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“Temporal Dynamics of Chronic Ethanol-Induced Pro-Inflammatory Phenotype and Senescence in Mouse Aortic Endothelial Cells”

Purpose: Cardiovascular disease remains a leading cause of morbidity and mortality worldwide, with chronic alcohol consumption serving as a major modifiable risk factor¹. The vascular endothelium plays an essential role in maintaining vascular tone and ensuring systemic cardiovascular health. Long-term alcohol exposure is known to damage endothelial cells, contributing to pathologies such as hypertension, coronary artery disease, and heart failure; however, the precise stepwise cellular mechanisms remain elusive. In this study, we investigated the temporal dynamics of alcohol-induced cellular injury by tracking how ethanol exposure drives oxidative stress, DNA damage, and pro-inflammatory senescence-associated signaling in primary mouse aortic endothelial cells.

Methods: Primary mouse aortic endothelial cells (MAECs) were cultured and subjected to 50mM ethanol or isosmotic vehicle control for 3 days, 5 days, or 7 days. Quantitative PCR (RT-qPCR) was performed across three time points to map the transcriptional changes of key cell-cycle checkpoint inhibitors (*Cdkn1a/p21*, *Cdkn2a/p16*, and *p53*) and Senescence-Associated Secretory Phenotype (SASP) factors (*Cxcl1*, *Il1a*, and *Il6*). Based on these time-resolved expression profiles, single-cell phenotypic confirmation was performed on the consolidated 7-day chronic exposure model. Cellular proliferation and permanent cell-cycle arrest were assessed by Ki-67 immunofluorescence, while double-strand DNA damage was evaluated and co-localized via phosphorylated histone H2Ax staining.

Results: Gene expression analysis revealed that 3- and 5-day ethanol exposures induce an early, transitional stress response characterized by limited transcriptional alterations. Extending continuous ethanol exposure up to 7 days, however, resulted in a severe pro-inflammatory phenotype in MAECs, characterized by robust transcriptional upregulation of core cell-cycle arrest markers: p21 ($p < 0.001$), p16 ($p < 0.0001$), and p53. This coincided with a pronounced pro-inflammatory SASP profile, as evidenced by significant increases in *Cxcl1* ($p < 0.01$) and *Il6* ($p < 0.01$) mRNA levels in cells, while *Il1a* remained unchanged relative to controls. Phenotypic validation on Day 7 by co-localization immunocytochemistry confirmed that chronic ethanol exposure dramatically increased the population of cell cycle-arrested, highly DNA-damaged (Ki-67 negative / H2Ax positive) senescent MAECs compared with controls ($p < 0.01$).

Conclusion: Chronic ethanol exposure impairs endothelial cell health through a distinct, time-dependent cascade that evolves from an early stress response at Day 3 into a stable state of DNA damage-associated cellular senescence and pro-inflammatory SASP activation by Day 7. These findings suggest that long-term alcohol misuse locks the vascular wall into a chronic pro-

inflammatory phenotype, advancing endothelial senescence, which may subsequently drive downstream vascular dysfunction and cardiovascular disease. Future studies will leverage this temporal framework to elucidate the chronic impact of ethanol exposure on the vasculature and identify potential therapeutics to ameliorate the adverse effects of alcohol misuse on advancing cellular senescence.