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Pro-inflammatory properties for thiazolidinediones

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Abstract

Thiazolidinediones (TZDs) are pharmacological ligands of the peroxisome proliferator-activated receptor (PPAR)-γ that are extensively used in the treatment of type II diabetes. Recently, an anti-inflammatory potential for TZDs has been suggested, based on observations that these compounds may inhibit pro-inflammatory cytokine expression in vitro and may attenuate the inflammatory response in vivo. Here, we show that the TZDs rosiglitazone (RSG) and troglitazone (TRO) do not inhibit the inflammatory response to tumor necrosis factor (TNF)-α in various epithelial cell types. On the contrary, both RSG and TRO significantly potentiated TNF-α-induced production of granulocyte/macrophage-colony-stimulating factor, interleukin (IL)-6 and/or IL-8 in these cells. This increase in pro-inflammatory cytokine expression was functionally significant as supernatants from cells co-treated with TNF-α and TZDs displayed increased neutrophil pro-survival activity when compared with supernatants from cells treated with TNF-α alone. Additionally, it was shown that TZDs enhance cytokine expression at the transcriptional level, but that the pro-inflammatory effects of TZDs are independent on PPARγ, nuclear factor κB or mitogen-activated protein kinase activation. Our study shows that TZDs may potentiate the inflammatory response in epithelial cells, a previously unappreciated effect of these compounds.

Keywords: Cytokines; Inflammation; Mitogen-activated protein kinases; Nuclear factor-κB; Peroxisome proliferator-activated receptor γ; Thiazolidinediones

1. Introduction

Thiazolidinediones (TZDs) are pharmacological agents that improve glucose homeostasis in type 2 diabetes by increasing insulin sensitivity. They were shown to reduce cardiovascular risk factors associated with this condition [1]. The TZDs are a group of structurally related compounds characterised by a thiazolidinedione ring, to which divergent molecular moieties are attached. Troglitazone (TRO) was the first TZD approved for treating type 2 diabetes but was withdrawn from the market due to hepatic toxicity [2]. Two other TZDs, rosiglitazone (RSG, also referred to as BRL49653) and pioglitazone, are now available and show no hepatic side effects [3,4]. Most of the beneficial effects of TZDs in the treatment of type 2 diabetes were attributed to the potential of these molecules to activate the nuclear receptor Peroxisome proliferator-activated receptor γ (PPARγ), a critical regulator of lipid metabolism and glucose homeostasis [5–10].

More recently, PPARγ has been suggested to play a downregulatory role in inflammatory processes, raising the hypothesis that PPARγ ligands, such as the TZDs, could be efficient in the treatment of inflammatory disorders [11,12]. The first support to the hypothesis of an

Abbreviations: AP-1, activator protein-1; HBEC, human bronchial epithelial cells; c/EBP, CAAT enhancer binding protein; CREB, cAMP responsive element binding protein; ERK, extracellular signal-regulated protein kinase; GM-CSF, granulocyte/macrophage-colony-stimulating factor; IL, interleukin; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; NF-AT, nuclear factor of activated T cells; NF-κB, nuclear factor-κB; PPARγ, peroxisome proliferator-activated receptor γ; RSG, rosiglitazone; TNF, tumor necrosis factor; TRO, troglitazone; TZD, thiazolidinedione

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anti-inflammatory potential for TZDs was provided by the observation that they inhibit monocyte/macrophage activation and expression of inflammatory molecules, i.e. interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF-α), inducible nitric oxide synthase (iNOS) and gelatinase B [13,14]. Later, these observations were extended to other inflammatory molecules (e.g. IL-2, IL-8, interferon-γ) and cell types (e.g. endothelial cells, colon cells, lymphocytes) in vitro [15–17]. Beneficial effects were also reported in vivo in animal models of human inflammatory disorders like inflammatory bowel disease [15], rheumatoid arthritis [18], multiple sclerosis [19] and asthma [20], suggesting a general anti-inflammatory potential for TZDs.

Here, we show that the TZDs RSG and TRO do not inhibit the cytokinic response to the potent pro-inflammatory cytokine TNF-α in various epithelial cell types. On the contrary, we show that both RSG and TRO may strongly increase TNF-α-induced pro-inflammatory cytokine expression. Insights into the mechanisms mediating this previously unappreciated effect of TZDs are also provided.

