load. Circumferential RS can be estimated ex-vivo via the opening angle (OA), by subjecting an aortic ring to a radial cut. Although the underlying mechanism for RS remains poorly understood, our recent efforts have demonstrated strong correlations between the amounts of sulfated glycosaminoglycans (sGAG) and the OA in healthy aortas. In addition, advanced glycation end products (AGEs) are known to accumulate with age and create crosslinks within the extracellular matrix. Since ruptures are more prevalent in older tissue, the purpose of this study was to compare the effect of sGAG on the OA in intact and glycated aortic tissue.

METHODS AND RESULTS: Sets of four adjacent aortic rings were excised from the upper thoracic regions of 9 porcine aortas. One ring served as a control, while a second ring underwent enzymatic sGAG depletion, a third underwent glycation, and the fourth ring underwent a combination of glycation followed by enzymatic sGAG depletion. A 100mM ammonium acetate buffer, pH 7.0 was used. Glycation and sGAG depletion were induced by incubating samples in 700mM of ribose, and 15U/mL hyaluronidase, 0.075U/mL chondroitinase ABC, 0.75U/mL heparinase for 48 hours at 37oC, respectively. The OA was then measured and the sGAG and general AGE contents were quantified. The quantification of sGAG contents in control and treated samples confirmed the successful removal of sGAG, with reductions by 94±2.5% (average ± standard deviation). In addition, a 93±27% increase in general AGEs was achieved. Statistically significant differences were found between the OAs of control and sGAG depleted, glycated, and glycated combined with sGAG depleted samples (paired sample t-test, $p < 0.001$). Specifically, the OA was reduced by 34±12% after sGAG depletion, 14±8% after glycation, and 45±15% after combined glycation and sGAG depletion. In addition, the OAs in sGAG depleted samples, as well as in combined glycated and sGAG depleted samples, were found to be significantly smaller than samples that underwent glycation only (paired sample t-test, $p=0.002$), being 22±15% and 34±18% smaller respectively.

CONCLUSION: These findings supported that sGAG depletion causes a reduction in the OA, in both intact and glycated tissue, and that AGEs may also affect the magnitude of the RS.

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P006
EICOSAPENTAENOIC ACID (EPA) DECREASES CYTOKINE RELEASE AND EXPRESSION OF INFLAMMATORY AND PRO-THROMBOTIC PROTEINS IN BRAIN VASCULAR ENDOTHELIUM

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BACKGROUND: Brain vascular endothelial cell (EC) dysfunction contributes to ischemic stroke due to inflammation and release of pro-thrombotic factors. Treatment with icosapent ethyl (IPE), the ethyl ester of the omega-3 fatty acid, eicosapentaenoic acid (EPA), reduced first and total ischemic strokes each by 36%, in statin-treated patients with elevated cardiovascular risk (REDUCE-IT). We tested the effects of EPA on cytokine release and expression of inflammatory proteins from brain microvascular ECs during inflammation.

METHODS AND RESULTS: Human brain microvascular endothelial cells were pretreated with the cytokine IL-6 at 12 ng/ml for 2 h before treatment with EPA (40 μM) for 24 h. Proteomic analysis was performed using LC/MS to capture relative expression levels. Only significant changes in protein expression between treatment groups >1-fold were analyzed. Levels of soluble intercellular adhesion molecule-1 (sICAM-1) and tumor necrosis factor-α (TNF-α) were measured by immunochemistry (ELISA). IL-6 exposure produced increased levels of sICAM-1 and TNF-α by 102% and 147% ($p < 0.001$), respectively, in brain ECs compared with vehicle. EPA treatment reduced release of sICAM-1 by 43% ($p < 0.001$) and TNF-α by 52% ($p < 0.001$) compared to IL-6 alone. EPA also decreased expression of 43 proteins involved in the "neutrophil degranulation" pathway in brain ECs (p-adjusted = 2.63×10-12). EPA also decreased expression of prothrombin by 1.3-fold ($p = 2.10 \times 10$-24) relative to IL-6 alone.

CONCLUSION: EPA significantly reduced cytokine release and expression of inflammatory and pro-thrombotic proteins in brain ECs during inflammation. The ability of EPA to reverse brain EC dysfunction and inflammation may contribute to reductions in stroke risk, as demonstrated in large outcome trials.

Amarin Pharma Inc., Elucida Research LLC

Young Investigator Award Winner —Basic Science

P007
ENDOTHELIAL COLONY-FORMING CELL-DERIVED EXTRACELLULAR VESICLES AND CARDIAC REPAIR AFTER MYOCARDIAL INFARCTION

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BACKGROUND: Despite improvements in therapeutics, ischemic heart disease remains a leading cause of death. Cardiac remodeling after myocardial infarction (MI), predominantly due to loss of cardiomyocytes and coronary vasculature, leads to a progressive decline in cardiac function resulting in heart failure. Current therapies for cardiac repair and heart failure are of limited benefit. Cell transplantation therapy upon MI is a very promising therapeutic strategy to replace dead myocardium, reducing scarring and improving cardiac performance.

