3-ITD (Internal Tandem Duplication) (Tyrosine Kinase Domain) mutations are known to confer unfavorable risk whereas NPM1 and CEBPA mutations are known to be favorable risk markers. The purpose of this study is to analyze the combination of NPM1 and CEBPA mutations in presence or absence of FLT3 mutations on prognosis of AML pts who have been managed at our institution which is considered the State’s largest tertiary care center. Patients and Method: We performed a retrospective chart review of all pts with AML evaluated at University of Oklahoma Health Sciences Center between January 2000 and December 2010. Patient’s age, gender, race, laboratory and clinical data as well as bone marrow biopsy and aspirate findings were reported. PCR and Fragment Analysis were conducted on all available DNA preserved bone marrow materials to test the FLT3, NPM1 and CEBPA mutations. For statistical analysis, Kaplan-Meyer curve was used.
Results: A total of 239 pts were evaluated. Male to female ratio was 2/1. Median age at diagnosis was 46 years. 21 out of the 239 pts were less than 18 year-old. DNA samples were present on 132 pts and mutation analyses for FLT3, CEBPA and NPM1 were performed. Correlation between mutations and AML prognosis was determined. 67/132 (50.8 %) patients were categorized into intermediate-risk group (majority of patients had normal cytogenetics). 14/67 (20.9%) pts were FLT3+ (FLT3-ITD or FLT3-TKD mutation), 17/67 (23.9%) were NPM1+. 7/67 (10.4%) were CEBPA+. Kaplan-Meier curve was used to identify cumulative proportion surviving over time. FLT3 presence or absence itself was not identified to be statistically significant (p = 0.416) in terms of overall survival. Interestingly, presence or absence of combined NPM1/CEBPA mutation in FLT3 negative pts, among intermediate risk group, was found to be statistically significant (p < 0.05) in determining overall survival. In this subgroup, presence of NPM1/CEBPA combination (NPM1+/CEBPA+) was associated with poor prognosis, while absence of NPM1/CEBPA combination (NPM1-/CEBPA-) carried a better prognosis.

Conclusion: Results of our study highlight the importance of performing combinations of mutation analysis in evaluation of overall prognosis in AML pts. FLT3-NPM1+ profile in pts with normal cytogenetics is thought to confer a favorable prognosis. We demonstrated in this study that using combination mutation analysis in pts with FLT3+ can change the risk stratification in patients with intermediate-risk group and might affect therapeutic interventions in this patient population. Larger prospective studies are needed in the future for further validation of our findings.

CONTROL ID: 1292050
NEURODEVELOPMENTAL IMPACT OF CONGENITAL HEART DEFECTS IN DOWN SYNDROME
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CURRENT CATEGORY: Genetic & Molecular Medicine
ABSTRACT BODY: Purpose: Down syndrome (DS) is the most common genetic cause of intellectual disability/mental retardation with an incidence of 1 in 691 live births. Nearly half of all children with DS are born with a congenital heart defect (CHD). Atrioventricular septal defect (AVSD), the most common form of CHD in DS, occurs in 31–61% of children with DS and CHD, yet, virtually no studies have examined their neurodevelopmental outcomes. This study is the first to characterize the early developmental profiles and home environment of children with DS and AVSD (DS + AVSD) compared to age-matched children with DS without CHD (DS – CHD).

Methods: Participants consist of 2 groups: 12 subjects with DS + AVSD (mean age 14.5 ± 7.3 months) and 18 subjects with DS – CHD (mean age 14.1 ± 8.4 months). The Bayley Scales of Infant and Toddler Development III (cognitive, language, and motor) was administered by a psychometrician who was blind to each subject’s cardiac status. Additionally, parental stress and home visit were captured using the Parenting Stress Index (PSI) and Home Observation for Measurement of the Environment (HOME) scale. The Bayley III, PSI, and HOME were administered to the DS – AVSD group after cardiac repair.

Results: The DS + AVSD cases exhibited lower composite scores in all domains relative to DS – CHD controls. Although the motor domain was the only domain that showed a statistically significant difference between groups (p < 0.05), both cognitive scores (p = 0.63) and language composite scores (p = 0.10) were marginally lower in the DS + AVSD cases compared with the DS – CHD controls. The DS + AVSD had higher PSI scores compared to DS – CHD group, indicating high parental stress levels persist after cardiac repair. The relationship between infant temperament and caregiver responsiveness in the DS + AVSD was significant, with caregiver demonstrating low levels of responsiveness when their child has a CHD.

Conclusions: Children with comorbid DS and CHD increasingly survive cardiac surgery, characterization of their early developmental trajectories, parental stress, and home environment is critical for designing early intervention to maximize individual potential. Our preliminary cross-sectional data document that children with DS + AVSD have greater developmental delays with higher parental stress level and mothers’ lack of responsibility compared to children with DS – CHD. These factors may contribute to greater developmental delays noted in the DS + AVSD group. Since this is the first study to examine the early developmental outcomes of children with DS and CHD, our findings may be useful for clinicians in providing anticipatory guidance and developmental surveillance.
We primarily use RT-PCR to identify changes in splicing in the Drosophila Orb2 mRNA. An isoform of Drosophila Orb2 that is necessary for stable response to various behavioral stimuli. Additionally, to identify trans acting regulatory mechanism and the putative trans-acting RNA binding protein could have implications for human memory formation.

CONTROL ID: 1313752
EFFECTS ON IPSILATERAL LOWER LIMB MUSCLES REVEALED WITH STIMULUS TRIGGERED AVERAGING OF EMG ACTIVITY IN MACAQUE MONKEYS
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CURRENT CATEGORY: Geriatrics
ABSTRACT BODY: Stimulus triggered averaging (STA) of EMG activity is well established as an effective method for identifying both excitatory and inhibitory linkages between motor cortex cells and motoneurons (Cheney and Fetz, 1985). Using this approach, work from our laboratory has yielded a rich new knowledge of the synaptic organization between cortical cells and motoneurons of upper and lower limb muscles (Boudrias et al, 2009; Griffin et al, 2009; Park et al, 2000; Hudson et al, 2010). Although output effects on muscle activity from contralateral primary motor cortex (M1) have been extensively documented using a variety of methods, studies of output effects from ipsilateral cortex have been much more limited. It is known that approximately 10% of the corticospinal axons descend ipsilaterally in the spinal cord (Hutchins et al, 1988; Lacroix et al, 2004; Dunn and Strick, 1966). These ipsilaterally projecting axons have been shown to terminate in intermediate layers of the spinal cord and also directly in the motor nuclei of distal muscles suggesting a relatively direct pathway by which ipsilateral stimulation might produce effects on muscle activity. Ipsilateral deficits associated with hemiparetic stroke demonstrate the potential functional importance of the ipsilateral motor cortex (Lewis and Brindley, 1965, Colebatch and Gandevia, 1989, Yarosh et al, 2004). The finding that bilateral pyramidotomy yields more severe deficits in limb movement than unilateral pyramidotomy also suggests a contribution of the ipsilateral corticospinal system to the control of limb movement (Porter and Lemon, 1993). Moreover, recovery of a patient from a unilateral section of the pyramidal tract was partially attributed to intact ipsilateral connections (Bucy et al, 1964). Despite the potential functional and clinical importance of the ipsilateral corticospinal projection, relatively little is known about the normal functional properties of this projection. The goal of this study was to use STA of EMG activity to investigate the properties (sign, strength, latency and muscle distribution) of poststimulus effects on lower limb muscle activity elicited from ipsilateral M1 cortex.

We compiled STA of EMG activity from 22 muscles of the hip, knee, ankle, and foot in rhesus monkeys (Macaca mulatta) during performance of a task which required the monkey to grip a manipulandum with its foot and produce leg extension-flexion movements against a light load. Compared to contralateral M1, mirror image sites in ipsilateral cortex produced effects that were broadly similar in sign and muscle distribution pattern. The minimum latencies of effects elicited from ipsilateral cortex were similar to those from contralateral cortex. The average magnitude of poststimulus facilitation from ipsilateral cortex was profoundly weaker than facilitation from contralateral cortex (9% vs 30%, peak increase over baseline at 120 µA). Double stimulus pulses were tested and increased ipsilateral facilitation by 50–75%. Although ipsilateral effects are much weaker, our latency data suggest that these effects are mediated, at least in part, through direct corticospinal connections.
were heterozygous for FVL gene mutation versus 8 heterozygous cases (21.05%) in the 38 patients with 3 sporadic pregnancy losses, p = 0.0045, versus 6 heterozygous and 2 homozygous cases (20%) in 41 patients with 4 pregnancy losses, (p = 0.0072).

When the thrombophilic physiologic hypercoagulability of pregnancy is superimposed on the thrombophilic Factor V Leiden mutation, the risk of thrombosis is increased 80 fold above normal, and placental ischemia with resultant fetal loss can occur because of thrombi obliterating the spiral arteries of the uterus. Caucasian women with recurrent, otherwise unexplained pregnancy loss should be screened for the FVL G1691A gene mutation. Obtaining pregnancy outcome history during medical evaluation of women is extremely important, because it provides crucial information for possible increased coagulation risk. Women with recurrent pregnancy loss, found to be hetero- or homozygous for the Factor V Leiden mutation can, in subsequent pregnancies be provided with the option to prospectively optimize subsequent live birth outcomes with low molecular weight heparin thromboprophylaxis, a treatable etiology for recurrent sporadic pregnancy loss. Identification of Factor V Leiden hetero- or homozygosity arising from the simple, inexpensive expedient of taking an accurate pregnancy outcome history has major diagnostic value for women’s first degree relatives, as well as for the proband woman herself.

CONTROL ID: 1344835
TARGETING HISTONE DEACETYLASES AS A NEW STRATEGY FOR GRAFT VERSUS HOST DISEASE PREVENTION
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CURRENT CATEGORY: Hematology and Oncology

ABSTRACT BODY: Background: Histone deacetylase inhibitors (HDACi) have emerged as an important class of anti-cancer agents. We and others have recently demonstrated novel immune regulatory effects by HDAC inhibition in experimental murine models. HDACi have been shown to: suppress pro-inflammatory cytokine production, (Reddy et al JCI 2008), activate STAT -3 acetylation, which is critical for the induction of IDO expression and histone H3/H4 are acetylated; (2) Tumor necrosis factor (TNF)-α is significantly reduced (p=0.04); and (3) Regulatory T cells are significantly increased (p=0.05), whereas absolute T cell numbers are not significantly different (day 30 CD4+, p=0.18; CD8+, p=0.25).

Results: In summary, the administration of vorinostat in the post-HCT setting appears safe and feasible and we have not identified any dose-limiting toxicities. All cases of acute GVHD have responded well to first-line treatment with steroids and there have been no cases of steroid-refractory GVHD during the study period. Furthermore, there have been no cases of severe grade 4 GVHD. The clinical results combined with the correlative studies suggest that vorinostat has activity in the prevention of GVHD, thereby exemplifying bench-to-bedside translation. Furthermore, these findings could lead to the development of an entirely new class of immunomodulatory therapy for GVHD and could have significant impact and potentially wide-ranging clinical applications in several other immunological diseases, including solid organ allograft rejection and autoimmune diseases such as lupus and diabetes.

CONTROL ID: 1344844
GH POTENTIATES ESTROGEN-DEPENDENT BREAST CANCER CELL PROLIFERATION IN AN IGFR-IR-INDEPENDENT BUT EGFIR-DEPENDENT MANNER
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CURRENT CATEGORY: Hematology and Oncology

ABSTRACT BODY: Breast cancer is a major public health problem in the U.S., causing the most cancer deaths in women second only to lung cancer. Estrogen receptor (ER) and Insulin-like Growth Factor-I Receptor (IGF-IR) interact and activate one another in breast cancer cells, and ER can up-regulate components of the IGF-I signaling pathway. Therefore, therapeutic strategies co-targeting ER and IGF-IR in ER-positive breast tumors have been developed. However, clinical trials of IGF-IR inhibitors have not been as successful as had been hoped. Growth hormone (GH) is implicated in having a role in breast cancer, but its effects are often attributed to the actions of IGF-I. Since inhibition of IGF-IR activity results in a marked increase in circulating levels of GH, we considered the possibility that GH itself may act directly on the tumor thus explaining the limited efficacy of IGF-IR antagonists in breast cancer. Using T47D human breast cancer cells, we find that GH not only acts in an IGF-I-independent manner, but also bypasses IGF-IR blockade to potentiate E2-stimulated proliferation. GH activates ERK via EGFIR (but not via IGF-IR), and requires ERK to potentiate E2-stimulated proliferation and transcription of ER target genes. We suggest that increased GH levels contribute to continued tumor growth and limited efficacy of IGF-IR inhibitors. This could have serious clinical implications for breast cancer patients directed toward IGF-IR-targeted treatments. Thus as researchers and clinicians we will behave us to regard GH and IGF-I as having both dependent and distinct functions in breast cancer biology, and to continue to investigate this critical issue. Ultimately, understanding the mechanisms by which GH bypasses IGF-IR inhibition may offer women new avenues for effective breast cancer therapy.

CONTROL ID: 1343707
ROLE OF MUC4 IN LUNG CANCER
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CURRENT CATEGORY: Hematology and Oncology

ABSTRACT BODY: Introduction: MUC4 is a high molecular weight type-I transmembrane protein composed to two subunits. The mucin-like subunit MUC4α has a N-terminal domain, unique 16-mer tandem repeat (TR) domain of variable length, a nidogen-like (NID) domain and an adhesion-associated domain (AMOP). This dimerizes with the growth-factor like subunit MUC4β carrying von-Willebrand factor, three epidermal growth factor (EGF) and EGF-like domains, a 21-mer transmembrane domain and
a short (22-mer) cytoplasmic tail (CT). MUC4 is believed to alter the prolifera- 
tion, differentiation, or cell-adhesion status of epithelial cells. MUC4 expression 
is seen in the trachea, main bronchi, lobar bronchi and alveolar epithelial cells. This 
study evaluates the role of MUC4 in non-small cell lung cancer (NSCLC).

**Methods:** Human NSCLC cell lines NCI-H292 and A549 were used. Stable clones of NCI-H292 in which MUC4 was down regulated using small-hairpin RNA against target sequence (5'-CAGCGAGCTGAGGGA CA-3') located 151 bp downstream of ATG (H292-shMUC4) and scramble 
transfected control sublines expressing endogenous MUC4 (H292-scr) were 
established as described previously. Stable clones of min-MUC4 transfected 
A549 (A549-MUC4) overexpressing an estimated 320 kDa MUC4 protein 
containing all domains of wild-type MUC4 but only 10% of the tandem 
repeats and empty vector control (A549-PsecTagC) were established. These 
cells were analyzed for proliferation, migration (scratch) motility (PET 
chamber), cell invasion (Matrigel) and apoptosis (Annexin). A lung cancer tissue 
microarray with 100 tissues (20 cases each of squamous cell carcinoma and 
adenoacinaroma, 10 cases each of small cell carcinoma and alveolar cell 
carcinoma, 10 cases of metastatic squamous cell carcinoma, 5 cases each of 
carcinoid, inflammatory pseudotumor, tuberculosis, cancer adjacent tissue, 
cancer adjacent normal tissue and normal lung tissue) was stained for MUC4 
expression. All experiments were performed in triplicates and analyzed with 
Student’s t-test or Mann-Whitney test. P-value <0.05 was considered to 
be statistically significant.

