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Effects And Mechanisms Of Cyclin-Dependent Kinase Inhibitor (CDKi) Drugs On Human Neutrophil Survival Induced By Dimethyloxaloylglycine (DMOG) And Deferoxiamine (DFO)

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Introduction: Inflammation is essential for dealing with injury and infection. However, when dysregulated this normally beneficial response results in worsening tissue damage and fibrosis, often exacerbated by prolonged neutrophil lifespan and function (1). Hypoxia, which occurs at sites of inflammation, prolongs neutrophil survival by delaying apoptosis (2)(3). Neutrophil apoptosis and their subsequent non-inflammatory clearance by phagocytes such as macrophages and dendritic cells are considered critical for the successful resolution of inflammation (4). We hypothesised that neutrophil survival induced by chemicals (DMOG and DFO) which mimic biochemical conditions occurring during hypoxic conditions may be overridden by CDKi drugs (e.g., AT7519, flavopiridol and R-roscovitine) (4). We also aimed to investigate the potential underlying mechanisms involved.

Methods: Human neutrophils were isolated from peripheral venous blood of healthy donors (3) (Lothian Research Ethics Committee, 08/S1103/38) and treated with DMOG (100μM) or DFO (100μM) with or without AT7519 (10μM), flavopiridol (10μM) and R-roscovitine (20μM); where appropriate, PBS/DMSO vehicle controls were used throughout the study. Cell survival and apoptosis were determined by morphological analysis of cytocentrifuge preparations and annexinV/propidium iodide binding assessed by flow cytometry (5). Western blotting was performed to investigate expression levels of the key intracellular anti-apoptotic protein, induced myeloid leukaemia cell differentiation protein (Mcl-1) (4).

Results: DMOG and DFO induced neutrophil survival by delaying apoptosis following 20 h culture in comparison to the control neutrophils (DMOG = 53.2% viable cells, SD = 8.1 vs. control = 38.0% viable cells, SD = 6.3, n = 5, $P<0.05$). R-roscovitine, flavopiridol and AT7519 all overrode neutrophil survival induced by DMOG and DFO (DMOG + AT7519 = 13.3% viable cells, SD = 8.2, n = 5, $P<0.001$). The NF-κB inhibitor, gliotoxin, attenuated DMOG-mediated neutrophil survival suggesting a role for this transcription factor in the observed delay in neutrophil apoptosis (gliotoxin + DMOG = 14.8% viable cells, SD = 4.0, n = 4, $P<0.001$). Results were analysed by two-way analysis of variance with a Tukey post-hoc test. Furthermore, AT7519 was found to override the pro-survival effect of DMOG by down-regulating Mcl-1 expression, concomitantly increasing the expression of cleaved caspase 3.

Conclusion: We have demonstrated that CDKi drugs override the delayed apoptosis induced by conditions that mimic hypoxia. DMOG may work by increasing expression of the anti-apoptotic protein Mcl-1 and is also likely to effect NF-κB activation. The ability of CDKi drugs to override DMOG-mediated neutrophil survival appears to be dependent on the down-regulation of Mcl-1. Inflammatory

environments, where tissue hypoxia occurs, may therefore be targeted by neutrophil apoptosis-inducing CDKi drugs to enhance inflammation resolution.

References:

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