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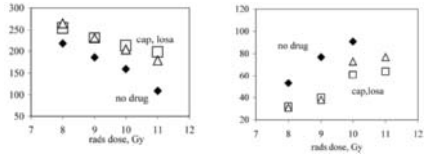
MITIGATION OF RADIATION NEPHROPATHY. E.P. Cohen, B.L. Fish, J.E. Moulder, Center for Medical Countermeasures Against Radiation, Milwaukee, WI.

Mitigation and treatment of experimental radiation nephropathy have been shown using a multifraction total body irradiation (TBI) model with angiotensin-converting-enzyme (ACE) inhibitors and angiotensin II (AII) blockers. The ACE inhibitors have been used at a range of doses, and the AII blocker, L-158,809, is not approved for human use. Efficacy of these mitigating agents for use in radiation accident or terrorist event must be tested after single exposure and at doses approved for human use. Eighty-eight barrier-maintained WAG/Rij/MCW rats underwent single fraction TBI followed by syngeneic bone marrow transplant (BMT). TBI was 8, 9, 10, or 11 Gy. Captopril, 150 mg/L , or losartan, 100 mg/L, was added to the drinking water in half of the rats in each group. Rats were followed for renal function and survival. BUN data shown are at 17 weeks after TBI. At the 10 and 11 Gy doses, survival was significantly prolonged by captopril and losartan ($p < .05$). The 17-week BUN was less for the drug-treated rats at all radiation doses. We expect that complete 26-week follow-up will show significant function differences in all groups. We conclude that captopril and losartan are effective in mitigating experimental radiation nephropathy at doses compatible with human use.



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THE EFFECTS OF QUINIDINE AND ITS CHIRAL ISOLATES ON ERG-1SM POTASSIUM CURRENTS AND CORRELATION WITH GASTROINTESTINAL AUGMENTATION. I. Cvetanovic, C. Lin, V. Ranade, J.C. Somberg, Department of Pharmacology, Rush University Medical Center, Chicago, IL.

Erg-1sm potassium channel has been recently reported to participate in modulation of gastrointestinal contractility. Since quinidine inhibits cardiac potassium channel and augments gastrointestinal contractility, it was thought that quinidine (Q) may affect erg-1sm. Studies were undertaken to evaluate the effects of Q and its chiral isolates on gastrointestinal erg-1sm potassium current and correlate these effects with colon contractility. Chiral separation (HPLC technique), mass spectrometry, and optical rotation determination were performed. The erg-1sm potassium channel was expressed in Xenopus oocytes and the two-electrode patch clamp technique was employed for recording. An isolated rat colon preparation was employed to measure changes in contractility. As a result of chiral separation, two peaks were obtained with elution times of 8.31 and 8.66 minutes all with a MW of 324; the optical rotations of racemate, isolates X and Y were + 258°, ± 0°; + 217°, respectively. The percentage changes in amplitudes of colon contraction (from baseline) were determined at different concentrations of Q and the two isolates in five experiments in each group. Quinidine 0.1, 1, and 10 μM increased contractility by 79 ± 34, 125 ± 42, 217 ± 51 ($p \leq .05$) for isolate X and 70 ± 20, 115 ± 32, 272 ± 32 ($p \leq .05$), and 22 ± 12, 46 ± 17, 59 ± 22 for isolate Y. The inhibition of erg-1sm currents by Q was 19 ± 4, 21 ± 5, and 48 ± 6 ($p \leq .05$), respectively, for isolate X, 20 ± 4, 23 ± 5, 39 ± 7 ($p \leq .05$), and for isolate Y, 22 ± 4, 21 [± 4, 31 ± 6. One chiral isolate and Q markedly augment contractility, while Q and the two chiral isolates inhibit the erg-1sm potassium currents to a similar extent. These results suggest that erg-1sm inhibition does not explain GI contractile augmentation caused by the Q racemate and its chiral isolates.

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A TWO-STEP TAGGING SINGLE NUCLEOTIDE POLYMORPHISM APPROACH FOR THE IDENTIFICATION OF GENETIC VARIANTS OF INTERLEUKIN-6 ASSOCIATED WITH ACUTE LUNG INJURY. C. Flores,[1] S.F. Ma,[1] J. Finigan,[2] L. Gao,[3] K. Maresso,[1] J. Villar,[4] J.G.N. Garcia,[1] [1]Section of Pulmonary and Critical Care Medicine, The University of Chicago, Chicago, IL; [2]Critical Care, Johns Hopkins University; [3]Allergy and Immunology, Johns Hopkins University, Baltimore, MD; [4]Instituto Canario de Investigacion Biomedica, Tenerife, Spain.