**Results:** We found that MUC4 attenuated in vitro proliferation of NSCLC 
cells. H292-shMUC4 grew and similarly, A549-PsecTagC C cells grew faster 
than A549-MUC4. Cell cycle analyses after synchronization with double- 
thymidine block showed higher percent cells in S-phase in H292-shMUC4 
and A549-PsecTagC cells compared to H292 and A549-MUC4. H292- 
shMUC4 and A549-vector cells exhibited higher apoptotic cell death. In 
addition, MUC4 abrogated proliferative signals. In H292shMUC4, with 
MUC4 down-regulation, significant increase in phosphorylation of Akt 
(Ser473) was observed with slight increase in total Akt level. We observed 
elevated levels of inactive p21GSK3β and Cyclin D and A as well as 
decrease in p21Cip1 and p27Kip1 in both H292-shMUC4 and A549- 
PsecTagC, compared to their MUC4 expressing sublines. In a scratch assay, 
H292-shMUC4 cells covered the scratch faster than the control. Similarly in 
the Boyden chamber assay, H292-shMUC4 and A549-PsecTagC cells 
showed decreased motility (4 and 3 fold, respectively) compared to H292-scr 
and A549-MUC4. We observed high levels of lysyl oxidase (LOX) in A549- 
PsecTagC compared to reduced levels in A549-MUC4. Similarly, over 
expression of LOX and decrease in LKB1 at transcript level was observed 
in H292-shMUC4 compared to control. HIF1α levels were increased in 
H292-shMUC4 supporting LOX upregulation. In the tissue microarray, 
MUC4 expression gradually decreased with increasing stage of malignancy 
(Mean composite score: Stage I = 2.4, Stage II=1.8, Stage III= 1.4 and 
Metastatic= 1.2, p= 0.0093).

**Conclusion:** MUC4 appears to have a novel tumor suppressor function in 
NSCLC.

**CONTROL ID: 1309173**

**AMAUROSIS FUGAX: PATHOETOLOGIC REVERSIBLE 
ASSOCIATIONS WITH THROMBOPHILIA IN PATIENTS 
WITHOUT CAROTID ARTERY ATHEROSCLEROSIS 
OR ATRIAL SEPTAL DEFECTS**

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**CURRENT CATEGORY:** Hematology and Oncology

**ABSTRACT BODY:** In patients with amaurosis fugax (AF) without carotid artery 
atherosclerosis or atrial septal defect, assess thrombophilia as a major treat- 
able pathoetiology with a goal to prevent permanent vision loss from retinal 
artery thrombosis.

**Design:** Nine patients with AF without carotid artery atherosclerosis or atrial 
septal defects were assessed for thrombophilia and hyperhomocysteinemia. 
PCR Studies were done for the Factor V Leiden, Prothrombin, MTHFR (C677T- 
A1298C), and plasminogen activator inhibitor-1 (PAI-1) 4G4G gene muta- 
tions. Serologic studies were done for resistance to activated protein C, 
proteins C S (total and free), antithrombin III, homocysteine, Factors VIII and 
XI, anticardiolipin antibodies ACL IgG and LgM, lupus anticoagulant, 
plasminogen activator inhibitor activity (PAI-Fx), and Lp(a).

**Setting:** Cholesterol Center Jewish Hospital.

**Patients:** Nine patients, six women, three men, age range from 27 to 
78 years old with AF documented by retinal specialist ophthalmologists.

**Results:** Of the Nine patients, one had high ACLA IgG and LgM, results 
were normal. The patients were normotensive, normocholesterolemic, and 
10 patients did not have any other thrombotic disorders. One patient was 
positive for lupus anticoagulant. The study could not demonstrate a 
positive association with thrombophilia and AF in the absence of athero- 
sclerosis or atrial septal defect.

**Discussion:** Amaurosis fugax is a neurovascular event caused by 
transient occlusion of the carotid artery. Amaurosis fugax is a major cause 
of transient visual loss, and is associated with a high risk of stroke. 
Thrombophilia is a leading risk factor for ischemic stroke. Thrombophilia 
is associated with a higher risk of AF. This study investigated the association 
between thrombophilia and AF in the absence of carotid artery atherosclerosis 
or atrial septal defect. The study findings suggest that thrombophilia may 
play a role in the pathogenesis of AF in the absence of carotid artery 
atherosclerosis or atrial septal defect.
4G4G homozygosity, and high levels of PAI-Fx, the PAI-1 gene product. Another patient had MTHFR C677T heterozygosity and elevated homocysteine and Methylmalonic Acid (MMA). One patient had high factor IX, one patient had PAI-1 4G4G homozygosity and one patient had high D-dimer and PAI-1a homozygosity. Because of increasing frequency of AF with longer duration of uni-ateral blindness, the patient with protein S deficiency was anticoagulated with coumadin under enoxaparin cover and maintained on coumadin, with a resultant sharp reduction in frequency of AF. Because of AF occurring approximatively 12 times per day, often accompanied by transient ischemic attacks, the patient with the prothrombin gene mutation was anticoagulated with enoxaparin, then coumadin, then Pradaxa with reduction of frequency of AF. Because of concurrent pulmonary embolii, the patient with PAI-1 gene 4G4G, high PAI-Fx, and MTHFR C677T homozygosity was anticoagulated with coumadin, with resolution of AF. The patient with elevated homocysteine and methylmalonic acid levels was treated with Vitamin B12, Folic acid and Vitamin B8 by normalizing of his methylmalonic acid and homocysteine levels and resolution of AF.

Conclusions: In patients free of carotid atherosclerosis or paradoxical emboli, familial thrombophilia is a pre-eminently treatable cause of recurrent AF. When AF is recurrent in patients with familial thrombophilia, with pro-gressive duration of loss of vision, to prevent retinal artery thrombosis and loss of vision, anticoagulation is therapeutic and prophylactic.

CONTROL ID: 1315562
CENTRAL RETINAL ARTERY AND VEIN THROMBOSIS: REVERSIBLE ASSOCIATIONS WITH THROMBOPHILIA
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ABSTRACT BODY: ABSTRACT BODY: In 133 patients with central retinal vein thrombosis (CRVO), and in 15 with central retinal artery thrombosis (CRAO) we assessed thrombophilia as a major treatable pathoetiology with a goal to prevent permanent vision loss.

Design: 133 patients with CRVO and 15 with CRAO (no carotid artery atherosclerosis, no atrial septal defects), and 105 healthy controls without ocular thrombosis were assessed for thrombophilia and hyperfibrinolysis. PCR Studies were done for the Factor V Leiden, Prothrombin, MTHFR (C677T-A1298C), and plasminogen activator inhibitor-1 (PAI-1) gene mu-tations. Serologic studies were done for resistance to activated protein C proteins C S (total and free), antithrombin III, homocysteine, Factor VIII and X, antitrombicidropin antibodies ACA IgG and ACA IgM, lupus anticoagulant, plasminogen activator inhibitor activity (PAI-Fx), and Lp(a).

Setting: Cholesterol Center Cincinnati, Cincinnati, OH.

Patients: CRVO and CRAO documented by retinal specialist opthalmologists. Of 133 CRVO patients, 122 were white, 6 black, 5 other, 55 men, 78 women, mean ± SD age 58 ± 14 yrs. Of 15 CRAO patients, 14 white, 1 black, 7 men, 8 women, mean ± SD age 52 ± 16 yrs.

Results: CRVO patients were more likely than controls to have the following:
1. high homocysteine 20/130 (15%) vs 2/102 (2%), OR = 9.1, 95% CI 2.1–39.9, X2 = 12, p = .0005;
2. high ACA IgM 15/128 (12%) vs 2/104 (2%), OR = 6.8, 95% CI 1.5–30.3, X2 = 8, p = .004;
3. high Factor VIII 23/117 (20%) vs 7/98 (7%), OR = 3.2, 95% CI 1.3–7.8, X2 = 7.0, p = .008;
4. low free protein S11/121 (9%), vs 2/92 (2%), OR = 4.5, 95% CI 1–21, X2 = 4.4, p = .037.

CRAO patients differed from controls:
1. Having higher homocysteine 5/15 (33%) vs 2/102 (2%), OR = 25.0, 95% CI 4.3–146, p = .0003;
2. Having lower protein C 4/15 (27%), vs 6/92 (7%), OR = 5.2, 95% CI 1.3–21.4, p = .03.

Based on age-gender-specific lipid distribution from healthy general populations in the Lipid Research Clinics Prevalence Study, the mean percentiles in 133 CRVO patients were 34% for total cholesterol (TC), 51% for triglyceride (TG), 49% for HDLC and 29% for LDL-C. In 15 CRAO patients, mean percentiles were 28% for TC, 40% for TG, 42% for HDLC, and 24% for LDL-C. Of the 133 CRVO patients, 7% had type 2 diabetes, 45% hyper-tension, 17% smoked compared to US population estimates of 8%, 24%, and 20%. Of the 15 patients with CRAO, 29% had type 2 diabetes, 43% had hypertension, and 20% smoked.

Conclusions: Treatable familial thrombophilia is common in patients with CRVO and with CRAO (free of carotid atherosclerosis or paradoxical emboli). High homocysteine, easily normalized by folic acid 5 mg, vitamin B6 100 mg, and vitamin B12 1 mg orally in both CRVO and CRAO. Hyper-tension, but not hyperlipidemia, is common in CRVO and CRAO, and type 2 diabetes is common in CRAO. Early diagnosis of thrombophilia in CRVO and CRAO should facilitate prevention of recurrent ocular thrombosis, protect vision, facilitate targeted family screening, and enable prevention of other thrombotic events.

CONTROL ID: 1344904
DEVELOPMENT OF NATURAL KILLER CELLS FROM HUMAN PLURIPOTENT STEM CELLS IN A FEEDER FREE SYSTEM AMENABLE TO CLINICAL TRANSLATION
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CURRENT CATEGORY: Hematology and Oncology

ABSTRACT BODY: Human natural killer (NK) cells are an attractive source of lymphocytes for adoptive immunotherapy and can achieve durable remissions in patients with poor-prognosis acute myelogenous leukemia (AML). In order to generate NK cells that are effective against a broader range of malignancies, our lab has focused on the generation of NK cells from human pluripotent stem cells. We have previously demonstrated the potency of NK cells derived from human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) both in vitro and in vivo. These previous studies utilized a stromal cell co-culture system to derive hematopoietic progenitor cells (CD34+CD45+ cells) in both CRVO and CRAO. Hyper-tension, but not hyperlipidemia, is common in CRVO and CRAO. Early diagnosis of thrombophilia in CRVO and CRAO should facilitate prevention of recurrent ocular thrombosis, protect vision, facilitate targeted family screening, and enable prevention of other thrombotic events.

CONTROL ID: 1314412
VARIATION IN DNA METHYLATION BETWEEN AFRICAN AND EUROPEAN HAPMAP POPULATIONS REVEAL POTENTIAL EPIGENETIC CONTRIBUTION TO RACIAL DISPARITY IN CANCER RISK

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Midwestern Regional Program Abstracts

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CURRENT CATEGORY: Hematology and Oncology

ABSTRACT BODY: Background: DNA methylation plays an important role in regulating gene transcription, as well as downstream phenotypes (e.g., disease risks, individual drug response). In order to understand how DNA methylation is altered in diseases such as cancer, we first need to comprehensively investigate genome-wide methylation patterns. As a complex trait itself, there is considerable inter-individual variability in DNA methylation patterns. In this study, we have gathered genome-wide DNA methylation data on individual CpG methylation sites in a collection of human lymphoblastoid cell lines (LCLs) from the HamMap Project. We evaluated the population differences in DNA methylation and their potential relationships with racial disparity in complex diseases (e.g., cancer).

Methods: We profiled DNA methylation in 74 unrelated African (YRI - Yoruba people from Ibadan, Nigeria) and 60 Caucasian (CEU - Caucausians from Utah, US) LCLs using the Illumina Human Methylation 450 BeadChip. Thischip interrogates >485,000 CpG methylation sites per sample at single-nucleotide resolution and assigns each site an average beta value, which is the fraction of signal obtained from the methylated bead signals over the sum of methylated and unmethylated bead signals. We corrected for potential batch effects due to array hybridization using COMBAT, and removed probes that contained known SNPs or mapped ambiguously to the reference genome. In our final analysis dataset, we included 46,236 probes on autosomes that showed relatively high variation (top 25% percentile) across the LCL samples based on coefficient of variation. The Wilcoxon rank sum test was performed to identify differentially methylated sites between the YRI and CEU samples. The cancer-related genes represented in this group of 988 differentially methylated sites, suggesting that promoter methylation patterns are more conserved between different ethnic populations. The differentially methylated sites were enriched in pathways and Gene Ontology terms such as "cell adhesion", "ECM-receptor interaction", and pathways involved in development such as WNT and Hedgehog.

Conclusions: A substantial number of CpG methylation sites were differentially methylated between individuals of African and European ancestry. These differentially methylated CpG sites appeared to be enriched in certain genomic regions and pathways. Significant population differences have been observed in a number of cancer-related genes, indicating the role of DNA methylation in determining racial disparities in these diseases. Future integration of this dataset with other available resources of these samples (e.g., genotypes, mRNA expression) will help elucidate the complex networks of genetic and epigenetic regulation of glycolysis in glioblastoma and indicate that anti-glycolytic therapies may be useful in treating malignancies that demonstrate AEG-1-overexpression.

CONTROL ID: 1310408
ENDOTHELIAL COLONY FORMING CELLS (ECFC) IN MULTIPLE MYELOMA
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CURRENT CATEGORY: Hematology and Oncology

ABSTRACT BODY: Introduction: Endothelial progenitor cells (EPCs) have been linked to angiogenesis in many cancers including multiple myeloma (MM). Most published reports on EPCs are based upon myeloid lineage cells, which can support angiogenesis, but cannot form blood vessels on their own. A recently described subpopulation of EPCs called endothelial colony forming cells (ECFCs) have a high clonogenic potential, an ability to differentiate into more mature endothelial cells, as well as in vitro and in vivo vasculogenesis. The role of ECFCs in the pathobiology of MM is unknown.

Objective: Determine the in vitro interaction of ECFCs and MM cells by using a matrigel assay, an MTS proliferation assay, and cell cycle analysis.

