014 APPA INHIBITS NEUTROPHIL PRO-INFLAMMATORY FUNCTIONS WITHOUT IMPAIRING HOST DEFENCE: IS THIS A POTENTIAL NEW THERAPY FOR ARTHRITIS?

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Background: APPA (a fixed dose combination of synthetic apocynin, AP and paeonol, PA) has shown efficacy in several animal models of osteoarthritis (OA) and is currently in Phase I evaluation for the treatment of OA. Its efficacy is thought to be mediated through synergistic effects of constituent components, AP and PA, on the regulation of NF-κB transcription factor activation. NF-κB plays a key role in both constitutive and inducible gene expression, particularly in immune cells, and is therefore an attractive therapeutic target. Neutrophils are key to innate host defence via phagocytosis of pathogens and activation of granule enzymes and reactive oxygen species (ROS). Neutrophil function during inflammation is regulated by the activities of a number of cytokines, including TNFα and GM-CSF. Neutrophils are potent producers of ROS (activators of NF-κB) via NADPH oxidase (NOX2) and many inflammatory functions, including delayed apoptosis, are regulated by NF-κB activation. The purpose of this study was to investigate the effects of APPA on key neutrophil functions and explore its potential use in treating inflammation.
Methods: Healthy subject blood neutrophils ($n = 8$) were used to assay a variety of neutrophil functions. All tests were performed in the absence and presence of APPA (600 μM). Inflammatory signalling pathways were activated using TNFα, GM-CSF & IL-6 and the effects of APPA on signal transduction measured using Western blotting. Formation of neutrophil extracellular traps (NETs), phagocytosis of latex beads, killing of *S. aureus*, chemotaxis of neutrophils, cell surface receptor expression (integrins and FcγRs) and ROS production were also measured. Expression of cytokine/chemokine genes was measured using qPCR.
Results: APPA did not significantly affect neutrophil host defence functions including apoptosis, receptor expression, phagocytosis and bacterial killing, but was a potent ROS scavenger. APPA significantly ($p < 0.05$) inhibited NET formation and decreased neutrophil degranulation. TNFα-induced NF-κB signalling was inhibited by APPA (600 μM and above), as was GM-CSF and IL-6 signalling via ERK1/2 and STAT3, respectively. APPA decreased TNFα-activated expression of IL-8 and TNFα mRNA but upregulated NRF2, an anti-inflammatory regulator of antioxidant proteins. APPA was also an effective inhibitor of IL-6 and chemokine expression (CCL3, CCL4) induced by the TLR8 agonist and chromatin re-modelling agent, R848 ($p < 0.05$). This agonist triggers the expression of these genes via endogenous TNFα secretion and activation of neutrophils; APPA was as effective as the biologic Infliximab in this inhibition.
Conclusion: APPA does not significantly impair host defence neutrophil functions and may have significant anti-inflammatory potential in diseases characterised by dysregulation of cytokine expression or oxidative stress, such as rheumatoid arthritis. These data also describe the novel finding that APPA is as effective as biologic drugs in inhibiting the effects of endogenous TNFα on immune cell activation. Thus it may have therapeutic potential in TNFα-driven inflammatory conditions.
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