was used to subtract head movement (translation and rotation) from the facial markers. The analyses in this study were restricted to two markers: midline lower lip and a virtually calculated midline jaw marker. A marker at the top of the nose bridge was used as the origin point. The following kinematic variables were obtained from each lip-jaw movement time-series: peak movement speed (mm/s), and displacement (mm). Each patient was instructed to perform 10 repetitions of the phrase "buy bobby a puppy" at his or her typical speaking rate and volume. Sentence-level intelligibility was obtained using the Sentence Intelligibility Test (SIT) and word-level intelligibility was obtained using the Word Intelligibility Test, using standard procedures. Intelligibility, measured in percentage of words correctly transcribed, and speaking rate, measured in words per minute (wpm), was derived from the SIT sentences for each patient. Intelligibility, measured in percentage of words correctly chosen via multiple choice was derived from the Word Intelligibility Test. RESULTS/ANTICIPATED RESULTS: Effect sizes (Cohen's d) across the 10 trials of "buy bobby a puppy" were computed to assess the effects of recovery time on range of motion and speed of the lower lip alone, the jaw alone, and the lower lip and jaw together for both range of motion and for speed. The largest effect sizes were observed for increased range of motion and increased speed of the articulators for participants within 24 months of surgery. Smaller effect sizes were observed for these parameters for the participants in the later stages of recovery, with some participants showing declines in range of motion and speed of some but not all articulators. Descriptive statistics indicate that both speech and word intelligibility improvements are most notable in the first two years following transplantation and appear to plateau during the later stages of recovery. Only two out of five of our participants achieved "normal" speech intelligibility (i.e., >97%) at five years post-transplantation. DISCUSSION/SIGNIFICANCE OF IMPACT: Biomechanical assessment revealed that kinematic recovery of articulator range of motion and speed appears most significant in the first two years following surgery, but that improvement continues to some degree as far as five-years post-transplant. Clinically-based assessments suggest that gains in intelligibility appear to plateau by 3-years post-surgery.

3559

Mechanisms of sebaceous skin microbial community remodeling through microenvironment modulation.

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OBJECTIVES/SPECIFIC AIMS: To understand the mechanisms of how a non-antimicrobial can reshape a commensal microbe community to cure a ubiquitous human disease. METHODS/STUDY POPULATION: Whole genome sequencing of bacterial isolates, metabolomic investigations of previously collected skin microbe isolates from patients, and structural investigations of a protein from these skin microbes. RESULTS/ANTICIPATED RESULTS: Metabolic pathways associated with adaptation to a changing skin microenvironment, novel antimicrobial characterization, and a structural understanding of a novel nutrient acquisition protein. DISCUSSION/SIGNIFICANCE OF IMPACT: Multiple angles of this investigation are poised to improve current non-antimicrobial dermatologic treatments and they have the potential to impact microbe-related diseases in other human microenvironments.

3496

Mesenchymal Stem Cell Extracellular Vesicle Delivery in a Shear-Thinning Hydrogel For Therapy in an Acute Myocardial Infarction Model: A Comparative Analysis