Methods: ECFCs derived from cord blood, or peripheral blood, of normal volunteers and MM patients were combined with human MM cells. MM cells and ECFCs were cultured alone or in co-culture and studied for in vitro tubulogenesis and were evaluated using a 2-dimensional matrigel assay, with images captured at 6 and 24 hours post culture, and analyzed using Image J. Vascular tubules regressed after 24 hours of culture. Therefore, to evaluate the interaction of ECFCs and MM cells over a longer duration, GFP-expressed ECFCs and cherry red expressing MM cells were cultured either alone or in co-culture for 3-5 days. T-tests were used to evaluate the differences between the 2 groups.

Results: In matrigel culture, MM cells preferentially associated with ECFCs as over 90% of MM cells migrated towards the newly formed vascular tubes. No statistical differences in the number of closed units, vascular area and branch length was observed between ECFCs cultured alone or with MM
cells. There was also no detectable difference in the proliferation of ECFCs or MM cells. In longer term cultures (3-5 days), the growth of MM cells was significantly reduced in the MM-ECFC co-culture compared to MM cells cultured alone (1.7 Vs. 2.8 fold of baseline, p = 0.008). ECFC growth was largely unaffected by MM cells.

Summary: Cell cycle analysis showed an increased G1 phase and decreased S phase when MM cells were co-cultured with ECFCs compared to MM cells cultured alone.

Conclusion: We demonstrated a tropism of MM cells for ECFCs. In contrast to the proliferation reported when HUVECs are co-cultured with MM cells, ECFCs keep MM cells in a quiescent state by causing G1 arrest. We hypothesize that ECFCs may induce a vascular niche for MM cells. The quiescent state could theoretically result in resistance to chemotherapeutics, which tend to target dividing cells. MM cells could subsequently acquire other molecular events allowing them to dislodge from the vascular niche and contribute to relapse. Additional work to understand the mechanisms underlying the interactions of ECFCs and MM cells, and whether this interaction could be targeted for therapy, are warranted.

CONTROL ID: 1344938
TARGETED THERAPY INDUCED LEUKEMIA: AN UNUSUAL PRESENTATION
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CURRENT CATEGORY: Hematology and Oncology
ABSTRACT BODY: Introduction: Head and neck cancers (HNC) account for about 3% of all cancers. Combined-modality treatment with chemoradiation or surgery/radiation is typically used for patients with locally advanced disease. Cetuximab, a novel recombinant human/mouse chimeric monoclonal antibody, is recently approved as a radio-sensitizer in locally advanced HNC. Cetuximab is associated with various hematologic/non-hematologic adverse effects, but no secondary malignancies are reported to date. We describe a case of high-risk myelodysplastic syndrome (MDS) that developed few months after cetuximab therapy.

Case Presentation: An 82 year-old white male patient, with significant history for locally advanced base of tongue squamous cell carcinoma, presents with persistent fatigue and loss of energy around 4 months after completion of cetuximab infusions. Weekly cetuximab was given concurrently with radiation with curative intent for 6 cycles. Chemoradiation therapy was poorly tolerated with severe local reactions (oral sores, esophagitis, skin erythema/ulcers, and weight loss). However, complete response was achieved with resolution of acute toxicity. Upon regular follow-up 4 months after cetuximab, workup for fatigue unexpectedly showed pancytopenia with macrocytosis (WBC 2.6 K/cmm; Hemoglobin 11.6 g/dl.; Platelets 80 K/cmm). Vitamin B12/folate were normal. Baseline counts prior to chemoradiation were normal except for slightly low platelets (119 K/cmm). Bone marrow biopsy was then performed; results were consistent with MDS, refractory anemia with excess blasts (RAEB-2) versus therapy-related MDS (t-MDS), with normal cytogenetic/molecular studies. Allogeneic transplant is the only curative therapy for RAEB-2 MDS. However, as our patient was poor transplant candidate, palliative standard therapy with azacitidine; unfortunately, after one cycle his condition rapidly deteriorated for which he decided to receive hospice. Patient died within 6 weeks of diagnosis.

Discussion: Therapy-related myeloid neoplasms (t-MN), including t-MDS, are well recognized complications that develop after exposure to cytotoxic agents or radiation. The latency period between first exposure and development of t-MN ranges from one to 10 years. Of the commonly implicating causes, alkylating agents and radiation typically cause t-MN after a latency period of 5 to 7 years. Topoisomerase II inhibitors have shorter latency period of 1 to 3 years. Around 90% of t-MNs have abnormal cytogenetics. Overall, elderly patients with t-MDS has poor prognosis with limited treatment options. To the best of our knowledge, cetuximab, an epidermal growth factor receptor inhibitor, has not been reported to cause t-MN. Our patient presents with high-risk MDS after around 5 months from starting chemoradiation. Radiation is unlikely cause for t-MDS given the shorter latency period. Hence, we speculate that cetuximab may be a potential cause for t-MDS in our case. Close monitoring of patients receiving cetuximab and reporting similar cases will be of crucial importance to prove causal relationship between cetuximab and t-MN.

Infectious Disease
CONTROL ID: 1310551
INDUCTION OF ANTIVIRAL CHEMOKINES IN MONOCYTE-DERIVED MACROPHAGES BY THE HTLV-2 TRANSACTIVATING PROTEIN, TAX2
G. Balistrieri, C.S. Barrios, L. Castillo, M.A. Beike Infectious Disease Division, Department of Medicine, Medical College of Wisconsin, Milwaukee, WI.
CURRENT CATEGORY: Infectious Disease
ABSTRACT BODY: Background: Members of the CC-chemokine family play a major role in innate immune responses against viral infections, including HIV-1. It has been previously shown that the human T lymphotropic virus type 2 (HTLV-2) transcriptional activator, known as Tax2 is a potent mediator of CC-chemokine expression in vivo and in vitro. This observation has clinical relevance in the clinical scenario of co-infections with HIV-1 and HTLV-2, based on recent data suggesting that HTLV-2 delays HIV-1 disease progression to AIDS. It is hypothesized that HTLV-2 infection of lymphocytes and macrophages could provide a source of chemokines that result in an antiviral response against HIV-1 infection. While previous literature provides strong preliminary evidence of HIV-1 infection in lymphocytic systems to down-regulate HIV-1 replication, this study examined the effect of Tax2 on antiviral chemokine production in monocyte-derived macrophages (MDMs).

Methods: Monocytes isolated from donor-derived peripheral blood mononuclear cells were cultured in vitro, allowed to mature into macrophages for 14 days, and treated with Tax2 or Tax1 at 100 pM, 10 pM, or 1 pM concentrations daily thereafter. Extracellular bacterial extract (ebe) and untreated samples were used as controls. Supernatant was collected from each sample every 1, 3, 5, 7, 10, 14, and 21 post-maturation and evaluated for MIP1a/CCL3, MIP-1b/CCL4, and RANTES/CCL5 by enzyme-linked immunosorbent assay. Two-way analysis of variance (ANOVA) and Tukey’s Honestly Significant Difference (Tukey HSD) tests were used to analyze the results.

Results: MDMs expressed significantly higher concentrations of chemokines MIP-1a, MIP-1b, and RANTES when cultured in the presence of Tax2 or Tax1 at all tested concentrations as compared to controls. These results were significant (p<0.001) by ANOVA and Tukey HSD at all measured days post-treatment. There was no consistently significant difference in chemokine expression between Tax1- and Tax2-treated samples, between ebe-treated and untreated samples, or between samples treated with different concentrations of Tax protein.

Conclusions: The increased expression of MIP-1a, MIP-1b, and RANTES suggests that alterations in innate immune responses via stimulation of antiviral chemokine production and release may play an important role and should guide the development of therapies to treat HTLV-2 infected individuals. Further work will be needed to determine the exact nature of HIV-1/HTLV-2 interactions in macrophagic reservoirs of HIV-1.
Control ID: 1318357

Bacillus anthracis lethal toxin impairs alveolar epithelial barrier function

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Current category: Infectious Disease

Abstract Body: Rationale: The lung is the site of entry for Bacillus anthracis (Ba) in inhalation anthrax, the deadliest form of the disease. Ba produces virulence toxins that are necessary for clinical disease in higher animal models, including lethal toxin (LT). Prior to studies previously reported from our laboratory, it was thought that human alveolar macrophages (AM) were the primary target of the Ba LT because mouse macrophages are killed by apoptosis through LT-induced MAP kinase kinase (MEK) cleavage. We have reported that human AM are not a target of LT, as they do not contain receptors for the binding component of the toxin, protective antigen (PA), and do not undergo MEK cleavage or undergo apoptosis in response to the toxin. We sought to determine whether the cells that line the functional respiratory units of the lung, alveolar epithelial cells (AEC), are a target of the B. anthracis virulence factor lethal toxin (LT).

Methods: We measured the effect of Ba LT on primary human AEC MEK cleavage by immunoblot, barrier function by measurement of trans-epithelial resistance (TER), and cell viability by propidium iodide staining and intrinsic dehydrogenase activity. The effect of LT exposure on AEC subcellular morphology was also assessed by electron microscopy (EM).

Results: In contrast to our findings for AM, human AEC were sensitive to LT. Epithelial barrier function was significantly impaired as measured by a 30% drop in TER. Consistent with these results, AEC, unlike AM expressed significant amounts of the anthrax toxin receptor TEM1/ANTXR1. The receptor was functional as LT exposure resulted in cleavage of multiple MEKs, as assessed by immunoblot, and as assessed by measurement of binding of PA to AEC. Surprisingly, despite these effects, LT exposure did not affect AEC viability as determined by propidium iodide staining or intrinsic dehydrogenase activity. LT exposure did, however, impair formation of tight junctions as assessed by EM.

Conclusions: These findings show that in contrast to AM, human AEC are a target of the B. anthracis virulence factor LT. Compromise of alveolar epithelial barrier function by LT may play a major role in the pathogenesis of inhalation anthrax by facilitating the dissemination of B. anthracis from the lung in early phases of illness, and by promoting barrier disruption and subsequent lung edema that occurs in terminal phases of the disease.

Control ID: 1336743

Prevalence of vancomycin heteroresistance in Staphylococcus aureus in a tertiary care teaching hospital

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Current category: Infectious Disease

Abstract Body: Background: Heteroresistance refers to presence of subpopulations with lesser degree of antibiotic susceptibility within a larger population of fully susceptible bacterial population. Over the last few years, Staphylococci have emerged as predominant bacteria known to have such resistant subpopulations. Staphylococci with heteroresistance to Vancomycin associated with VISA have been isolated from all types of cultures. hVISA have minimum inhibitory concentrations (MICs) for vancomycin in the susceptible or intermediately susceptible range (MIC between 0.5 and 4) and likely represent a step on the path to the development of vancomycin-intermediate Staphylococcus aureus (VISA). hVISA subpopulations are present at frequencies of only 10-5 to 10-6 in standard inocula.

AIM: Estimation of the prevalence of hVISA in Mid-Atlantic Medical Center in Springfield, IL. To determine if it is cost effective to routinely perform the test in the clinical microbiology laboratory.

Methods: Population analysis profile / area under the curve (PAP/AUC) method is the gold standard being a labor intensive process it is not suitable for the clinical microbiology laboratory. Other methods like broth micro dilution method, macro E test and Glycopeptide resistance detection (GRD) E test method have been in use. In our study, we used GRD E test strips approved by the FDA for detection of hVISA. A total of 288 isolates of MRSA from patients admitted at Memorial Medical Center (MMC) during 2009 and 2010 were tested for heteroresistance. Suspension of several well isolated colonies from overnight blood agar plate was prepared in Mueller-Hinton broth and turbidity was adjusted to 0.5 McFarland standards. Using a sterile, non-toxic swab inocula were streaked on 5% Blood agar plates. Isolates were tested with GRD E test strip validated with Nema CH8 applicator. The plates were read at 24 and 48 hours after incubation. ATCC 700698 strain was used for quality control.

Results: The prevalence of hVISA isolates in Memorial Medical Center was 2.7%. The prevalence of hVISA varies in the different parts of USA from 0.3% to 8.3%. A recent study done in 2010 with 4210 isolates, estimates the prevalence of hVISA in United States at about 0.3 % on an average. Other studies have noted that 10.5% of hVISA isolates had MIC of 2 μg/ml to vancomycin and only 0.1% of isolates had a MIC of 1 μg/ml to vancomycin. 100% of our heteroresistant strains had an MIC of >2, which were read to be completely susceptible by the standard tests used in the microbiology laboratory. Thirdly, all the isolates from our study were from a device related infection.

Conclusion: Using this value of prevalence of hVISA at Memorial Medical Center, a cost analysis showed benefit of routinely employing Etest GRD for hVISA detection in patients with Bone and Joint Infections with and without hardware.

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CONTROL ID: 1308179
IMPACT OF CATHETER TYPE ON MICROBIOLOGY OF CENTRAL VENOUS CATHETER INFECTION

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CURRENT CATEGORY: Infectious Disease

ABSTRACT BODY: Background: Several types of Central Venous Catheter’s (CVC’s) are currently in use for various indications. CVC’s carry a risk of becoming infected which negatively impacts patient outcomes. Early and appropriate antimicrobial therapy based on suspected organism’s favors treatment success.

Methods: Retrospective review of medical records for hospital admissions due to CVC related complications from October 2008 to December 2010 was conducted. Data regarding the type of catheter used and the resulting microbiology was evaluated. Odds ratios and relative risk were calculated with Chi-square analysis determining significance of association.

Results: Using the Chi-square Continuity Correction, significant associations were made between peripherally inserted central catheters (PICC) and the occurrence of Staphylococcus aureus ($\chi^2 = 6.920, p = .005$) and other gram positive organism ($\chi^2 = 3.331, p = .034$). Results suggested individuals with a PICC were 2.378 times more likely to present with Staphylococcus aureus and .438 times more likely to present with other gram positive organism. Non-tunneled and implanted catheters were not associated with increased risk of any of the tracked microbiology variants (i.e Staphylococcus aureus, CONS, Gram negative bacilli, Candida species, or other gram positive organism). PICCs were not associated with increased risk of Gram negative bacilli, Candida species, or other gram positive organisms. Tunneled catheters were not associated with increased risk of CONS, Gram negative bacilli, or Candida species.

Conclusions: Findings demonstrate that the type of catheter can influence variations in the microbiology of CVC infections. These data have implications in choosing initial presumptive antimicrobial therapy and the decision to remove the CVC.

CONTROL ID: 1343319
INFLUENCE OF DIABETES MELLITUS ON MICROBIOLOGY OF CENTRAL VENOUS CATHETER INFECTION

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CURRENT CATEGORY: Infectious Disease

ABSTRACT BODY: Background: Central Venous Catheter (CVC) related infection have a great impact on morbidity and mortality of patients as well on the health care cost. Patients with diabetes mellitus remain at a higher risk for various types of infection. Early and appropriate antimicrobial therapy based on suspected organisms improves outcomes.