2. Materials and methods

2.1. Cell culture and reagents

A549, Hct-116, and OVCAR cells were obtained from the German Collection of Microorganisms and Cell Cultures. The cells were cultured in either Ham F-12 (A549 cells), McKoy (Hct-116 cells) or RPMI 1640 (OVCAR cells), supplemented with 10% FCS, 1% glutamine, 50 μg/ml streptomycin, and 50 IU/ml penicillin (GIBCO BRL). BEAS-2B cells from the American Type Culture Collection Cells and Human Bronchial Epithelial Cells (HBEC) from primary human explants were prepared and cultured as previously described [21]. Human blood neutrophils were obtained from buffy coats (Transfusion Center of Liege, Belgium). Neutrophils were separated from mononuclear cells by density centrifugation (Histopaque, Sigma). Contaminating erythrocytes were removed from the neutrophil fraction by hypotonic lysis. Neutrophil purity, as determined by counting of cytospin preparations stained with Diff-Quick (Dade Behring), was always >95%. Recombinant human TNF-α was purchased from Roche. Rosiglitazone was a generous gift from Laboratories Servier (Paris, France) and troglitazone was obtained from Biomol. Neutralizing antibodies directed against human GM-CSF or IL-6 were from R&D, actinomycin D was from Sigma and GW9662 was from Cayman Chemicals.

2.2. Immunoassays

The concentration of IL-8, IL-6, and GM-CSF in cell supernatants was measured using ELISA kits (Biosource).

2.3. Cell proliferation assays and detection of apoptosis and necrosis

Cell proliferation was assayed using the Cell Proliferation Reagent WST-1 (Roche) according to the manufacturer's instructions. Apoptosis and necrosis were assessed by staining with annexin-V-FITC and propidium iodide using the annexin-V-FLUOS staining kit (Roche), following the recommendations of the manufacturer. Flow cytometry analyses were performed with a FACStar Plus® (Becton Dickinson).

2.4. Quantitative polymerase chain reactions

Total RNA was extracted from cells using the Rneasy Mini kit according to manufacturer's instructions (Qiagen). Poly(A) RNA was primed with oligo(dT) and random hexamers (Roche) and reverse transcribed with the AMV reverse transcriptase (Roche) for 1 h at 42 °C. Sequences of the primers (Eurogentec) used in subsequent PCR were as follows—GM-CSF: cagcctcaccaagctcaag and ctgggttgcacaggaagttt; IL-6: cagccactcacctcttcaga and tcttgttacatgtctcctttctca; IL-8: tcaaagaactgagagtgattgaga and gagctctcttccatcagaaagc. Primer sequence was determined using the Primer 3 software [22]. Amplification reactions were performed in a final volume of 25 μl using SybrGreen reaction mix (Eurogentec) in the presence of 300 nM of the adequate primers and 0.5 μl of total cDNA. Real time PCR and fluorescence quantification were performed in a Lightcycler GeneAmp 5700 (Applied Biosystems). The level of β-actin mRNA was used as an internal control for normalization.

2.5. Transient transfections

The pcDNA3.1 (Invitrogen) expression vector coding for human PPARγ was generated by subcloning from a pSG5-PPARγ vector. The dominant negative form of the receptor (PPARγDN) was generated by deleting the 15-carboxy-terminal amino acids from the wild-type receptor [23]. The pTkpGL3 plasmid derived from a pGL3 vector (Promega) and luciferase reporter gene assays were previously described [24]. Transient transfection of A549 cells was performed using Fugene 6 (Roche) according to the manufacturer's instructions.

2.6. Nuclear protein extraction

Nuclear protein extracts were prepared as previously described [25]. Cytoplasmic buffer contained 10 mM Hepes, pH 7.9, 10 mM KCl, 2 mM $MgCl_2$, 0.1 mM EDTA, 0.2% (v/v) Nonidet P-40, and 1.6 mg/ml protease inhibitors (Complete, Roche). Pelleted nuclei were resuspended in 20 mM Hepes, pH 7.9, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.63 M NaCl, 25% (v/v) glycerol, and 1.6 mg/ml protease inhibitors (nuclear buffer), incubated for 20 min at 4 °C