IV SIMPÓSIO INTERNACIONAL EM INVESTIGAÇÕES QUÍMICO-FARMACÊUTICAS

Área: FAR

ANTI-INFLAMMATORY EFFECTS OF THE PARTIAL PPARγ AGONIST D3 IN MACROPHAGES (RAW 264.7)

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INTRODUCTION

Macrophages are sentinel cells capable of detecting lesions and responding to pathogen invasion during the inflammatory process, producing effector molecules for the recruitment of other cells. To resolve inflammation, macrophages engulf neutrophils through efferocytosis, undergo phenotypic changes, and restore homeostasis. However, during this process, an exacerbation of the pro-inflammatory response may occur, triggering chronic inflammation. Compound D3 was synthesized at UNIVALI to function as a partial agonist of PPARγ with anti-inflammatory activity; however, its effect on the activation of PPAR isoforms has not been experimentally confirmed. Therefore, the aim of this study was to analyze the activity of compound D3 on PPAR isoforms and on the anti-inflammatory activity of Raw 264.7 cells (macrophage) *in vitro* induced by lipopolysaccharide (LPS).

MATERIAL AND METHODS

A cell viability assay was conducted using Raw 264.7 cells (1×10^5) to evaluate the effect of D3. The experiment included 10% dimethyl sulfoxide (DMSO) and D3 (1 μM), with or without PPAR isoform antagonists. Cells were treated with MTT (5 mg/mL) and incubated for 21 hours. The reporter gene test was performed with HeLa cells (25×10^3), to evaluate the effect of D3 on the activation of PPAR isoforms. DMSO (vehicle), WY14643 (PPARα agonist, 100 μM), GW0742 (PPARβ/δ agonist, 0.1 μM), Beza (PPARβ/δ agonist, 100 μM), rosiglitazone (PPARγ agonist, 10 μM) and pioglitazone (PPARγ agonist, 10 μM) were used as controls. Raw macrophages were stimulated with LPS (1 μg/mL) and treated with D3 (1 μM) in the absence or presence of the antagonists GSK3787, GW9662 and T0070907 (10 μM), for 24 hours. Nitrite levels were evaluated in the culture supernatant using the Griess reaction (1% sulfanilamide with 0.1% α-naphthyl ethylenediamine).

RESULTS

The MTT assay confirmed that compound D3 had no impact on macrophage viability. In the reporter gene assay D3 exhibited partial activation of the PPARγ receptor compared to the control agonists, while also activating PPARα and PPARβ isoforms to a lesser extent. This suggests that D3 functions as a partial agonist of the nuclear receptor PPARγ, contributing to the repression of pro-inflammatory genes and the inhibition of inflammatory cytokines, as supported by nitric oxide data. D3 reduced nitrite release in LPS-stimulated macrophages, and its anti-inflammatory effects were reversed upon co-cultivation with the inhibitors T0070907 and GW9662. However, PPARβ activation did not affect the anti-inflammatory activity of D3, as the use of the inhibitor GSK3787 had no impact on its function.

CONCLUSIONS

Together, the data herein obtained demonstrate that compound D3 is a PPAR-pan agonist but presents PPARγ-dependent anti-inflammatory activity without presenting cytotoxicity to macrophages.

ACKNOWLEDGMENTS

UNIVALI, PIBIC, CAPES, CNPq, UnB, INCT Inovamed.