Methods: Retrospective review of medical records for hospital admissions due to CVC related complications from October 2008 to December 2010 was conducted. Data regarding resulting microbiology was evaluated in patients based on whether they had history of diabetes mellitus or not. Odds ratios and relative risk were calculated with Chi-square analysis determining significance of the association.

Results: Using the Chi-square Continuity Correction, a significant association was found between history of diabetes mellitus and the occurrence of Staphylococcus aureus ($\chi^2 = 2.743, p = .049$). Results suggested individuals with a history of diabetes mellitus were 2.743 times more likely to present with Staphylococcus aureus. No significant association was found between a history of diabetes mellitus and coagulase negative Staphylococci species ($\chi^2 = 911, p = .170$), gram negative bacilli ($\chi^2 = 576, p = .224$), Candida species ($\chi^2 = 1.641, p = .100$), or other gram positive organisms ($\chi^2 = .003, p = .955$).

Conclusions: Findings demonstrate that history of diabetes mellitus increases risk of Staphylococcus aureus related CVC infections but not the other organisms. Patients with diabetes, particularly those who inject insulin daily, often have asymptomatic nasal and skin colonization with Staphylococcus aureus. Colonization may predispose to cutaneous or incisional staphylococcal infections as well as transient bacteremia. These data have implications in choosing initial presumptive antimicrobial therapy and the decision to remove the CVC.

CONTROL ID: 1315033
AEROSOLIZED AMIKACIN, AN OPTION FOR TREATMENT OF MULTI-DRUG RESISTANT PNEUMONIA IN A PATIENT WITH CHRONIC KIDNEY DISEASE

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CURRENT CATEGORY: Infectious Disease

ABSTRACT BODY: Abstract: Multi-drug resistant pneumonia is a difficult entity to treat specially in a patient with chronic kidney disease (CKD) since it leaves physicians with only a handful of antibiotics to choose from. Aerosolized administration of antibiotics is one route that can be contemplated in a patient with poor renal function to avoid the nephrotoxic effect of the antibiotics.

Case Report: A 62-year-old female presented to the ER from a nursing home with complaints of increasing cough and sputum production. She was started on cefepime at the nursing home; however, her creatinine and blood urea nitrogen (BUN) kept rising, so she was transferred to the hospital for further management. Her past medical history was significant for diabetes mellitus, chronic kidney disease (CKD), and chronic obstructive pulmonary disease. On presentation her vitals were as follows: temperature 100.8°F, blood pressure 104/80 mmHg, pulse 117/min and respiration 20/min. Exam was remarkable for decreased breath sounds bilaterally with diffusely scattered rales and bilateral lower extremity stasis dermatitis.

Lab: WBC 15.2 Thou/mm3 with 82 % segments; hemoglobin 11 g/dl, BUN 31 mg/dl and creatinine 1.82 mg/dL. A CXR showed a left lower lobe infiltrate with effusion. Later, sputum cultures revealed Pseudomonas aeruginosa which was sensitive to amikacin only and resistant to rest of the antimicrobials. Patient was was started on intramuscular (IM) amikacin, 250 mg every 12 hours, keeping her CKD in mind. Amikacin peak and trough levels remained therapeutic. However, patients creatinine continued to worsen (2.3 mg/dl) and her urine output decreased with the treatment. A follow-up sputum culture again grew Pseudomonas aeruginosa with the same sensitivity and the clinical picture did not improve. Therefore, aerosolized amikacin therapy was begun at a dose of 250 mg in 2 mL of normal saline every 12 hours in an attempt to treat the pneumonia. Nebulized albuterol was used prior to the administration of amikacin to enhance delivery of the antibiotic to distal segments of the lung.

The patient’s symptoms and signs started to improve within 48 hours of aerosolized amikacin treatment. The white cell count started to trend down. A follow-up sputum culture after a week of therapy showed complete clearing of the pseudomonas. Blood levels of amikacin were undetectable (<2.5 mg/g/mL) while the patient was receiving aerosolized therapy. Renal function remained stable throughout the 10-day course of this treatment. The patient recovered from the illness and was ultimately discharged from the hospital. A follow-up CXR showed complete resolution of the left lower lobe infiltrate.

Conclusion: Aerosolized administration of antibiotics to treat multi-drug resistant pneumonia in critically ill patients is not recommended due to the lack of well designed clinical trials and possible development of microbial resistance. However, more recent studies show that aerosolized administration of antibiotics may be effective in patients with multi-drug resistant pneumonia without adversely altering the ICU bacterial resistance patterns and worsening renal function.
CURRENT CATEGORY: Nephrology

ABSTRACT BODY: We have shown the benefit of ACEi for treatment and for mitigation of experimental and clinical radiation nephropathy. Captopril was the most commonly used ACEi for this purpose. We tested whether this effect was shared by other ACEi, especially those that could be given once a day in clinical use. 115 male WAG/Rij/cmr rats were used. 45 were non-irradiated, 70 underwent 10 Gy single fraction total body irradiation (TBI) followed by syngeneic bone marrow transplant. This model reliably causes radiation nephropathy (rad np). All rats were on a 0.3 % salt diet. In non-irradiated rats, captopril 300 mg/L in the drinking water (DW), enalapril 30 mg/L fosinopril 50 mg/L, and ramipril 1.2 mg/L in the DW starting right after TBI caused equivalent increases in plasma renin activity (PRA) by 45 days after start of drug. Irradiation alone did not change the PRA. Irradiated rats that had the four ACEi at the same doses had the same PRA as 45 days after the start of drug, but divergent azotemia at 21 week after TBI. PRA are shown as means, BUN as geometric means.

These results confirm the lack of activation of the systemic renin-angiotensin system (RAS) in this model, they confirm the mitigation benefit of captopril, and they show that fosinopril does not mitigate radiation nephropathy. We have previously shown that captopril and enalapril mitigate radiation pneumonitis, but fosinopril is also ineffective in that model. We have not shown a role for bradykinin or acSDKP potentiation by use of captopril in experimental rad np. The superior mitigation benefit of captopril is not explained by its effect on the RAS alone.

CONTROL ID: 1076899

ACUTE ANGIOTENSIN II EFFECTS ON NCC ARE DEPENDENT ON WNK4

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CURRENT CATEGORY: Nephrology

ABSTRACT BODY: Acutely, angiotensin II (AII) is known to affect sodium chloride cotransporter (NCC) abundance and surface expression (SE). A possible role for WNK4 in mediating effects of AII on NCC has been described. To study this in a mammalian cell model, RNAi was used on mDCT15 cells to generate a WNK4 knock-down cell line (WNK4KD). Thiazide-sensitive 22Na+ uptake assays in mDCT15 cells showed a dose response to AII, peaking at 10-11M. mDCT15 cells showed a time dependent increase in activity, with peak response at 60 min (40-4%, p<0.01). An angiotensin receptor blocker eliminated this effect. WNK4 knock down in WNK4KD cells was measured via real-time PCR to be 50-3%. WNK4KD cells did not show a statistically significant increase in activity with AII. NCC SE as measured by biotinylation and densitometry increased in mDCT15 cells with AII, peaking at 30 min (100;14%, p<0.01). There was a modest but statistically significant increase in SE at 30 min in WNK4KD cells (43;13%, p<0.05). These findings indicate a critical role for WNK4 in mediating acute effects of AII on NCC and provide a foundation to examine the mechanisms by which this occurs.

PEDIATRICS

CONTROL ID: 1314566

ADOLESCENTS ARE AT GREATER RISK FOR COCAINE ADDICTION THAN ADULTS

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CURRENT CATEGORY: Pediatrics

ABSTRACT BODY: Adolescence is a period of heightened propensity to develop cocaine addiction in humans. To determine if such a finding is related to developmental differences in neurobiology, we performed a variety of studies employing different techniques. We first examined age differences in midbrain dopamine neuron activity in the ventral tegmental area; these neurons are critical players in addiction and reward.

Adolescent (postnatal day 42) and adult (~postnatal day 88) rats were compared for midbrain dopamine neuron activity using in vivo extracellular recording. Adolescents show greater activity of dopamine neurons than adults. Given that elevated activity of dopamine neurons is associated with elevated propensity to self-administer cocaine in rats, we tested whether the period of adolescence is associated with higher liability to self-administer cocaine relative to adulthood.

Adolescent and adult rats were compared for cocaine addiction liability using self-administration. We show that adolescent rats are more likely to acquire self-administration of cocaine than adults, show greater motivation and escalate in intake more readily. These findings parallel the greater addiction liability observed in human adolescents relative to adults.

The next question to ask is: are they more likely to remain addicted to cocaine compared with adults? In one experiment we asked how punishment
associated with drug intake affected subsequent drug use. To do this, cocaine was delivered together with an electric footshock during one of the self-administration sessions. Cocaine intake is suppressed for both ages on the day of the electric footshock. However, the next day adolescents resume cocaine taking whereas adults do not.

Finally, we examined how onset of cocaine use affects stress-induced relapse later on. Here, following self-administration, rats withdrew from cocaine in their home cages for over 40 days, a time when the adolescents had grown into adults. We then subjected rats to stressful experiences known to trigger relapse: electric footshock or injection of the stress hormone corticosterone. Both stressors trigger relapse, but this is far more pronounced if onset of cocaine use occurred during adolescence vs. adulthood.

Our results indicate that cocaine use during adolescence has critical consequences. Our research is the first to offer scientific evidence that when all opportunities to take drugs are equal, biology alone makes adolescents more likely to use cocaine compared to adults. We also show that adolescents do not refrain from taking cocaine after punishment, and they are more likely to relapse in response to a stressor later on in life than adults. These results have broad implications both for the attempts to steer adolescents away from drug use using punishments as well as for preventing relapse. Together our results indicate that adolescents are at greater risk for cocaine addiction than adults.

**Pulmonary/Critical Care**

**CONTROL ID:** 1315203

**MUTIPLE MICRORNAS REGULATE NONMUSCLE MYOSIN LIGHT CHAIN KINASE (NMMLCK) GENE EXPRESSION IN PULMONARY ENDOTHELIUM**

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**CURRENT CATEGORY:** Pulmonary/Critical Care

**ABSTRACT BODY:** RATIONALE: Increased vascular permeability is a cardinal feature of inflammation that occurs in such conditions as acute lung injury and sepsis. We previously demonstrated that non-muscle myosin light chain kinase (nmMLCK) plays a key role in agonist-induced pulmonary endothelial cell (EC) barrier regulation. MicroRNAs (miRNAs) regulate gene expression post-transcriptionally through binding to 3'UTR of mRNA and are linked to a variety of inflammatory conditions, cancer, and cardiovascular diseases. Preliminary in-silico analysis (Miranda- microrna.org, TargetScan) identified four miRNA candidates, hsa-miR-374a, hsa-miR-374b, hsa-miR-520c-3p and hsa-miR-1290 as potentially binding to the 3' UTR of nmMLCK. Whether these miRNAs participate in regulation of NMMLCK gene expression post-transcriptionally remains to be established.

**METHODS:** The function of hsa-miR-374a, hsa-miR-374b, hsa-miR-520c-3p and hsa-miR-1290 was studied in human pulmonary EC (HPAEC). Reporter constructs (SwitchGear Genomics, Menlo Park, CA) containing the luciferase gene fused to the MLCK 3' UTR (luc-MLCK-3'UTR) were used in dual luciferase assays to determine the effects of individual miRNAs on nmMLCK expression in TNF-a-stimulated EC.

**RESULTS:** In HPAEC transfected with the luc-MLCK-3'UTR reporter construct, TNF-a (24 hrs) increased luciferase activity 2.5±0.1 fold (4 replicate experiments) as compared with unstimulated cells. Cotransfections of EC with the luc-MLCK-3'UTR reporter construct and hsa-miR-374a, hsa-miR-374b, hsa-miR-520c-3p or hsa-miR-1290 decreased luciferase activity after TNF-a stimulation (± SD) for hsa-miR-374a, ~22±5% for hsa-miR-374b, ~50±7% for hsa-miR-520c-3p and ~55±4% for hsa-miR-1290, n=3) as compared with untreated EC. Moreover in this model transfection with hsa-miR-520c-3p or hsa-miR-1290 antagonists significantly increased luciferase activity (~75±5% for hsa-miR-520c-3p antagonist and ~82±4% for hsa-miR-1290 antagonist, n=3) as compared with transfection with a negative control oligonucleotide. Cotransfection with both hsa-miR-1290 and its antagonist significantly attenuated luciferase activity (~55±5%, n=3). TNF-a increased nmMLCK mRNA transcription levels 1.6±0.1 fold at 4 hrs post stimulation as measured by qRT PCR. This increase was attenuated by transfection of EC with hsa-miR-374a (50±3%, n=3), hsa-miR-520c-3p (~50±2%, n=3) or hsa-miR-1290 (20±3%, n=3).

**CONCLUSION:** These data demonstrate that hsa-miR-374a, hsa-miR-374b, hsa-miR-520c-3p and hsa-miR-1290 regulate nmMLCK expression in pulmonary EC after TNF-a stimulation and represent novel candidates for therapeutic modulation of this critical barrier regulatory gene.

**CONTROL ID:** 1315274

**DIFFERENTIAL ROLE OF EZRIN/RADIXIN/MOESIN PROTEINS IN REGULATION OF ENDOTHELIAL HYPER PERMEABILITY AFTER THROMBIN**

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**CURRENT CATEGORY:** Pulmonary/Critical Care

**ABSTRACT BODY:** RATIONALE: The pulmonary vascular endothelium serves as a semi-selective barrier between circulating blood and surrounding tissues. Endothelial cell (EC) barrier integrity is therefore critical to tissue and organ function. Disruption of the endothelial barrier leads to perturbation in vascular tone and increases risk of alveolar flooding, hypoxemia and pulmonary edema. Contraction of endothelial cells and intercellular gap formation is a primary reason for increased paracellular permeability. Compromised EC barrier function in response to inflammation mediators, such as thrombin, is accompanied by reversible cell rounding and paracellular gap formation. Thrombin challenge stimulates F-actin stress fiber formation, triggers actomyosin contraction and alters EC permeability through multiple mechanisms that include protein kinase C (PKC) activation. Reversible protein phosphorylation is a key mechanism in the regulation of many cellular processes. The ezrin, radixin, and moesin (ERM) family of actin-binding proteins link the actin cytoskeleton with plasma membrane proteins and serve to transduce signals from agonists to induce cytoskeletal remodeling. Phosphorylation of a conserved threonine residue in the C terminus of ERM proteins is considered a hallmark of ERM activation. Phosphorylation causes conformational changes in ERM, unmasking binding sites. In the present study we test the hypothesis that ERM proteins are phosphorylated on this critical threonine residue by thrombin-induced signaling events and explore the role of the ERM family in modulating thrombin-induced cytoskeletal rearrangement and EC barrier function.

**METHODS:** To study the involvement of ERM in EC barrier regulation in vitro, we used immunoblotting, immunocytochemistry, transendothelial monolayer resistance (TER) measurements (a sensitive indicator of EC barrier function), and RNA interference in cultured human pulmonary artery EC.