METHODS AND RESULTS: Our research focuses on endothelial colony-forming cell-derived exosomes (ECFC-exosomes), which are actively secreted endocytic nanovesicles (30-100

nm) that transport functional miRNAs, proteins, mRNAs, and lipids, playing a key role in paracrine intercellular communication. We identified a novel ability of ECFC-exosomes to promote angiogenesis and cardiac tissue repair. Administration of ECFCs to mice following experimental end-organ ischemia resulted in ECFC-exosome-dependent increase in angiogenesis. ECFC-derived exosomes were taken up by endothelial cells leading to their increased proliferation and migration, tube formation, and formation of new vessels. Administration of ECFC-exosome to a murine model of myocardial infarction prevented cardiac remodeling and heart failure. Next generation sequencing and bioinformatics analyses identified 136 miRNAs present in ECFC-exosome cargo, and factor inhibiting HIF-1α and PTEN as their potential targets in endothelial cells.

CONCLUSION: Our findings support the view that the ECFC-exosomes represent a novel therapeutic approach to improve cardiac repair and prevent the onset of heart failure after MI.

Canadian Institutes of Health Research (CIHR)

Trainee Research Award Finalist—Basic Science

P008
EXTRACELLULAR VESICLES FROM HUMAN HEART EXPLANT-DERIVED CELLS ATTENUATE ACTIVATION OF THE NLRP3 INFLAMMASOME IN MACROPHAGES

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BACKGROUND: Intramyocardial injection of heart explant-derived cells (EDCs) improves cardiac function in preclinical models of ischemic cardiomyopathy. This therapeutic benefit is partially attributable to the anti-inflammatory cargo (micro RNAs and proteins) enriched within the extracellular vesicles (EVs) released by EDCs. Recent work has shown that activation of the NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) inflammasome in both immune and non-immune cells plays a critical role in promoting cardiac inflammation and adverse remodeling. Although EDC EVs are known to modulate inflammatory mediators, their effects on the NLRP3 inflammasome are not known. Therefore, we explored the ability of EDC EVs to attenuate NLRP3 inflammasome activation in macrophages, the major pro-inflammatory cell type recruited to myocardium after an ischemic insult.

METHODS AND RESULTS: EVs were isolated from EDC conditioned media (ultracentrifugation) and characterized (Nanosight & antibody array). Monocytes (THP-1) were differentiated into macrophages (PMA; 3 days) and treated with EVs (20 hours) before priming (LPS; 4 hours) and activating (nigericin; 1 hour) the NLRP3 inflammasome. Secreted caspase-1 in the culture supernatants was measured by using a bioluminescent assay (Promega). The miRNA and protein cargo within EVs was profiled using miRNA detection (Nanostring) and liquid chromatography-mass spectrometry, respectively. miRNA and protein data were analyzed using appropriate bioinformatics tools (Tam 2.0, miRWalk, and Uniprot). EV size (160$\pm$2 nm) and markers (ICAM, ALIX, CD81, CD63, EPCAM, ANXAS, TSG101, FLOT-1) confirmed EV identity. Macrophages pretreated with EVs (4E+10 EVs/mL) showed a significant attenuation in NLRP3 inflammasome induced caspase-1 vs. LPS+nigericin-only treated cells (50% lower, n=4-6, p=0.02). EV cargo profiling revealed that EVs were enriched with 22 distinct anti-inflammatory miRNAs (Tam 2.0). Specifically, 3 miRNAs (miR-21, miR-100, miR-181a, n=3) and 5 proteins (Peroxiredoxin-1, Thioredoxin-1, Caveolin-1, Sequestosome-1, n=3) abundantly found within EVs were predicted to inhibit the NLRP3 inflammasome (miRWalk & Uniprot).

CONCLUSION: EDC EVs suppress activation of the NLRP3 inflammasome in macrophages via transfer of anti-inflammatory miRNAs and proteins.

Canadian Institutes of Health Research (CIHR), University of Ottawa Heart Institute - Strategic Endowed Research Fellowship

P009
HIGH-INTENSITY STATINS COMBINED WITH EICOSAPENTAENOIC ACID (EPA) IMPROVES ENDOTHELIAL FUNCTION DURING EXPOSURE TO OXIDIZED LDL

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BACKGROUND: During atherosclerosis, endothelial cell (EC) dysfunction results in reduced nitric oxide (NO) bioavailability and increased cytotoxic peroxynitrite (ONOO$-$). This loss of NO bioavailability results in abnormal vasodilation and inflammatory changes. Eicosapentaenoic acid (EPA) administered as icosapent ethyl (IPE) reduced cardiovascular (CV) events in high-risk patients treated with statins (REDUCE-IT). We tested the effects of high-intensity statins and EPA in ECs exposed to oxidized LDL (oxLDL).

METHODS AND RESULTS: Human umbilical vein ECs (HUVECs) were pretreated with 20 mg/dL oxLDL for 20 min, then treated with atorvastatin (active metabolite, ATM) and rosuvastatin (rosuva) at 1.0 μM $\pm$ EPA (10μM) for 1 hr. Cells were stimulated with calcium and assayed for the NO/ONOO$-$ release ratio using nanosensors. ECs exposed to oxLDL showed a 60% reduction in NO release compared with vehicle (386$\pm$29 to 156$\pm$18 nM, $p< 0.001$) concomitant with a pronounced increase in ONOO$-$ release (205$\pm$31 to 283$\pm$16 nM, $p< 0.001$), resulting in a >70% decrease in the NO/ONOO$-$ release ratio ($p < 0.001$). ECs treated with ATM had an improved NO/ONOO$-$ release