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EFFECT OF A NOVEL PPAR AGONIST ON NEUTROPHIL AND MACROPHAGE FUNCTION IN THE INFLAMMATORY RESPONSE

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INTRODUCTION

PPARɣ activation inhibits inflammatory cytokines in neutrophils and macrophages. This study evaluates the effects of a new thiazolidinedione (D3) and its role in inflammation.

MATERIAL AND METHODS

In-silico toxicity prediction (QSAR Toolbox, Swiss Adme) and molecular docking assessed D3's interaction with PPARγ and PPAR isoform activation. Neutrophils were stimulated with LPS (1 µg/mL) and treated with D3 (0.1, 1, 10 µM). RAW 264.7 macrophages received the same treatment, with or without GW9662 (10 µM), to assess PPARγ involvement. NO2- (Griess), TNF, IL-6, and IL-1β (ELISA) were measured in the supernatant, and COX-2 expression was analyzed by Western blot. D3's chemotaxis against fMLP (0.1 µM) was evaluated. An efferocytosis assay with apoptotic neutrophils measured TNF and IL-10 (ELISA). Human neutrophils and monocyte-derived macrophages were used in further studies. Neutrophils were stimulated with PMA (100 nM) to induce NETosis and treated with D3, followed by flow cytometry (DAPI, MPO, Cit-H3). Phagocytosis was assessed by incubating neutrophils with D3 and Escherichia coli. Flow cytometry analyzed neutrophil surface molecules (CD62L, CD11b, CD66b, CD33, CD49d, CD47, CD64, CD16, CXCR2) and macrophage polarization markers (CD206, CD163, CD86, CD80, CD64, CCR2, CCR5, CCR7) along with intracellular signaling (pSTAT3, NF-κB, p38 MAPK, IL-6). In vivo, a lung inflammation model was used. Mice received D3 (1, 10, or 30 mg/kg), dexamethasone (5 mg/kg), or pioglitazone (18 mg/kg). Inflammation was induced via intranasal LPS (4 mg/mL). After 24 hours, bronchoalveolar lavage (BAL) was analyzed for cellularity (CD45, LY6G, CD11B, CD62L). Animal experiments were approved by CEUA 024/21, and human studies by UCL Ethics Committee 1309/006.

RESULTS

In silico assays predicted good oral bioavailability for D3, a partial PPARγ agonist with slight PPARα and PPARβ/δ activation. Molecular docking showed strong PPARγ interaction. D3 reduced pro-inflammatory mediator release in neutrophils and macrophages via PPARγ. It decreased migration and NETosis but increased phagocytosis and efferocytosis. D3 modulated cell surface markers, shifted M1 to M2-like macrophages, regulated pSTAT3 and p38 MAPK, and inhibited COX-2, indicating immunomodulatory activity. In LPS-stimulated mice, D3 reduced BAL leukocytes and neutrophils and modulated CD11b expression.

CONCLUSIONS

The results obtained demonstrate that compound D3 is a promising anti-inflammatory molecule, with activity dependent on PPARɣ activation. The compound showed important effects on murine and human neutrophils and macrophages, demonstrating that in addition to reducing pro-inflammatory signaling, it favors the resolution of the inflammatory process, through changes in macrophage phenotypes and promotion of efferocytosis.

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