**RESULTS:** Our data demonstrate that thrombin (1 U/ml) promotes ERM phosphorylation on critical threonine residues (Ezrin-567, Radixin-564, Moesin-558). This phosphorylation peaks at 5 min after thrombin and is dependent upon activation of PKC isoforms. Immunofluorescent studies reveal that thrombin-mediated ERM phosphorylation occurs at the cell periphery. Thrombin-induced ERM phosphorylation is likely stimulated by protease-activated receptors (PARs), PAR1 and PAR2, because stimulation of EC with PAR1 (TFLLR-NH2) or PAR2 (SLIGRL-NH2) selective agonists alone induce ERM threonine phosphorylation. Importantly, stimulation of cells with a combination of these two agonists enhances this ERM phosphorylation, suggesting synergistic mediation by both PARs. Importantly, siRNA depletion of either moesin alone, or of all three ERM proteins, significantly attenuates thrombin-induced increase in EC barrier permeability (TER), cytoskeletal change, paracellular gap formation and accumulation of di-phospho-MLC. In contrast, radixin depletion has the opposite effect on barrier function as demonstrated by slightly enhanced thrombin-induced stress fiber formation, MLC phosphorylation and increase in permeability.

**CONCLUSIONS:** These data suggest that activation of ERM proteins plays an important role in the thrombin–induced modulation in EC permeability. Despite their structural similarities and reported functional redundancy, the ERM proteins differentially modulate thrombin-induced changes in lung EC cytoskeleton and permeability, with moesin promoting barrier dysfunction and radixin opposing it.
ABSTRACT BODY: RATIONALE: Regulation of the pulmonary endothelial barrier between the lung vascular and interstitial spaces is disrupted during the development of sepsis and acute lung injury (ALI). Barrier function is determined by actin cytoskeletal structure and rearrangements. Recent work indicates that dynamic peripheral actin events such as cell membrane protrusions and lamellipodia formation are critical to closure of gaps between endothelial cells (EC) to maintain barrier function and recovery from injury during vascular leak syndromes. We previously have described a critical role for the actin-binding protein cortactin in regulating EC cytoskeletal rearrangements and pulmonary barrier function. In this study we sought to further characterize the functional role of cortactin in dynamic peripheral cytoskeletal events and membrane dynamics.

METHODS: In vitro studies were performed using cultured human pulmonary artery endothelial cells (HPAEC) and human lung microvascular ECs. Pharmacological inhibitors of paxillin were employed to increase or decrease specific protein expression, with subsequent analysis by immunofluorescence in fixed cells and with live cell imaging techniques. Cell membrane protrusions were characterized in live cells by kymography.

RESULTS: We previously described a novel, ALI-associated single nucleotide polymorphism (SNP) in the human cortactin gene that encodes a serine to asparagine amino acid change at position 484 that alters phosphorylation at a critical regulatory tyrosine site. Lung microvascular cells overexpressing a mutant cortactin construct containing this SNP demonstrate decreased lamellipodia leading edge protrusion distance (1521.3 +/- 700.7 nm) and protrusion velocity (57.19 +/- 39.96 nm/sec) when compared to WT cortactin (2571.4 +/- 1364.2 nm and 68.3 +/- 26.6 nm/sec respectively). Reduced cortactin expression (via siRNA) in HPAEC decreases co-localization of actin with vascular endothelial cadherin (VE-cad) in preliminary studies, suggestive of altered junctional complex anchorage. Live cell imaging studies conducted in pulmonary EC overexpressing a GFP-tagged construct of the barrier regulatory protein myosin light chain kinase (nmMLCK) mutatated at the putative site of cortactin binding demonstrate a persistence of barrier disruptive actin stress fibers and a decreased translocation of nmMLCK to the cell periphery when compared to cells overexpressing a GFP-tagged WT construct. These findings suggest that inhibition of cortactin-nmMLCK results in an EC cytoskeletal pattern that produces barrier disruption.

CONCLUSION: These results support the hypothesis that cortactin is a key regulator of peripheral cytoskeletal structure and dynamic membrane events that determine pulmonary endothelial cell barrier function.

CONTROL ID: 1310857

INTEGRIN β4 MEDIATES CYCLIC STRETCH-INDUCED ENDOTHELIAL CELL INFLAMMATORY RESPONSES


CURRENT CATEGORY: Pulmonary/Critical Care

ABSTRACT BODY: Rationale: Simvastatin, an HMG-CoA reductase inhibitor, has potent lung vascular-protective effects that are associated with decreased bronchoalveolar lavage fluid cell counts compared to VILI controls. Subsequently, we overexpressed wildtype integrin β4 as a mediator EC protection in the context of excessive mechanical stretch at levels relevant to ventilator-induced lung injury (VILI).

Methods/Results: We initially confirmed simvastatin-mediated protection in a murine model of VILI (VT 30 ml/kg, 6 h). VILI-challenged animals pre-treated with simvastatin (20 mg/kg, 16 h) were found to have significantly decreased bronchoalveolar lavage fluid cell counts compared to VILI controls. Subsequently, we overexpressed wildtype integrin β4 as a mediator EC protection in the context of excessive mechanical stretch at levels relevant to ventilator-induced lung injury (VILI). The media was collected for measurement of inflammatory cytokines.

Controlled to VILI. Compared to EC transfected with the vector, overexpression of wildtype integrin β4 resulted in a significant increase in CS-induced IL-6, IL-8, MCP-1, and RANTES in the media while there was no effect on GM-CSF or VEGF. In contrast, pretreatment of untransfected EC with simvastatin (5 μM, 16 h) significantly inhibited CS-induced increases in inflammatory cytokines. To investigate the importance of specific integrin β4 tyrosine phosphorylation sites on these effects, we transfected various mutant integrin β4 constructs into EC prior to CS (18%, 6 h). Compared to cells overexpressing wildtype integrin β4, overexpression of a mutant integrin β4 lacking a cytoplasmic tail significantly attenuated CS-induced cytokine expression. Additionally, a similar effect was observed in EC overexpressing integrin β4 constructs with various tyrosine substitutions and pulmonary barrier function. In this study we sought to further characterize the functional role of cortactin in dynamic peripheral cytoskeletal events and membrane dynamics.

METHODS: In vitro studies were performed using cultured human pulmonary artery endothelial cells (HPAEC) and human lung microvascular ECs. Pharmacological inhibitors of paxillin were employed to increase or decrease specific protein expression, with subsequent analysis by immunofluorescence in fixed cells and with live cell imaging techniques. Cell membrane protrusions were characterized in live cells by kymography.

RESULTS: We previously described a novel, ALI-associated single nucleotide polymorphism (SNP) in the human cortactin gene that encodes a serine to asparagine amino acid change at position 484 that alters phosphorylation at a critical regulatory tyrosine site. Lung microvascular cells overexpressing a mutant cortactin construct containing this SNP demonstrate decreased lamellipodia leading edge protrusion distance (1521.3 +/- 700.7 nm) and protrusion velocity (57.19 +/- 39.96 nm/sec) when compared to WT cortactin (2571.4 +/- 1364.2 nm and 68.3 +/- 26.6 nm/sec respectively). Reduced cortactin expression (via siRNA) in HPAEC decreases co-localization of actin with vascular endothelial cadherin (VE-cad) in preliminary studies, suggestive of altered junctional complex anchorage. Live cell imaging studies conducted in pulmonary EC overexpressing a GFP-tagged construct of the barrier regulatory protein myosin light chain kinase (nmMLCK) mutatated at the putative site of cortactin binding demonstrate a persistence of barrier disruptive actin stress fibers and a decreased translocation of nmMLCK to the cell periphery when compared to cells overexpressing a GFP-tagged WT construct. These findings suggest that inhibition of cortactin-nmMLCK results in an EC cytoskeletal pattern that produces barrier disruption.

CONCLUSION: These results support the hypothesis that cortactin is a key regulator of peripheral cytoskeletal structure and dynamic membrane events that determine pulmonary endothelial cell barrier function.

CONTROL ID: 1310857

ABSTRACT BODY: Rationale: Simvastatin, an multi-domain scaffold protein, plays a pivotal role in regulating cell movement, migration and intracellular signal transduction. Focal adhesions provide a bi-directional linkage between the actin cytoskeleton and cell-extracellular interface and maintain endothelial barrier integrity. Paxillin is also phosphorylated at multiple serine/threonine and tyrosine residues; however, the role of paxillin and paxillin phosphorylation in LPS-induced endothelial barrier function is unclear. Here, we hypothesize that c-Abl dependent tyrosine phosphorylation of paxillin regulates LPS-mediated reactive oxygen species (ROS) production and barrier integrity of human lung microvascular endothelial cell (HLMVEC).

METHOD: HLMVECs were treated with LPS (100 ng/ml) for indicated time (0.5, 1, 2 and 6 h) and phosphorylation of paxillin and its interacting proteins were detected by immunoprecipitation with anti-phospho tyrosine, beta catenin and PI3 kinase antibodies. The effect of paxillin on LPS-induced endothelial permeability was assessed by measuring trans-endothelial resistance (TER) and leakage of FITC-labeled dextran (70 kDa). The role of tyrosine phosphorylation of paxillin on ROS generation and endothelial permeability was investigated by transfection of HLMVECs with paxillin Y31 and Y118 constructs. Interaction of paxillin with target proteins was studied by transfacing HEK 293 cells with GFP-paxillin fragments (LD1-5, LIM1-4).

Results: LPS challenge of HLMVECs resulted in enhanced tyrosine phosphorylation of paxillin at Y31 and Y118 with no change in Y180. LPS challenge increased association of paxillin with beta-catenin and p85 subunit of PI3 kinase as evidenced by western blotting of paxillin immunoprecipitates. Treatment of HLMVECs with LPS resulted in significant barrier dysfunction, which was attenuated by knockdown of paxillin with siRNA or by transfection of cells with paxillin mutants (paxillinY31F, paxillinY118F and double mutant paxillinY31F, Y118F). Studies with LD1-5, LIM1-4 fragments show enhanced interaction of β-catenin with paxillin LIM1-2 domain after LPS challenge. Furthermore, LPS induced ROS production in HLMVECs, which was attenuated by paxillin Y31 and Y118 mutants. Conclusion: Tyrosine phosphorylation of paxillin at Y31 and Y118 is critical for LPS-induced endothelial barrier dysfunction and ROS generation. Also, paxillin LIM1-2 domain is the major site for potential interaction with β-catenin.

CONTROL ID: 1315053

LYSOCARDIOLIPIN ACYLTRANSFERASE (LYCAT) PROTECTS BLEOMYCIN-INDUCED LUNG INFLAMMATION AND FIBROSIS IN MICE

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CURRENT CATEGORY: Pulmonary/Critical Care

ABSTRACT BODY: Rationale: Lysocardiolipin acyltransferase (LYCAT) protects bleomycin-induced lung inflammation and fibrosis in mice.
ABSTRACT BODY: RATIONALE: Idiopathic pulmonary fibrosis (IPF) is characterized by alveolar epithelial cell injury, areas of type II cell hyperplasia, accumulation of fibroblasts and myofibroblasts, and the deposition of extracellular matrix proteins such as collagen and fibronectin. Our previous genome-wide SNP association study in patients with IPF identified a novel candidate, lysocardiolipin acyltransferase (LYCAT), which encodes for a protein involved in the remodeling of cardiolipin. However, the role of LYCAT in bleomycin-induced pulmonary fibrosis and lung injury is unclear.

Here, we hypothesized that over-expression of LYCAT protects bleomycin induced lung inflammation and fibrosis in mice.

METHODS: Bleomycin (1.5-2 unit/kg) or vehicle (PBS) was administered intratracheally to C57BL6 mice. To examine the role of LYCAT, expression vector encoding hLYCAT was delivered intratracheally in to mice 3 days prior to bleomycin challenge and subsequently at 3 day intervals. Mice were sacrificed on day 7 or 14 post-bleomycin or vehicle administration; lungs were perfused and fixed for histology and immunohistochemical analysis.

RESULTS: Bleomycin challenge showed significant increase in fibrosis as measured by acid-soluble collagen, enhanced expression of α-SMA, collagen and fibronectin were analyzed by Western blot, and real time RT-PCR analysis.

Fibrosis was evaluated by trichrome staining for collagen in lung tissue. Expression of α-smooth muscle actin (α-SMA), collagen and fibronectin were analyzed by Western blot, and real time RT-PCR analysis.

CONCLUSION: These results demonstrate an important role for LYCAT in reducing bleomycin-induced lung fibrosis and suggest that LYCAT may be a potential novel therapeutic target for IPF.

This work was supported by P01 HL098050 to VN.

CONTROL ID: 1337339
TREFOIL FACTOR AUGMENTS ENDOTHELIAL CELL BARRIER FUNCTION AND ATTENUATES MURINE VENTILATOR-INDUCED LUNG INJURY

CURRENT CATEGORY: Pulmonary/Critical Care
ABSTRACT BODY: RATIONALE: Endothelial cell (EC) barrier dysfunction is a cardinal feature of acute lung injury (ALI) and ventilator-induced lung injury (VILI). We previously identified trefoil factor (TFF) as a gene that is downregulated in various animal models of VILI and confirmed decreased expression of TFF in EC subjected to excessive mechanical stress. Although its function is largely unknown, we hypothesized that TFF is a potential mediator of EC barrier regulation and may play a protective role in ALI/VILI.

METHODS: Phosphorylation of mitogen activated protein kinases (MAPK) including ERK, JNK, and p38 was assessed by Western blotting of human lung microvascular EC after treatment with LPS alone (10 µM, 4 h) or with TFF2 pretreatment (3 µM, 15 min before LPS). To assess the effects of TFF2 (10 µM) on LPS-induced (10 µM) EC barrier dysfunction we measured transendothelial electrical resistance (TEP). In separate studies, TFF2 knockout (KO) mice or wildtype animals (C57BL/6) were administered intratracheal LPS (1mg/kg, 18h) as well as subjected to mechanical ventilation (VT = 30 ml/kg or 20 ml/kg, 4 h) after which bronchoalveolar lavage (BAL) fluid was collected to assess protein levels and total cell counts.

RESULTS: Treatment of EC with recombinant human TFF2 significantly attenuated LPS-induced phosphorylation of ERK, JNK, and p38 MAPK. In addition, TFF2 significantly attenuated LPS-induced EC barrier disruption as measured by TEP. In the in vivo studies, all but one of the male TFF2 KO mice ventilated with VT = 30 ml/kg died at 2 h (n = 8) while all wildtype mice survived (n = 8). BAL fluid protein levels from the TFF2 KO mice (at death or at 4h) were significantly increased compared to wildtype ani-

mals (834 µg/ml vs 346 µg/ml, p < 0.05) although total cell counts did not significantly differ. These results demonstrate an important role for TFF2 in reducing bleomycin-induced lung fibrosis and suggest that TFF2 may represent a potential novel therapeutic target for IPF.