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OBJECTIVES/SPECIFIC AIMS: The primary aim is to assess differences in therapeutic effect between MSC and EPC EVs on acute ischemic rat hearts through delivery in a biocompatible and shear-thinning hydrogel. Primary outcomes for therapeutic assessment include an in-vitro angiogenesis assay and in-vivo hemodynamic analysis, mainly identifying differences in ejection fraction and contractility. Secondary hemodynamic outcomes include cardiac output, stroke volume, and end-diastolic pressure volume relationship (EDPVR). Secondary structural outcomes include post-mortem scar analysis and immunohistochemistry (IHC) staining for angiomyogenesis. METHODS/STUDY POPULATION: MSCs and EPCs will be cultured according to previously published protocols. EVs will be isolated from cultured cell lines through precipitation methods with polyethylene glycol. EVs will be qualitatively analyzed with nanoparticle tracking analysis (NTA) and flow cytometry. The shear thinning hydrogel (STG) will be constructed using a hyaluronic backbone conjugated to adamantane or beta-cyclodextrin, ultimately facilitating guest-host interactions with shear thinning properties. Controls and treatment groups mixed with the hydrogel will be injected into the border zone of infarcted Wistar rat hearts immediately following a left anterior descending artery ligation. Hemodynamic assessment will be performed at four weeks through left ventricular catheter based pressure-volume recordings. Ex-vivo analysis will include scar thickness assessment using Masson collagen staining and IHC stain for vessel (anti-vonWillebrand factor; anti-Isolectin) and myocyte formation (anti-cardiac Troponin I). RESULTS/ANTICIPATED RESULTS: We hypothesize that, in-vitro, MSC-EVs will demonstrate non-inferior angiogenic potential as compared to EPC-EVs. We posit that MSC-EVs will demonstrate superior therapeutic effect to EPC-EVs in-vivo as measured by functional hemodynamics and structural assessment. We have successfully isolated MSC and EPC EVs and have validated uniformity across EV populations (Figure 1). Preliminary data from the angiogenesis assay (n=3) demonstrated that MSC-EV and EPC-EV produce non-significantly different angiogenic potential as measured by number of vascular meshing extremes (p=0.144) and length of master vascular segment (p=0.193), with significant differences compared to either positive or negative controls. DISCUSSION/SIGNIFICANCE OF IMPACT: Novel regenerative therapies are needed for patients with a history of AMI given current limitations to therapy and sequelae of ischemic heart disease. Delivery of extracellular vesicles through a shear-thinning gel is a novel "off-the-shelf" translational approach to address the current clinical need.

3019

Metabolomic Markers of Methotrexate Response in Juvenile Idiopathic Arthritis

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OBJECTIVES/SPECIFIC AIMS: In this study, a semi-targeted metabolomics approach is used to identify metabolic markers of

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methotrexate (MTX) response in juvenile idiopathic arthritis (JIA) and in vitro. METHODS/STUDY POPULATION: A comparative metabolomic analysis was used to identify metabolomic markers and metabolic pathways associated with MTX activity in vitro and in vivo. Cell-based studies assessed metabolomic profiles in K562 erythroblastoid cells with or without MTX treatment. In vivo analysis utilized plasma samples from JIA patients treated with MTX (n=30) and included samples collected prior to the initiation of MTX and after 3-months of MTX treatment. Plasma samples were from an IRB-approved single center prospective cohort study of biomarkers of MTX response in patients with JIA and were stratified based on American College of Rheumatology pediatric (ACR Pedi) response criteria. Semi-targeted global metabolomic profiles including over 800 metabolites across three analytical platforms at the NIH West Coast Metabolomics Center at UC-Davis and were analyzed by univariate and multivariate analysis using MetaboAnalyst 3.0. RESULTS/ANTICIPATED RESULTS: In K562 cells, MTX treatment was associated with statistically significant changes in 550 of the 850 intracellular metabolites detected (false discovery rate less than 0.05). Major metabolic pathways inhibited by MTX included branched-chain amino acid metabolism, purine and pyrimidine biosynthesis, and lipid metabolism including the inhibition of arachidonic acid metabolism. In patients with JIA, far fewer plasma metabolites were significantly altered following the initiation of MTX and included only 15 of the 833 plasma metabolites detected. Interestingly, MTX treatment was associated with the inhibition of arachidonic acid synthesis, inhibition of purine metabolism, and a dramatic reduction in plasma levels of various exogenous metabolites. In particular, MTX treatment was associated reductions in known metabolic markers of intestinal microbiota metabolism, including: biotin and dehydrocholic acid. Further, stratification of patients based on ACR Pedi response demonstrated that clinical response was associated with a greater reduction in plasma dehydrocholic acid levels following the initiation of MTX. DISCUSSION/SIGNIFICANCE OF IMPACT: This work demonstrates that MTX therapy is associated with a number of biochemical changes in vitro and in vivo, including: inhibition of purine metabolism, inhibition of arachidonic acid metabolism, and an apparent inhibition of gut microbiota metabolism. Most notably, inhibition of gut microbiota metabolism appears to demonstrate a relationship with the observed clinical efficacy of MTX in JIA.