Rationale: Interleukin-6 (IL-6) is a key proinflammatory cytokine in the inflammatory diseases including acute lung injury (ALI) and sepsis. Genetic associations of IL-6 variants with disease have focused on the −174 G>C single nucleotide polymorphism (SNP) within the IL-6 promoter. However, it is not clear whether these associations are due to linkage disequilibrium to flanking regions or to other SNPs of the gene. **Methods:** Eighty-four healthy controls (H), 91 septic (S), and 64 ALI patients of European American descent (ED) were analyzed for association. A set of seven evenly distributed intergenic SNPs covering ≈74 kb plus the −174 SNP were chosen to evaluate the associations with the flanking regions of IL-6 gene. *TagIT* software (<http://www.genome.duke.edu/resourses/computation/software>) was used to select IL-6 cosmopolitan tagging SNPs (tSNPs) from 23 ED and 24 African American descent (AD) individuals. SNP dropping with resampling method was used to predict the properties of tSNPs covering variation. **Results:** Only the −174 G>C SNP was significantly associated with ALI (ALI vs H: OR = 0.28, 95% CI 0.08–0.96, $p = .039$; ALI vs S: OR = 0.26, 95% CI 0.08–0.87, $p = .038$). Additionally, a set of 14 cosmopolitan tSNPs were identified covering more than 85% of known and unknown ED or AD common variations in the IL-6 gene. **Conclusion:** Our data demonstrate that variants in the IL-6 gene, but not flanking regions, are involved in susceptibility to ALI. The analysis of haplotypes defined by tSNPs within the IL-6 gene may confirm whether genetic variants other than −174 G>C SNP may be associated with ALI. This approach will allow us to delimitate the search for causal variants in the IL-6 gene.

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COMPARISON OF METABOLIC EQUIVALENT WITH THE 6-MINUTE WALK TEST AS A MEASURE OF EXERCISE CAPACITY AND OUTCOME PREDICTOR IN PULMONARY ARTERIAL HYPERTENSION: RESULTS FROM THE INTRAVENOUS TREPROSTINIL STUDY. M. Gomberg-Maitland, D. Huo, V.F. Tapson,* V.V. McLaughlin,** R.L. Benza,*** R.J. Barst,****The University of Chicago, Chicago, IL; *Durham, NC; **Ann Arbor, MI; ***Birmingham, AL; ****New York, NY.

Purpose: The 6-Minute Walk Test (6MW) is the recommended test to assess exercise capacity in pulmonary arterial hypertension (PAH). Metabolic equivalent (MET) is the amount of oxygen used by a seated person and is a predictor of survival in the general population. We sought to determine if the two tests had good repeated reliability and determine their ability to predict functional class and/or hemodynamic parameters. **Methods:** Forty-seven PAH patients enrolled in a prospective open-label 12-week trial of intravenous treprostinil. At baseline, week 6, and week 12 both 6MW followed by Naughton-Balke treadmill tests were preformed (> 30 minutes apart) in addition to NYHA Functional Class (FC). Hemodynamic measures were recorded at baseline and week 12. Treadmill time in seconds was converted to MET based on the protocol. Baseline data are expressed as means ± SD. Pearson correlation coefficient (r) was calculated to express interrelationships between measures of exercise capacity. Intraclass correlation coefficient (ICC) was used to indicate the repeated reliability of exercise measures over time. Linear regression assessed the relationship between CO, mPA, and exercise capacity and logistic regression assessed the relationship between FC and exercise capacity. GEE population average modeling compared change in FC and/or hemodynamic measures with exercise outcomes. Two-sided p values < .05 were statistically significant. **Results:** Mean values at baseline: 404 ± 98 m, 495 ± 263 sec, 5.6 ± 2 MET and at week 12: 425 ± 88m, 563 ± 239 sec, 6.1 ± 1.9 MET. Values for both tests were normally distributed. Treadmill time and METS were highly correlative, $r = .99$ ($p < .001$). ICCs for 6MW, treadmill time, and MET were 0.77, 0.82, and 0.80, respectively. FC correlated with 6MW and MET at baseline and at week 12 (all $p < .01$). CO correlated with exercise at baseline (6MW, $p = .01$; MET, $p < .001$) but not at week 12; mPA did not correlate with either test. **Conclusions:** MET is a reliable exercise measure in PAH patients and correlates with 6MW. Exercise capacity measures highly correlated with FC but not consistently correlated with resting hemodynamics. One-year data will allow more comparative data.