CONTROL ID: 1344977
MITOTEMPOL TREATMENT TO TARGET MITOCHONDRIAL OXIDATIVE DAMAGE IN AN EXPERIMENTAL MODEL OF ASTHMA
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CURRENT CATEGORY: Pulmonary/Critical Care
ABSTRACT BODY: Introduction: Previous studies have suggested that free radical oxidative damage—particularly of lipids, nucleic acids, and proteins—is extensive in the lungs of ovalbumin (OVA)-sensitized asthmatic mice – however little is known about the molecular mechanisms by which increased reactive oxygen species (ROS) contribute to the asthma phenotype. Mitochondria derived ROS are key signaling molecules in the cell, though their role in allergic models of asthma is unknown. Mitotempol is a mitochondrial targeted peperidine nitroxide designed to sequester ROS produced from the mitochondria. We hypothesize that treatment with Mitotempol may decrease airway resistance in a mouse model of asthma.

Methods: Mice were pretreated with either saline or Mitotempol (5 mg/kg/day) through an osmotic minipump and subjected to an 18 day ovalbumin (OVA) sensitization protocol, an established model of allergic airway disease. On day 18 airway hyperreactivity (AHR) in response to methacholine was assessed using the Scireq Flexivent. Broncho-alveolar lavage (BAL) was collected for differential cell counts.

Results: Mice treated with Mitotempol had trend to decrease in AHR that was not statistically significant. The control group (n=7) AHR at 50mg/ml methacholine = 13.93 (7.31-20.6) (total lung resistance cmH2O/sec/ml) and the Mitotempol treated group (n=5) AHR at 50 mg/ml methacholine = 9.86 (4.66-15.06) (total lung resistance cmH2O/sec/ml). Mice treated with Mitotempol had a trend to decrease BAL eosinophil percentage that was not statistically significant. The control group (n=5) had 51% (+/- 24) eosinophils and the Mitotempol group (n=5) had 41% (+/- 27) eosinophils.

Conclusion: Our preliminary data suggest a trend towards decreased airway hyperreactivity in Mitotempol mice, suggesting that ROS produced from the mitochondria maybe a key signaling event in the progression of asthma. Therefore, targeting ROS production from the mitochondria may prove a novel treatment strategy for asthma.

CONTROL ID: 1314512
PATHOLOGIC STRETCH INCREASES LUNG ENDOTHELIAL MICROPARTICLE RELEASE
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CURRENT CATEGORY: Pulmonary/Critical Care
ABSTRACT BODY: Rationale: Ventilator-induced lung injury (VILI) results from pathologic over distention of the lung during high tidal volume mechanical ventilation, a process that induces pulmonary vascular endothelial cell dysfunction. Recent studies have implicated endothelial-derived microparticles (EMPs) as important mediators and markers of endothelial injury. EMPs are small membrane vesicles (0.1-1 µm) released from activated or apoptotic endothelial cells. We sought to characterize the effects of mechanical stretch on EMP release and composition from pulmonary endothelium.

Methods: Pathologic cyclic stretch (18%) was applied to human pulmonary artery endothelial cells (HPAECs) for 4 and 24 h. Static control cell cultures were maintained in parallel. EMPs released from EC were isolated from the media by differential centrifugation followed by flow cytometry quantification using annexin V and CD31 markers. EMPs were negatively stained with uranyl acetate solution, and sections were examined

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with transmission electron microscopy (TEM) to define the size and the shape of the particles. Mass spectrometry proteomic analysis (LC-MS) was performed to determine EMP composition. Results: HPAECs subjected to pathologic 18% cyclic stretch shed increased numbers of annexin-V-FITC and CD31 double positive MPs compared to static EC (three fold-increase). MP release was detected within 4h of stretching achieving maximal effect at 24h. TEM confirmed the presence of isolated EMPs and proteomic analysis indicated the presence of metabolic enzymes, chaperones, histones, annexins, cytoskeleton-associated proteins (actin, myosin, vimentin), glycoproteins (von Willebrand factor, fibrinectin, thrombospondin-1) and membrane proteins ( caveolin, integrins). Preliminary analysis revealed 16 unique proteins in stretch- derived EMPs and 15 in control EMPs, strongly suggesting that excessive mechanical stretch produces distinct populations of EMPs with unique compositions. Conclusion: These findings indicate that exposure to pathologic cyclic stretch results in significant release of EMPs with unique compositions which suggest novel VILI pathogenetic mechanisms.

Supported by NHLBI/NIH Grant HL058064 (JNG).

CONTROL ID: 1344949
RAC1 MITOCHONDRIAL IMPORT AND MITOCHONDRIAL H\textsubscript{2}O\textsubscript{2} GENERATION IS MODULATED BY GERANYLGERANYLATION OF THE CYSTEINE-189 RESIDUE
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CURRENT CATEGORY: Pulmonary/Critical Care

ABSTRACT BODY: Asbestos is a prototypical and important cause of pulmonary fibrosis. Exposure to asbestos results in lung injury and aberrant remodeling of lung tissue in a complex process that is poorly understood, but ultimately leads to fibrosis. The generation of reactive oxygen species (ROS), including H\textsubscript{2}O\textsubscript{2} by alveolar macrophages plays a critical role in pulmonary fibrosis by modulating extracellular matrix deposition. We have demonstrated that alveolar macrophages obtained from patients with pulmonary fibrosis produce high levels of H\textsubscript{2}O\textsubscript{2} and that the primary source of H\textsubscript{2}O\textsubscript{2} generation in these cells is the mitochondria. The small GTP-binding protein, Rac1, increases the generation of H\textsubscript{2}O\textsubscript{2} in multiple cell types, and we have shown that Rac1 is imported into the mitochondria in alveolar macrophages. This import requires the cysteine-189 residue of Rac1. This C-terminal residue is known to undergo geranylgeranylation, a post-translational modification that is necessary for activation of Rho GTPTases, including Rac1. We hypothesized that Rac1 import into the mitochondria is modulated by the geranylgeranylation of the cysteine-189 residue and is necessary for mitochondrial H\textsubscript{2}O\textsubscript{2} generation. We found that inhibition of geranylgeranylation-transferase (GGTase) with GGTI completely abrogated Rac1 import into the mitochondria. To determine if Rac1 mitochondrial import regulated mitochondrial H\textsubscript{2}O\textsubscript{2} levels, cells were treated with solvent (DMSO) or GGTI prior to asbestos exposure. GGTI inhibited mitochondrial H\textsubscript{2}O\textsubscript{2} generation in a dose-dependent manner compared to cells treated with DMSO. Because attenuation of alveolar macrophage H\textsubscript{2}O\textsubscript{2} levels modulates asbestos-induced pulmonary fibrosis, our data suggest that geranylgeranylation of Rac1 is necessary for mitochondrial import and H\textsubscript{2}O\textsubscript{2} generation in alveolar macrophages and may represent a therapeutic option to prevent the development of asbestos-induced pulmonary fibrosis.

CONTROL ID: 1343836
GENOTYPE-PHENOTYPE STUDIES ON THE ROLE OF CD177 IN SEPSIS
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CURRENT CATEGORY: Pulmonary/Critical Care

ABSTRACT BODY: Rationale: The events that lead to sepsis are incompletely understood. Neutrophils play a key role in both pathogen clearance and the host injury that follows. CD177 is a novel protein that is expressed on the surface of a subset of neutrophils in sepsis. In this study, we examined the phenotypic and genotypic role of CD177 in inflammation and sepsis. We asked if CD177 positive neutrophils are functionally distinct from CD177 negative neutrophils. Then, we asked if polymorphisms that affects CD177 surface expression correlate with the incidence of sepsis.

Methods and Materials: Cell based studies: Neutrophils were isolated from human blood. These cells were stained for CD177 and sorted into negative and positive populations with FACS Aria cell sorter. Differences in reactive oxygen species were measured against various stimuli using a Fluoroset Omega luminescence machine. Migration properties across airway epithelia grown at the air-liquid interface were examined by placing neutrophils at the basolateral side and adding an activating serum (ZAS) to the apical side. SNP analysis: We examined the most common Single Nucleotide Polymorphism (SNP) associated CD177 surface expression. Genomic DNA of 2049 subjects obtained from the VALID (Validation of biomarkers in Acute Lung Injury Diagnos-tudy) database was genotyped using TaqMan SNP genotyping assays on an Applied Biosystems 7900HT Fast Real-Time PCR System. Allelic discrimination was performed using the Applied Biosystems SDS software. The clinical relevance of C49G polymorphism in relation to sepsis was examined by comparing genotype frequency with presence or absence of sepsis.

Results: All subjects tested had two neutrophil populations circulating in the peripheral blood, a CD177 positive and a CD177 negative population. The percentages of these two populations varied across human subjects. CD177 positive cells have impaired reactive oxygen species (ROS) in response to ZAS when compared with their CD177 negative counterparts. In addition, when stimulated by ZAS, CD177 positive cells migrate more effectively across airway epithelia than CD177 negative cells. Chi Square analysis revealed a significant association of this missense variant with sepsis in the African American population (χ\textsuperscript{2} = 7.63 and p = 0.0057) and Logistic Regression analysis showed that subjects with the CC genotype are highly susceptible to sepsis [odds ratio (OR) = 3.05, 95% confidence interval (1.33-7.00)]. The European American population did not show a similar association or susceptibility.

Conclusions: CD177 positive neutrophils produce less ROS in response to stimulation. In addition, CD177 positive neutrophils migrate across airway epithelia cells more efficiently. African Americans with the CC genotype (C49G SNP) appear to be significantly susceptible to sepsis. These differences could be mechanisms for local modulation of the inflammatory cascade and suggest a new and important role for CD177 in neutrophil function and sepsis.

CONTROL ID: 1315102
PULMONARY HYPERTENSION IN MICE AS A CONSEQUENCE OF INFARCTION-INDUCED CARDIAC DYSFUNCTION
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CURRENT CATEGORY: Pulmonary/Critical Care

ABSTRACT BODY: Introduction: The effects of myocardial infarction (MI) on the development of pulmon-ary hypertension (PH) and right ventricular (RV) remodeling remain unclear.

Purpose: Accepting that PH in mice can be evaluated based on end systolic RV pressure (ESRVP), which mimics pulmonary artery pressure in the absence of pulmonary valve stenosis, we sought to study morphological and functional post-MI changes on both LV and RV to show that PH could be a consequence of infarction-induced cardiac dysfunction.

Methods: A permanent ligation of the left anterior descending coronary artery produced around 45% infarct size in healthy wild type A/J middle-aged (1 year old) male mice (N=41). Biventricular function was assessed using the Millar pressure-volume conductance system in vivo in the left ventricle and RV via a right carotid arterial catheter and RV via a right jugular vein at time points corresponding to early LV remodeling (3 wk), to late compensatory LV hypertrophy (5 wk) and to a dilated decompensatory state leading to HF (7 wk).

Results: Post-MI hearts developed significant total cardiac hypertrophy over time compared to controls verified by percentage of heart weight over body weight (3 wk=31% increase, 5 wk=63%, 7 wk=72%) as well as H&E staining indicated progressive LV dilation and RV enlargement. ESRVP significantly increased over time compared to controls (3 wk=32±3*, 5 wk=38±5*, 7 wk=40±4* vs. 0 wk=23±2 mmHg, *p<0.01) and with significantly worsening LV remodeling as demonstrated by cardiac output (O\textsubscript{c}: 3 wk=3±1*, 5 wk=5156±484*, 7 wk=4274±233* vs. 0 wk=9183±64 μl/min, *p<0.01). Also, load-independent parameters of LV contractility such as end systolic pressure volume relationship significantly and progressively decreased with
These data demonstrated that a permanent ligation of coronary artery could be a feasible and reliable approach to evaluate not only cardiovascular, but pulmonary changes following MI over time. In this model, ESRV reflected RV dysfunction following MI, as an indirect measurement of RV afterload, which is typically a main cause of death in PH. This approach could be helpful to better understand and design experiments to delay and/or prevent PH attributed to LV dysfunction in mice.

CONTROL ID: 1315173

INTESTINAL EXPRESSION OF CFTR ALLEVIATES THE MECONIUM ILEUS PHENOTYPE IN CYSTIC FIBROSIS PIGS


CURRENT CATEGORY: Pulmonary/Critical Care

ABSTRACT BODY: In order to better understand cystic fibrosis (CF) disease pathogenesis, we recently developed a porcine model of CF. CF pigs display many of the same features as humans with CF including meconium ileus, exocrine pancreatic destruction, focal biliary cirrhosis, micro-gallbladder, vas deferens abnormalities, and airway disease. In contrast to humans, there is a 100% penetrance of meconium ileus in both CFTR-/- and CFTRJ508F/508F newborn piglets. Features of the CF pig meconium ileus closely replicate that observed in humans with CF including distal bowel obstruction, atretic intestinal segments, and microc Normal-sized spiral colon. In contrast, piglets from the three other lines (1b, 1c, 1d) had evidence of severe meconium ileus at birth. Both ileal CFTR mRNA and protein levels and CFTR-mediated Cl− transport were greater in the intestines of lines 1a and 1b compared to 1c and 1d. We found that approximately 20-30% of wild-type CFTR expression levels was sufficient to alleviate the meconium ileus phenotype. In all of the lines studied, we observed a similar degree of pancreatic destruction, micro-gallbladder, and focal biliary cirrhosis compared to CFTR-/- pigs suggesting that the pathogenesis of meconium ileus is not dependent upon abnormal pancreatic or gallbladder function. Importantly, in Ussing chamber studies there was no evidence of CFTR-mediated Cl− transport in either freshly excised tracheal tissue or cultured tracheal and nasal turbinate epithelial samples. Similar to older CFTR-/- and CFTRJ508F/508F pigs, the older gut-corrected pigs in this study also developed varying severity of meconium ileus phenotype at birth. In these animals, there was a relatively normal-appearing small intestine, no evidence of atretic intestinal segments, and a normal-sized spiral colon. In contrast, piglets from the other three lines (1b, 1c, 1d) had evidence of severe meconium ileus at birth. Both ileal CFTR mRNA and CFTR-mediated Cl− transport were greater in the intestines of lines 1a and 1b compared to 1c and 1d. We found that approximately 20-30% of wild-type CFTR expression levels was sufficient to alleviate the meconium ileus phenotype. In all of the lines studied, we observed a similar degree of pancreatic destruction, micro-gallbladder, and focal biliary cirrhosis compared to CFTR-/- pigs suggesting that the pathogenesis of meconium ileus is not dependent upon abnormal pancreatic or gallbladder function. Importantly, in Ussing chamber studies there was no evidence of CFTR-mediated Cl− transport in either freshly excised tracheal tissue or cultured tracheal and nasal turbinate epithelial samples. Similar to older CFTR-/- and CFTRJ508F/508F pigs, the older gut-corrected pigs in this study also developed varying severity of CF-like lung disease. These data demonstrate a successful genetic approach to correction of meconium ileus in CF pigs and provide valuable insights into the underlying pathogenesis of meconium ileus in CF. These gut-corrected CF pigs may be a useful model to study CF lung disease pathogenesis over time and the effects of therapeutics.

