ratio (53%) that increased in combination with EPA by 216% ($p < 0.01$). Similar results were observed for EPA in combination with rosuvastatin. When either statin was combined with EPA, there was also decreased ONOO− release compared to statin alone ($p < 0.01$).

CONCLUSION: In combination with high intensity statins, EPA enhanced NO bioavailability in dysfunctional human ECs. The ability of EPA to reverse vascular EC dysfunction may lead to reduced ischemic events in statin-treated patients, as evidenced in outcome trials.

Amarin Pharma Inc., Elucida Research LLC

P010
IMMUNE ANALYSIS OF TISSUE ENGINEERED PORCINE AORTIC VALVE LEAFLETS AFTER ALPHA-GALACTOSE CLEAVAGE

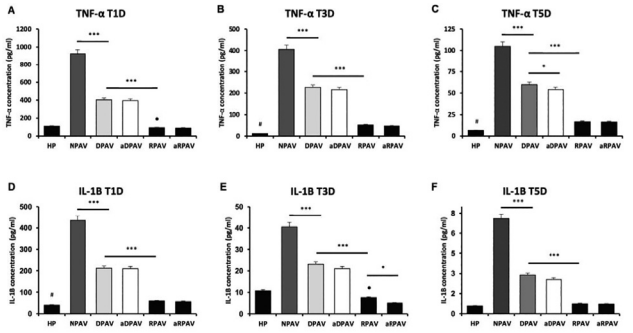
S Bozso, R EL-Andari, J Kang, J Nagendran

Edmonton, Alberta

BACKGROUND: Bioprosthetic heart valves are prone to structural valve deterioration (SVD) due to an inflammatory immune response resulting in calcification, stenosis, and ultimately valve failure. The antigenicity of xenografts is thought to underlie the immune response, with galactose-α-1,3-galactose (alpha-gal) the principal antigen of investigation. The objective of this study is to further characterize the role of alpha-gal in SVD and determine whether the addition of alpha-gal cleavage to tissue engineered porcine aortic valve (PAV) leaflets will attenuate the xenoreactive humoral immune response.

METHODS AND RESULTS: Samples of human pericardium, bone marrow, and whole blood were collected from patients undergoing elective cardiac surgery. PAV leaflets were excised from the hearts of female juvenile Yorkshire pigs, decellularized with or without alpha-gal cleavage via green coffee bean alpha-galactosidase, and recellularized with allogeneic human mesenchymal progenitor cells (hMSCs). These tissue-engineered constructs, as well as native PAV leaflets and autologous human pericardium, were exposed to human blood. At 1, 3, and 5 days proinflammatory cytokine production was quantified via enzyme-linked immunosorbent assays. On days 1, 3, and 5 there was a significant reduction in TNF-α and IL1-β concentration in the serum exposed to decellularized and recellularized PAV leaflets as compared to native PAV leaflets, as well as a significant reduction in the recellularized PAV tissue compared to the decellularized tissue. Compared to the decellularized tissue, the addition of alpha-gal cleavage reduced the TNF-α concentration on day 5. Similarly, as compared to the recellularized tissue, the addition of alpha-gal cleavage reduced the IL1-β concentration on day 3.

CONCLUSION: Allogenic recellularization of PAV tissue with hMSCs attenuates the xenoreactive immune response. Independent of the decellularization and recellularization processes, alpha-gal cleavage reduced the xenoreactive immune response to PAV tissue only at select time points. Therefore, other antigenic epitopes on xenogeneic tissue in addition to alpha-gal likely contribute to the immune-mediated SVD affecting bioprosthetic heart valves.



TNF-a production after (A) 1 day of blood exposure; (B) 3 days of blood exposure; (C) 5 days of blood exposure (n = 6). IL-1B production after (D) 1 day of blood exposure; (E) 3 days of blood exposure; (F) 5 days of blood exposure (n = 6). HP: human pericardium; NPAV: native porcine aortic valve; DPAV: decellularized porcine aortic valve; aDAPV: alpha-gal cleaved decellularized porcine aortic valve; RPAV: recellularized porcine aortic valve; aRPAV: alpha-gal cleaved recellularized porcine aortic valve. * Indicates P = 0.005; *** indicates P < 0.0001; # indicates HP < RPAV, P < 0.05; ' indicates RPAV < HP, P <0.05.

Alberta Innovates Health Solutions (AIHS), University Hospital Foundation

P011
IMMUNOENGINEERING APPLICATION OF MXENE FOR PREVENTION OF TRANSPLANT VASCULOPATHY

W Yan, A Rafieerad, K Alagarsamy, L Saleth, R Arora, S Dhingra

Winnipeg, Manitoba

BACKGROUND: Cardiac allograft vasculopathy is an aggressive form of atherosclerosis and a major cause of mortality among patients with heart transplants. Blood vessel endothelial cells stimulate alloreactive T-lymphocytes to result in sustained inflammation. MXenes are an emerging class of nanomaterials that have significantly outperformed several existing biomaterials as anti-cancer agents, biosensors, and in anti-microbial therapies. Herein, we report the first application of titanium carbide ($Ti_3C_2T_x$) MXene nanosheets for prevention of allograft vasculopathy.