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THE LOSS OF LIMK1 PROTECTS ENDOTHELIAL BARRIER FUNCTION. M. Gorovoy, R.F Neamu, D. Predescu, T. Voyno-Yasenetskaya, Department of Pharmacology, College of Medicine, University of Illinois at Chicago, Chicago, IL.

Introduction: Acute lung injury (ALI) is a syndrome of acute respiratory failure that results from acute pulmonary edema and inflammation. The development of ALI is associated with direct pulmonary injury from pneumonia and aspiration as well as indirect pulmonary injury from trauma and sepsis. LIMK1 is a serine/threonine kinase that is involved in cytoskeleton dynamics. **Methods:** The role of LIMK1 in the regulation of endothelial permeability was evaluated using in vivo lung perfusion studies, transendothelial resistance of cell culture measurements, Western blotting, and electron and confocal microscopy. **Results:** As enhanced pulmonary vascular permeability is a hallmark of acute lung injury, we examined the lung microcirculation in LIMK1 knockout mice. We found that endothelial permeability in the lungs of LIMK1 −/− mice was lower than that of wild-type mice. Notably, the endothelial permeability of the lungs of LIMK1 −/− mice after PAR1 peptide perfusion was significantly lower than that of wild type. Down-regulation of endogenous LIMK1 with siRNA in HUVECs resulted in increased transendothelial resistance. The overexpression of w.t. LIMK1 in HUVECs led to the decreased transendothelial resistance and opening of tight junctions as was revealed by confocal microscopy with the staining for ZO-1 and VE-cadherin. To study endotoxin-induced acute lung injury, anesthetized mice received LPS (ip) and the wet to dry ratio of the lungs of wild-type mice was compared to that of LIMK1 −/− mice. We found a decreased edema formation in LIMK1 −/− mice upon LPS treatment. **Conclusions:** We suggest that the loss of LIMK1 protein leads to less permeable pulmonary blood vessels. These results favor the possibility that the inhibition of LIMK1 function may attenuate acute lung injury.

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HIV DRUG RESISTANCE: RAMAN CRYSTALLOGRAPHY STUDIES OF THE "FLOPPY FLAP" IN HIV PROTEASE. M.S. Helfand,[1,2] P. Pattaniak,[2] M.A. Taracila,[1] M.P. Carey,[2] P.R. Carey,[2] F. van den Akker,[2] [1]Cleveland VAMC and [2]CASE School of Medicine, Cleveland, OH.

Background: Drug resistance mutations are commonly found in HIV protease (PR) and result from many factors allowing specific mutations to predominate, eg, single base pair changes resulting in functional yet drug-resistant enzymes. Drug exposure drives evolution by selecting for energetically stable and functional proteins. Flap region (residues 36–63) mutations in PR are of particular interest because they are distal from the active site and as they accumulate contribute significantly to resistance while preserving enzymatic function. The structural and protein dynamical aspects of how this occurs are poorly understood. We hypothesize that the flap is stuck in a partially open conformation in the resistant forms, which may improve protein stability even in the absence of bound PI while simultaneously impeding PI binding. We are developing a new structural biology technique, Raman crystallography, to study PR flap mutations. We present Raman spectroscopic and x-ray crystallographic data showing how the Phe 53 flap residue can be used to determine the flap position. **Methods:** "Wild-type" PR was crystallized and used in our experiments. Nonresonance Raman difference spectra were obtained with indinavir by soaking the crystals in inhibitor solution for 10 minutes. Crystals for x-ray crystallography were prepared as for Raman, and 2–3 Å resolution data obtained for the apo-structure and the indinavir-inhibited structure. Structures were solved by molecular replacement. Ab initio calculations were used to model the Raman spectra. **Results:** The intensity of the peaks in the Raman spectra is very sensitive to the orientation of the crystals. The phenylalanine transitions at ≈1,000–1,008 cm^{-1} in the difference spectra are especially informative. The frequency and intensity of these peaks are signatures of open—>closed conformational changes in the Phe53s on binding of inhibitor. There is also a contribution from the methylphenyl side chain of the bound inhibitor. Other PI transitions are also sensitive to crystal orientation. The preliminary crystal structure data indicate that the flap region is highly disordered in the apo-crystal (some open, some closed) and shows bound inhibitor when it is added. The Phe 53 orientation is still being refined. **Conclusions:** The ≈1,000–1,008 cm^{-1} region in Raman spectra of PR can be used to assess the flap conformation in WT PR on PI binding. Peak widths and intensity can give information of the stability of the complexes. We will study resistant PR next. Raman spectroscopy is a powerful tool to study flap mutations in PR.

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