CONTROL ID: 1310246

ASSOCIATION OF BIOMARKER SPHINGOSINE-1-PHOSPHATE RECEPTOR 3 GENETIC VARIANTS WITH HUMAN SUSCEPTIBILITY TO SEPSIS-INDUCED ACUTE LUNG INJURY

X. Sun, S. Ma, P.A. Singleton, M.S. Wade, J.G. Garcia Medicine, University of Illinois at Chicago, Chicago, IL.

CURRENT CATEGORY: Pulmonary/Critical Care

ABSTRACT BODY: RATIONALE: Acute lung injury (ALI) is characterized by profound inflammation and increased vascular permeability with mortality rate 30-40%. The genetic mechanisms underlying ALI are poorly understood. We have previously demonstrated that sphingosine 1-phosphatase (SIP) and its receptor, SIPR3, are intimately involved in lung inflammatory responses including vascular barrier regulation. Here we explored genetic roles of SIPR3 in sepsis-induced ALI. METHODS: SIPR3 expression levels were detected in vivo in 66 ICU patients by ELISA. Posttranslational modification of SIPR3 in human lung endothelial cells was studied by immunoprecipitation. The genetic roles of SIPR3 in ALI A were investigated by combination of SIPR3 gene re-sequencing for SNP discovery, case-control association, gene promoter functional and transcription factor binding study. We identified common SIPR3 gene variants (80 SIPR3 variants, 51 novel) by direct DNA sequencing of a multicase panel of 27 samples, and SNPs with MAF > 5% were selected for subsequent genotyping. We then performed association studies in case-control samples of unrelated individuals from both African and European American population in Chicago (218 cases and 378 controls). SIPR3 promoter was cloned into a luciferase reporter vector and assessed for functionality by luciferase assay in transduced human lung endothelial cells (EC). Transcription factors binding to the SIPR3 promoter was detected by electrophoretic mobility shift assay. RESULTS: SIPR3 expression in ALI was significantly increased in patients with severe sepsis-induced ALI and associated with increased mortality rate of ALI. In lung endothelial cells, SIPR3 was nitrated and shed into medium as microparticles following LPS stimulation. In European Americans, SIPR3 promoter SNPs rs7022797 (-1899 T/G) and rs11137480 (-1785 G/C) conferred decreased susceptibility of both severe sepsis and sepsis-induced acute lung injury (p < 0.05). Compared to TNF-stimulated EC SIPR3 promoter activity containing -1899 T and -1785 G, promoter activity with either SNP-1899 G, -1785 C or both SNPs was significantly decreased luciferase promoter activity in (60%, 50% or 80% decrease, respectively (p < 0.05)). Binding of the transcription factors CXD1 and EBF1 to the SIPR3 promoter was significantly interrupted by SNP-1899 T-G and -1785 G-C, respectively. SIPR3 protective promoter SNPs, -1899 G and -1785 C, each significantly associated with decreased SIPR3 protein expression in ICU patients. Conclusion: These results suggest that increased SIPR3, which was nitrated and released into microparticle during ALI, linked to severity and outcome of ALI. Functional SIPR3 promoter variants (rs7022797 and rs11137480) significantly decrease the risk of sepsis and sepsis-induced ALI.

CONTROL ID: 1310284

A PROMOTER POLYMORPHISM OF MYLK ASSOCIATED WITH SEVERE ASTHMA UPREGULATES SMOOTH MUSCLE MYOSIN LIGHT CHAIN KINASE ISOFORM X. Sun, S. Ma, Y.J. Han, M.S. Wade, J.G. Garcia Medicine, University of Illinois at Chicago. Chicago, IL.

CURRENT CATEGORY: Pulmonary/Critical Care

ABSTRACT BODY: RATIONALE: Myosin light chain kinase (MLCK) is a central cytoskeletal regulator encoded by MYLK gene, and associated with disruption of vascular barrier integrity, plays a key role in complex diseases leading asthma, a complex inflammatory disorder strongly influenced by environmental and genetic factors. We studied the potential regulatory roles of MYLK SNP (rs57186134), which were previously reported to be associated with asthma risk. METHODS: The location and potential function of MYLK SNP was studied by in silico analysis. MYLK promoter for smooth muscle MLCK (smMLCK) was cloned into a luciferase reporter vector and assessed for functionality by luciferase assay in transduced human lung endothelium. The DNA fragment with SNP was generated by site-mutagenesis. Transcription factors binding to MYLK promoter was detected by electrophoretic mobility shift assay. RESULTS: In silico, MYLK SNP (rs57186134) located in 5′1′5′ upstream of TSS, the promoter region of MYLK for smMLCK. In luciferase assay, compared to one with rs57186134 (-551A), promoter with rs57186134 (-551G), a SNP associated with increased blood eosinophil and asthma risk, significantly increased promoter activity in endothelial cells (60% increase, p < 0.05). Comparing to SNP rs57186134 (-551A), the binding of MYLK promoter to the growth factor independent 1 transcription repressor (GFI1) was significantly interrupted by rs57186134 (-551G). CONCLUSIONS: These functional insights into the contribution of MYLK SNP further strengthen the concept that MYLK contributes to inflammatory disease susceptibility and represents an attractive molecular target in complex lung disorders. Funded by HL58064 and HL91889 (JNG).
CONTROL ID: 1310277
SIMVASTATIN INCREASES SPHINGOSINE–1–PHOSPHATE RECEPTOR 1 GENE EXPRESSION IN HUMAN LUNG ENDOTHEL BY KRUPPEL-LIKE FACTOR 2
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CURRENT CATEGORY: Pulmonary/Critical Care
ABSTRACT BODY: BACKGROUND: We demonstrated that simvastatin and sphingosine-1-phosphate (S1P) each enhance endothelial cell (EC) barrier function, resulting in an attenuation of increased vascular permeability in acute lung injury or ischemia reperfusion injury. We hypothesized an interaction between these barrier regulatory agonists through upregulation of the S1P receptor 1 (S1PR1) by statins either at the mRNA or the protein level. As statins are recognized to affect differential gene expression via modulation of specific transcription factors, we further hypothesized that simvastatin enhances EC barrier via increased promoter activity of S1PR1 gene (S1PR1) through transcription factors. METHODS: S1PR1 promoter regulation by transcription factors tested with in silico analysis. The S1PR1 promoter region from the human genome was isolated, amplified, and inserted into a luciferase reporter vector, which was transfected into human lung endothelial cells (HPAEC) and tested for promoter activation by a luciferase activity assay following simvastatin challenge. siRNA for knocking down proteins was transfected into HPAEC by DharmaECT 1. Microarray for mRNA changes was used after mice were treated with simvastatin in vivo.
RESULTS: S1PR1 promoter regulatory by transcription factors lung Kruppel-like factor (KLF2) in silico. Simvastatin significantly increased endogenous S1PR1, which was enhanced by KLF2 overexpression in HPAEC. Compared to basal levels, S1PR1 promoter activity was dramatically increased in response to simvastatin (120% increase, p < 0.05), which was significantly attenuated by siRNA KLF2. Simvastatin significantly increased endogenous KLF2 expression in HPAEC. Microarray assay demonstrated simvastatin significantly increased KLF2 and S1PR1 gene expression in vivo. COMPARISON: Further in vivo mechanistic studies are required, these results suggest that simvastatin upregulates S1PR1 expression via enhanced activity of the S1PR1 promoter, through transcription factor KLF2. These data suggest a potential synergistic effect of simvastatin and S1P in endothelial signaling and barrier function which may lead to novel combinatorial therapeutic strategies for lung inflammatory syndromes.

CONTROL ID: 1315256
FTY720 S-PHOSPHONATE ENHANCES ENDOTHELIAL BARRIER FUNCTION VIA CYTOSKELETAL REARRANGEMENT AND PROTECTS AGAINST BLEOMYCIN-INDUCED ACUTE LUNG INJURY IN MICE
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CURRENT CATEGORY: Pulmonary/Critical Care
ABSTRACT BODY: Rationale: A significant and sustained increase in vascular permeability is a hallmark of acute inflammatory diseases such as acute lung injury (ALI), but effective therapies for preserving or reconstituting the vascular barrier are lacking. Prior work has demonstrated that FTY720 (S-phosphonate Tysomyphosphate), an analog of plasma sphingosine 1-phosphate (SIP) and FTY720, allows potent pulmonary barrier protective effects than those agents in vitro and in the LPS mouse model of mouse ALI. Moreover, Tys preserves expression of the barrier promoting S1PR1 receptor (S1PR1), whereas SIP and FTY720 induce its ubiquitination and degradation. Therefore, we hypothesized that Tys would exhibit superior protection in a more prolonged model of ALI. In this report, we further characterize the novel barrier promoting effects of Tys in the bleomyicin model of ALI as well as its effects on intracellular signaling and junctional assembly function in cultured human pulmonary endothelial cells (EC).
Methods/Results: Mice received intratracheal bleomyicin or vehicle control and then were administered Tys or FTY720 (0.5 mg/kg IP) every other day for 1 week. In both vehicle-treated and bleomyicin-injured mice, Tys maintained significantly higher S1PR1 lung expression (detected by Western blot) compared to FTY720. Importantly, while FTY720 failed to protect against bleomyicin-induced ALI in mice, Tys significantly decreased multiple indices of lung leak and inflammation. Further mechanistic experiments were performed in vitro using cultured human pulmonary EC. Reduced S1PR1 expression via siRNA significantly attenuated transendothelial electrical resistance (TER) elevation by Tys. Inhibition of cytoskeleton rearrangement by depolymerizing actin with cytochalasin blocked Tys-induced TER elevation. Furthermore, Tys significantly increased Rac1 activity, while inhibition of this Rac1 activity by pharmacological inhibition significantly attenuated Tys-induced TER elevation. Although reduced expression (via siRNA) of the adherence junction proteins VE-cadherin (p-catenin decreased basal EC barrier function, neither inhibited subsequent Tys-induced barrier enhancement. In contrast, claudin 5, ZO-1, or ZO-2 siRNA inhibited Tys-induced TER elevation, suggesting that tight junctions are involved in this process.
Conclusion: FTY720 S-phosphonate exhibits superior barrier protection to FTY720 in bleomyicin-induced acute lung injury via preservation of endothelial S1PR1. Cytoskeleton rearrangement, Rac1 signaling and tight junctions are involved in Tys-mediated barrier protection.

CONTROL ID: 1314984
CYCLIC STRETCH INDUCES TYPE 2 DEODINASE EXPRESSION IN LUNG ENDOTHELIAL CELLS
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CURRENT CATEGORY: Pulmonary/Critical Care
ABSTRACT BODY: Rationale: Critically ill patients, including those with ALI, exhibit reduced serum concentrations of the prohormone, thyroxine (T4) and the active hormone, 3, 3, 5-triiodothyronine (T3). We recently reported that the Type 2 deiodinase, a enzyme converting T4 to bioactive T3, is a novel gene linking thyroid metabolism to protective responses to acute inflammatory lung injury, including ventilator-induced lung injury (VILI). While the molecular mechanism of D2 gene expression regulation remains unclear, cyclic stretch of endothelial cells was applied as an in vitro VILI model to investigate the transcriptional regulation of the D2 gene.
Methods: Immunohistochemical staining was applied to locate D2 protein expression in lung tissue from control mice and mice exposed to VILI (40ml/ kg, 4 hrs). Human pulmonary artery endothelial cell (HPAECs) were used at passage 5-8 in in vitro assays. Monolayers of HPAECs were exposed to 18% cyclic stretch for the specified time. D2 gene promoter region (2.2kb) was amplified by PCR and cloned to pGJ3 luciferase reporter plasmid. DNA transfection were mediated by Jetprime. D2 promoter activity was detected by Dual-Luciferase Reporter Assay System (Promega). D2 gene expression levels were evaluated by qPCR and western blotting.
Results: Immunohistochemical staining of D2 revealed markedly increased D2 expression in pulmonary endothelium and alveolar epithelium in VILI mice. In vitro cyclic stretch induced a rapid expression of D2 gene in HPAEC, mRNA and protein increase were detected in 1 hour and was sustained for 4 hours. D2 promoter activity was significantly increased upon 4h cyclic stretch, suggested a mechanical stress responsive element in the 2.2kb D2 promoter region.
Conclusion: D2 expression is increased in endothelial and epithelial cells in VILI mouse lung tissues. D2 expression was induced in HPAEC upon cyclic stretch in a quick response pattern, there are mechanical stress responsive elements in D2 promoter region between upstream 2.2 kb and transcriptional start site.
glycogen synthase kinase 3β (GSK3β) phosphorylates ST2L to mediate its elimination.

Methods and Results: IL-33 induced ST2L serine phosphorylation and degradation in models of type IV collagen (MLE12). GSK3β is activated in response to IL-33 treatment. Over-expression of GSK3β wild type and a constitutively active form (GSK3βS9A) induced phosphorylation and degradation of ST2L. Knockdown of GSK3β by GSK3β siRNA or inhibition of GSK3β by its inhibitor (TWS119) effectively attenuated IL-33-induced serine phosphorylation and degradation of ST2L. Overexpression of a serine442 mutant of ST2L (ST2LS442A) diminished GSK3β-mediated ST2L phosphorylation and IL-33-induced ST2L degradation. Intratracheal administration of endotoxin increased IL-33 levels in bronchoalveolar lavage fluid and decreased ST2L levels in lung tissues. Intratracheal administration of IL-33 induces ST2L degradation, phosphorylation of GSK3β, and enhanced endo-toxin-induced lung inflammation.

Conclusion: These results suggest that modulation of the IL-33-ST2L axis by GSK3β might serve as a unique strategy to lessen pulmonary inflammation.

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CONTROL ID: 1314929
F-BOX PROTEIN FBXL19 PROTECTS AGAINST LIPOPOLYSACCHARIDE-INDUCED LUNG INFLAMMATION AND INJURY
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CURRENT CATEGORY: Pulmonary/Critical Care
ABSTRACT BODY: Acute lung injury (ALI) is a condition of acute respiratory failure resulting from acute pulmonary inflammation. The alveolar epithelium provides a complex physical and biochemical barrier to inhaled particles, allergens, and environmental toxins and thus plays a vital role in host defense. Ubiquitin-proteasome system regulates turnover of majority of intracellular proteins. Here, we show a new orphan protein that exhibits the prototypical behavior of a SCF E3 ligase subunit, termed FBXL19 (F-box protein 19, SCFFBXL19) regulates lung inflammation and injury. Over-expression of FBXL19 attenuates LPS-induced IL-6 release and apoptosis of lung epithelial cells. To investigate the effect of FBXL19 in LPS-induced lung injury, we expressed a FBXL19-overexpressed fusion protein or a lenti-control-shRNA construct in mouse and in vivo expression of these constructs in lung tissue was analyzed by fluorescence scanning. TUNEL assays show that FBXL19 overexpression in mice blocked LPS-i.t. challenge-induced cell death. Further, FBXL19 administration effectively attenuated LPS-induced pulmonary inflammation histologically, alveolar protein leak, and reduced IL-6 and TNFα levels in BAL fluid. These results suggest that FBXL19 exhibits an anti-inflammatory property and protects against LPS-induced lung inflammation and injury.