METHODS AND RESULTS: To infer mechanisms and to ensure reproducibility of results, detailed physicochemical characterization of $Ti_3C_2T_x$ MXene nanosheets was performed using scanning/transmission electron microscopy, x-ray diffraction, and x-ray photoelectron spectroscopy. In vitro studies were carried out using co-cultures of human umbilical vein endothelial cells (HUVECs) with allogeneic peripheral blood mononuclear cells, and immunomodulatory function was assessed using flow cytometry and RNA sequencing. A rat aortic transplantation model was used for in vivo validation of safety and immunomodulatory function. $Ti_3C_2T_x$ MXene nanosheets were 2 to 5 μm in size and enriched with biologically active surface groups, including carboxyl, hydroxyl, and fluorine. In vitro, MXene nanosheets interacted with

HUVECs and downregulated the expression of genes involved in alloantigen presentation through the class I major histocompatibility complex (IRF1, TAP1, B2M), and reduced the activation and pro-inflammatory polarization of co-cultured allogeneic lymphocytes. Consequently, RNA-Seq analysis of these lymphocytes showed that treatment with MXene nanosheets downregulated genes responsible for transplant-induced T-cell activation, cell-mediated rejection, and development of allograft vasculopathy. Furthermore, gene set enrichment analysis revealed significant negative enrichment of genes involved in interferon alpha/beta and interferon gamma signaling. Finally, in an in vivo rat model of allograft vasculopathy, treatment with Ti3C2Tx MXene nanosheets reduced lymphocyte infiltration and preserved medial smooth muscle cell integrity within transplanted aortic allografts.

CONCLUSION: These findings support the potential of Ti3C2Tx MXene nanosheets for prevention and treatment of allograft vasculopathy and other inflammatory diseases. This research also opens the door to development of Ti3C2Tx MXene technologies for other immune-sensitive regenerative medicine applications.

Canadian Institutes of Health Research - Fellowship, Canadian Institutes of Health Research (CIHR)

P012
INCREASED EICOSAPENTAENOIC ACID TO ARACHIDONIC ACID (EPA/AA) RATIO IN BRAIN ENDOTHELIAL CELLS ASSOCIATED WITH REDUCED EXPRESSION OF NEUTROPHIL DEGRANULATION PROTEINS DURING INFLAMMATION

S Sherratt, P Libby, D Bhatt, P Mason

Beverly, Massachusetts

BACKGROUND: Endothelial cell (EC) dysfunction results in inflammation and increased atherothrombotic risk, including stroke. The omega-3 fatty acid (FA) eicosapentaenoic acid (EPA) improves arterial compliance in a manner predicted by circulating EPA/AA ratios, an indicator of cardiovascular (CV) risk. Treatment with icosapent ethyl (IPE), a formulation of highly purified EPA, reduced composite CV events, including stroke, in REDUCE-IT that correlated with on-treatment EPA levels. We measured the effects of EPA on the EPA/AA ratio and protein expression in endothelial cells from brain ECs (BECs) following cytokine challenge.

METHODS AND RESULTS: BECs were challenged with IL-6 (12 ng/mL) for 2 hours and then treated with EPA (40 μM) or equivolume vehicle and incubated for 24 hours. Samples were allocated for FA analysis or proteomic analysis. Total cellular FAs were extracted, derivatized to FA methyl esters (FAME), and analyzed using gas chromatography. Total protein content was also measured and used to normalize fatty acid content. Global proteomic analysis was performed using LC/MS to measure relative expression levels of proteins between treatment groups. Significant ($p < 0.05$) changes in expression between treatment groups (>1-fold) were analyzed using differential enrichment analysis of proteomics data (DEP). Biological pathways were analyzed using proteins that survived the cutoff criteria via gene set enrichment analysis (GSEA). EPA treatment significantly modulated proteins in the "neutrophil degranulation" pathway (GO:0043312) with pathway adjusted-p values of $3.68 \times 10\text{-}13$. EPA significantly altered expression of 66 proteins in this pathway, including increasing peroxiredoxin-6 and heat shock protein 90, relative to IL-6. These changes in protein expression in BECs correlated with large, significant increases in the EPA/AA ratio from 0.025 ± 0.002 to 1.72 ± 0.20 (>67-fold increase, $p < 0.001$).

CONCLUSION: EPA favorably modulated proteins in brain ECs related to neutrophil degranulation in a manner that correlated with increases in the EPA/AA ratio. The anti-inflammatory benefits may contribute to reduced stroke risk with IPE as demonstrated in outcome trials.

Amarin Pharma Inc., Elucida Research LLC

P013
INTESTINAL ADAPTATION TO SHORT-TERM, EXTREME FAT CONSUMPTION ALTERS TRIGLCYERIDE-RICH LIPOPROTEIN SECRETION AND INTESTINAL LIPID HANDLING IN MALE AND FEMALE MICE

N Morrow, C Locatelli, N Trzaskalski, C Klein, A Hanson, I Tripathi, A Clément, I Lorenzen-Schmidt, E Mulvihill

Ottawa, Ontario

BACKGROUND: Dysregulated postprandial metabolism including elevated chylomicron remnants, contributes to atherogenesis. The ketogenic diet is a patient-accessible metabolic health intervention whereby nutritional ketosis is achieved by consuming predominantly fat (>70% of kcal) with restricted carbohydrates and protein. It has been established that intestinal enterocytes package dietary triglycerides (TG) into chylomicrons for transport into circulation and store excess TGs as cytosolic lipid droplets (CLDs) such that nutrient absorption is maximized.

METHODS AND RESULTS: The current study aimed to characterize the impact of diet composition on postprandial intestinal lipid handling in male and female wild-type mice. We evaluated intestinal cytosolic lipid droplets, TG secretion rates, and intestinal morphology with short-term, high-fat Western diet (WD) and an extreme fat, low protein, carbohydrate-restricted ketogenic diet (KD) before developing obesity. For three weeks, mice previously fed a grain-based standard laboratory diet (GBD) were switched to a WD or KD. Both male and female KD-fed mice displayed significantly higher plasma TG levels in response to an olive oil gavage than WD- and GBD-fed mice. At fasting, intestinal TG mass was significantly higher in both male and female