3280

Mycobacterium bovis Bacille-Calmette-Guérin infection aggravates atherosclerosis

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OBJECTIVES/SPECIFIC AIMS: The study aimed at assessing whether M. bovis BCG infection and inflammation exacerbates the development of atherosclerosis in Ldlr-/- mice. METHODS/STUDY POPULATION: Twelve-week old male Ldlr-/- mice (n=10) were infected with M. bovis BCG (0.3–3.0x10^6 colony-forming units (CFUs)) via the intranasal route, to simulate a natural respiratory route of infection. Mice were subsequently fed a western-type diet (WD) containing 21% fat and 0.2% cholesterol for 16 weeks. Age-matched uninfected Ldlr-/- mice (n=10) fed with an identical WD served as controls. Mice were euthanized after 16 weeks of WD to examine atherosclerotic lesions in aortic root sections and en face aorta using Oil Red O staining. Plasma cholesterol and triglyceride levels were measured by enzymatic assays and lipoprotein distribution was assessed using fast protein liquid chromatography. Because of the important role of T cells and monocytes in atherosclerosis development, we assessed these cell subsets in blood using flow cytometry at 8 and 16 weeks. Experiments were conducted in duplicate. We used unpaired Student's t-test for group comparisons of numeric variables and flow cytometry data. RESULTS/ANTICIPATED RESULTS: M. bovis BCG infection significantly increased atherosclerotic lesions in en face aorta (plaque size per aorta area ratio; 0.15±0.13 vs. 0.06±0.02; $P<0.01$), but not in the aortic root. There were no significant differences in plasma cholesterol (1,160 mg/dL vs. 1,278 mg/dL; $P = 0.36$), triglycerides (340 mg/dL vs. 413 mg/dL; $P = 0.28$), or lipoprotein profiles between infected vs. uninfected mice at 16 weeks. M. bovis BCG increased circulating T lymphocytes (1,490 cells/uL vs. 1,227 cells/uL; $P = 0.03$) and monocytes (901 cells/uL vs. 414 cells/uL; $P<0.01$) within 8 weeks post-infection. When we assessed T lymphocyte subsets, M. bovis BCG infection increased total CD4+ T cell counts (556 cells/uL vs. 416 cells/uL; $P<0.01$) but not CD8+ T cells. No differences in the proportion of CD44+CD25+ activated T lymphocytes were noted between groups. When we assessed monocyte subsets, M. bovis BCG infection increased the numbers of Ly6Chigh (709 cells/uL vs. 362 cells/uL; $P<0.01$) and Ly6Clow (145 cells/uL vs. 35 cells/uL; $P<0.01$) monocytes. Infection was associated with an increased proportion of Ly6Clow monocytes at week 8 (17% vs. 8%; $P<0.01$) and week 16 (19% vs. 5%; $P<0.01$), compared to uninfected mice. DISCUSSION/SIGNIFICANCE OF IMPACT: M. bovis BCG infection increased the extent of atherosclerosis formation in the aortas of WD-fed hyperlipidemic Ldlr-/- mice after 16 weeks. Lipid profiles were similar between infected and uninfected mice, and therefore do not explain the observed differences in atherosclerosis. Compared to uninfected controls, M. bovis BCG-infected mice exhibited increased CD4+ T cell and monocyte driven inflammation. Interestingly, M. bovis BCG-infected mice had a higher proportion of non-classical Ly6Clow monocytes, suggesting a pro-atherogenic contribution of these cells in our model. Overall, our results support a pathogenic role of mycobacterial infection in atherosclerosis development and ASCVD.

3000

Olfactory habituation in schizophrenia

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OBJECTIVES/SPECIFIC AIMS: The aim objective of this research work is the study of neurocognitive endophenotypes in the new classifications of mental disorders. METHODS/STUDY POPULATION: Neuropsychological tests,such as the UPSIT test were used. The population was composed by a sample of patients with a diagnosis of schizophrenia RESULTS/ANTICIPATED RESULTS: Olfactory discrimination is a potencial neurocognitive endophenotype in the study of schizophrenia research DISCUSSION/SIGNIFICANCE OF IMPACT: The aim impact of this research work is the study of Dimensional classifications of mental disorders.

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