The study is supported by NIH RO1 HL091916 (to YZ) and American Heart Association Science Developmental Grant (to ZJ).

CONTROL ID: 1315024
THE INCREASE IN PULMONARY ENDOTHELIAL PERMEABILITY INDUCED BY GROUP V PHOSPHOLIPASE A2 (gVPLA2) DOES NOT REQUIRE MEMBRANE HYDROLYSIS PRODUCTS OR DOWNSTREAM INTRACELLULAR SIGNALING
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CURRENT CATEGORY: Pulmonary/Critical Care
ABSTRACT BODY: Acute lung injury (ALI) is characterized by inflammation-induced disruption of the alveolar-vascular barrier resulting in airspace flooding and severe respiratory compromise. Inhibition of the intercellular messenger protein Group V Phospholipase A2 (gVPLA2) blocks vascular permeability caused by LPS both in vivo and in vitro. gVPLA2 increases permeability of cultured human pulmonary endothelial cells (EC) by an undefined mechanism. We previously reported that the prototypical behavior of a SCF E3 ligase subunit, termed FBXL19 (F-box protein 19, SCFFBXL19) regulates lung inflammation and injury. Overexpression of FBXL19 attenuates LPS-induced IL-6 release and apoptosis of lung epithelial cells. To investigate the effect of FBXL19 in LPS-induced lung injury, we expressed a FBXL19-overexpressed fusion protein or a lenti-control-shRNA construct in mouse and in vivo expression of these constructs in lung tissue was analyzed by fluorescence scanning. TUNEL assays show that FBXL19 overexpression in mice blocked LPS-i.t. challenge-induced cell death. Further, FBXL19 administration effectively attenuated LPS-induced pulmonary inflammation histologically, alveolar protein leak, and reduced IL-6 and TNFα levels in BAL fluid. These results suggest that FBXL19 exhibits an anti-inflammatory property and protects against LPS-induced lung inflammation and injury.

The study is supported by NIH RO1 HL091916 (to YZ) and American Heart Association Science Developmental Grant (to ZJ).

CONTROL ID: 1314997
TUMSTATIN, A FRAGMENT OF COLLAGEN IV, ATTENUATES THROMBIN-INDUCED FAK PHOSPHORYLATION AND ENDOTHELIAL BARRIER DISRUPTION
T. Zhou, W. Chen, J. Garcia, J. Jacobson Medicine, UIC, Chicago, IL.
CURRENT CATEGORY: Pulmonary/Critical Care
ABSTRACT BODY: Acute lung injury (ALI) is a challenging clinical problem encountered in the intensive care unit and is associated with significant morbidity and mortality. A cardinal feature of ALI is an increase in lung vascular permeability precipitated by an exuberant inflammatory response with subsequent lung endothelial cell (EC) barrier disruption. EC responses to mechanical and inflammatory stimuli in ALI are mediated, in response with subsequent lung endothelial cell (EC) barrier disruption. EC responses to mechanical and inflammatory stimuli in ALI are mediated, in

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Conclusion: gVPLA2 mediates the critical pathophysiologic event of increased vascular permeability that occurs in ALL. These data further support the hypothesis that gVPLA2 increases pulmonary EC permeability through direct action at the EC membrane that does not require membrane hydrolysis products or downstream intracellular signaling. Supported by NIH HL81144, HL85779, GSX Center of Excellence.

Rheumatology/Immunology/Allergy
CONTROL ID: 1339799
THE ROLE OF FACTOR V LEIDEN MUTATION IN OSTEONECROSIS OF THE HIP
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CURRENT CATEGORY: Rheumatology/Immunology/Allergy
ABSTRACT BODY: Background: We examined the hypothesis that the thrombophilic Factor V Leiden mutation is a common pathophysiologic cause of osteonecrosis of the hip.
Methods: We prospectively evaluated 244 consecutively referred adults with osteonecrosis, 161 idiopathic and 83 secondary (steroids 64, trauma 6, alcohol 4, combination 9). We compared the Factor V Leiden mutation in the 244 patients with 104 normal controls who did not differ from patients by race.
Results: Of the 244 patients with osteonecrosis, 23 (9.4%) were heterozygous for the Factor V Leiden mutation versus 2/104 normal controls (1.9%), p=0.013, risk ratio = 4.90, 95% confidence interval 1.18 to 20.4. Of 161 patients with idiopathic osteonecrosis, 15 (9.3%) were heterozygous for the Factor V Leiden mutation versus 2/104 normal controls (1.9%), p=0.017, risk ratio = 4.84, 95% confidence interval 1.13 to 20.8. Of 83 patients with secondary osteonecrosis, 8 (9.6%) were heterozygous for the Factor V Leiden mutation versus 2/104 normal controls (1.9%), p=0.024, risk ratio = 5.01, 95% confidence interval 1.09 to 23.0.
Conclusions: The thrombophilic Factor V Leiden mutation appears to be a common, pathophysiologic cause of osteonecrosis of the hip.

CONTROL ID: 1344810
TYPE I AND TYPE II TGF BETA RECEPTORS ARE PRESENT WITHIN THE NUCLEI OF HUMAN AIRWAY EPITHELIAL CELLS
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CURRENT CATEGORY: Rheumatology/Immunology/Allergy
ABSTRACT BODY: Structural changes after lung injury are observed in patients with asthma, chronic obstructive pulmonary disease (COPD) and pulmonary fibrosis. Transforming growth factor (TGF)-β plays a multifunctional and prominent role in the process of tissue repair by participating in the fate of monocytes including cell proliferation and migration, extracellular matrix turnover, and regulation of gene expression. The levels of TGFβ are higher in the lungs of subjects with asthma and COPD compared to non-affected subjects. Binding of TGFβ to the type I (TβRI) and type II (TβRII) receptors localized at the cell membrane activate these serine/threonine kinases, which leads to phosphorylation and activation of the transducers proteins Smad 2/3. The phospho-Smad2/3 complex associates with Smad4 and translocates to the nucleus to regulate transcriptional activity of TGFβ-responsive genes. Using confocal microscopy and cell fractionation we demonstrated that cultured airway smooth muscle cells and lung parenchyma fibroblasts exhibit cytoplasmic as well as nuclear localization of TβRI and TβRII. In this study we hypothesize that TβRI and TβRII are also present in the nuclei of other cell types. Western analysis of nuclear (NE) and cytosolic (CE) extracts of human airway epithelial (HAE1o-) cells, human embryonic kidney (293HEK) and acute myeloid leukemia (AML) cells reveal different degrees of nuclear abundance of TβRI and TβRII. Immunoprecipitation of NE and CE proteins with anti-TβRI or anti-TβRII antibodies but not with control IgG shows that Smad 2/3 associates with TβRII receptors within the NE and cytosolic compartments of HAE1o- cells, suggesting that Smad 2/3 participates in the signaling of TβRII and TβRII into the nucleus. Treatment of quiescent HAE1o- cells with TGFβ increases the relative abundance of TβRII but not TβRII within the nuclei. IL-10 stimulation has no effect on either receptor’s cellular distribution. Administration of leptomycin B, an inhibitor of nuclear export, induces retention of both TβRII receptors within the nuclear compartment of serum-fed HAE1o- cells. We conclude that TβRII and TβRII are present in a variety of cell types and that TβRI and TβRII are bound to Smad 3 inside the nucleus. Moreover, intracellular localization of TβRIs can be modulated by cytokines exposure and is augmented by inhibiting the exportin 1 / crm1-dependent nuclear export pathway.

CONTROL ID: 134900
BASELINE SERUM INTERFERON ALPHA/BETA RATIO PREDICTS RESPONSE TO TUMOR NECROSIS FACTOR ALPHA INHIBITION IN RHEUMATOID ARTHRITIS
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CURRENT CATEGORY: Rheumatology/Immunology/Allergy
ABSTRACT BODY: Background: Response to tumor necrosis factor alpha (TNFα) inhibition is heterogeneous in rheumatoid arthritis (RA). Previous studies have suggested that circulating type I interferon (IFN) levels may predict treatment response to TNFα inhibitors and other biological agents in RA. Prediction of likely responders prior to initiating therapy would represent a major advance in biological treatment strategies for RA.
Methods: We studied sera from 32 RA patients from the ABCON Consortium pre-treatment and 4-6 weeks after beginning treatment with TNFα inhibitors, selecting patients who either had a good response or no response at 14 weeks by EULAR criteria. 27 of the 32 subjects were of European ancestry. Total serum type I IFN activity as well as IFNα vs. IFNβ activity were measured using a functional reporter cell assay. Data were available regarding baseline and follow up disease activity score (DAS), EULAR response criteria at 14 weeks, anti-CCP antibody titer, and type of TNFα inhibitor used.
Results: An increased ratio of IFNβ/IFNα > 1.3 in the pre-treatment serum sample was associated with lack of response by EULAR criteria at 14 weeks (p=0.009). Similarly, higher IFNα/IFNβ ratio was positively correlated with higher DAS score at 14 weeks (Spearman’s rho = 0.57, p = 0.005). Anti-CCP antibody titer and type of TNFα inhibitor did not influence this relationship. In follow up sera at 4-6 weeks, the EULAR non-responders were more likely to have increased total type I IFN activity than good responders (p=0.008), and this increase was characterized by a shift toward increased IFNα as compared to good responders (p=0.039).
Conclusions: Increased pre-treatment serum IFNβ/IFNα ratio was strongly associated with non-response to TNFα inhibition by EULAR criteria at 14 weeks. This study supports the potential utility of serum type I IFN in predicting outcome of TNFα inhibition in RA.

CONTROL ID: 1314575
RHABDOMYOLYSIS: A LESS KNOWN SIDE EFFECT OF A WELL KNOWN DRUG
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CURRENT CATEGORY: Rheumatology/Immunology/Allergy
ABSTRACT BODY: Introduction: Isoniazid is the drug of choice for the treatment of latent tuberculosis. The commonly reported adverse effects include liver toxicity, skin rash, peripheral neuropathy, anemia and central nervous system adverse effects. We are reporting a case where a young patient developed acute rhabdomyolysis from isoniazid therapy.
Case: 21-year-old Caucasian male with complaints of generalized weakness, muscle aches and voiding dark brown urine after his routine exercise activity. He took the last dose of isoniazid for his latent tuberculosis therapy a day earlier. He denied any history of recent trauma, seizures or similar symptoms with exercise in the past. The patient denied tobacco, alcohol or recreational drug abuse and was not on any other prescription medication as well. Patient’s mother and brother, who were also being treated with isoniazid for latent tuberculosis at the same time, did not report similar symptoms. At the time of presentation, he was hemodynamically stable and the physical examination was unremarkable except for generalized muscles tenderness. Urinalysis showed large amount of blood but microscopic examination did not show erythrocytes. Urine myoglobin was 33 mg/L. Initial laboratory tests showed elevated serum Creatinine Kinase (179564 IU/L), Myoglobin (11119 ng/ml), ALT (278 IU/L), AST (748 IU/L), LDH

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(2486 IU/L) and Aldolase (757.9 U/L). The serum electrolyte panel including creatinine was normal. Blood and urine toxicology was negative for any recreational drug. Hepatitis panel was negative for hepatitis A, B and C viruses. ESR, CRP and autoimmune diseases workup were all negative. Aggressive hydration with intravenous fluid was initiated. The patient remained hemodynamically stable and his serum Creatinine Kinase and Myoglobin levels gradually normalized and his symptoms of generalized weakness and muscle tenderness resolved as well. Patient was discharged from the hospital 72 hours later. It was concluded that he developed acute rhabdomyolysis due to isoniazid and the exercise-induced dehydration was probably a contributing factor. On two and four weeks follow up visits the muscle enzymes level remained within normal range.

Summary: To conclude, our patient developed rhabdomyolysis after many months of being treatment with isoniazid and discontinuation of isoniazid led to improvement of muscle enzymes which supports the causative relationship. Rhabdomyolysis could be secondary to direct toxic effect of isoniazid or its metabolite on the muscle, however, more work need to be done to better understand the patho-physiology of rhabdomyolyis due to isoniazid use.

Blood Clotting

CONTROL ID: 1344777
THE EFFECT OF FRACTIONATED Calf THYMUS HISTONE ON PROTHROMBIN TIME OF HUMAN LEVEL 1 PLASMA
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CURRENT CATEGORY: Hematology and Oncology
ABSTRACT BODY: A.S. Brecher, J.R. Catazaro Bowling Green State University, Bowling Green, OH. Calf thymus histone is a basic polypeptide bearing a resemblance to protamine, a low M.W. peptide which is employed clinically to neutralize the anticoagulant effects of heparin. Utilizing fractionated histone in these studies, a 3-4 sec. increase in prothrombin time (PT) was observed over a range from 0.94-37.6 μg of histone added to human level I plasma. Heparin (0.238U) prolonged PT from a control of 12.8±:5 sec. (n=5) to 59.3±4 sec. (n=5; p<0.05). When heparin (0.238U) and histone (2.5 μg) were added successively to plasma, a PT of 26.3±1.5 sec. (n=5; p<0.05) was obtained indicating a major neutralization of the anticoagulant effect of heparin by histone was attained. When histone (2.5 μg) and acetaldehyde (AcH) (44.7 mM) were added successively to plasma, a PT of 29.6±2 sec. (n=5; p<0.05) was observed. Relative to a 2.1 sec. increase for histone alone and a 11.1 sec. increase for AcH alone, the 29.6 sec. increase represented a 16.8±1.5 sec. increase (n=5; p<0.05) corresponding to a slightly synergistic effect of the histone and AcH upon PT. Whereas AcH (44.7 mM) prolonged PT by 23.9±1 sec. (n=5; p<0.05), successive additions of heparin and AcH to plasma yielded a PT of 68.8±6 sec. (n=5; p<0.05) exemplifying an additive effect of the two components upon PT. These data constitute a pattern of interaction by histone which is similar to that of protamine in that both exhibit a slight but significant anticoagulant effect on clotting. Both neutralize heparin effects on clotting. Both have an additive or synergistic effect on the anticoagulant effect of AcH. (The authors express their appreciation to Prof. Robert Harr, Chair of the Medical Technology Program at BGSU, for generously supplying samples of plasma and thromboplastin.)